FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GLOB2007bF

Product name: Observer Pro

Chemical active substances:

Zoxamide, 67.5 g/L
Propamocarb-HCl, 450 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Globachem NV

Submission date: November 2023

Update: July 2024

MS Finalisation date: 31/10/2024

Version history

When	What
November 2023	Initial dossier submission by applicant for approval of new product
December 2023	Submission to the Polish Ministry of Agriculture and Rural Development
March 2024	Submission to the evaluation unit
July 2024	Applicant revision 01 to address zRMS initial comments
July 2024	zRMS finalised evaluation
October 2024	zRMS finalised evaluation after commenting period

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9 Ecotoxicology (KCP 10)

Review Comments:

This application was submitted by Globachem NV for approval of the formulation Observer Pro (Product code: GLOB2007bF) containing 67.5 g/L of zoxamide and 450 g/L of propamocarb-HCl a suspension concentrate for use as a fungicide on: potato (seed potato, ware and starch potato).

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

Since this document is based on the information provided by the Applicant, all review comments, additions, and corrections have been made using commenting boxes or highlighted in grey. Any incorrect data or text not evaluated by the zRMS has been crossed out.

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 saf-ener/ 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)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CZ, HU, IE, PL, RO, SK, DE, BE, NL	Potato	F	PHYTIN	Downwards spraying	BBCH 21-79	a) 3 b) 3	7	a) 2 b) 2	a) 135 Zoxamide + 900 Propamocarb b) 405 Zoxamide + 2700 Propamocarb	150-300	7	/							
2	CZ, HU, IE, PL, RO, SK DE, BE, NL	Potato	F	PHYTIN	Downwards spraying	BBCH 21-79	a) 3 **b) 3**	7	a) 1.9 b) 5.7	a) 130 Zoxamide + 855 Propamocarb b) 390 Zoxamide + 2565 Propamocarb	150-300	7	Alternative GAP with a slightly lower dose rate in order to maintain a mitigation of maximum 10 m VFS only where necessary							

* 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

Review Comments:

GAP presented in the Table 9.1-1 of this document is revised with consideration of the outcome of the evaluation performed in area of ecotoxicology.

9.1.1 Overall conclusions

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

Birds
The risk assessment for birds and mammals was carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

The TER_a value is greater than the Annex VI trigger of 10, indicating low acute risk to birds from zoxamide and metabolites following application of GLOB2007bF at the intended GAP. The TER_{lt} value for zoxamide and metabolites is greater than the Annex VI trigger of 5, indicating that GLOB2007bF presents no unacceptable long-term risk to birds when applied according to the proposed GAP.

According to the first-tier assessment for potato the TER_a and TER_{lt} for propamocarb-HCL are greater than the Annex VI trigger of 5 and 10, respectively, indicating that GLOB2007bF presents an acceptable acute and long-term risk to birds according to the intended uses on potato.

The risk assessment for secondary poisoning, required for zoxamide and its metabolites, showed that the risk for earthworm-eating and fish-eating birds is acceptable following use of GLOB2007bF according to the proposed use pattern. Furthermore, the risk assessment for exposure to zoxamide *via* drinking water also showed an acceptable risk. Furthermore, for mixture toxicity acceptable risk could be demonstrated.

Mammals
The risk assessments for mammals indicated that the TER_a and TER_{lt} values for zoxamide (screening step) and for propamocarb-HCl (First-tier), are greater than the Annex VI trigger of 10 or 5 respectively indicating that the use of GLOB2007bF in potato according to the proposed GAP poses a low acute and long-term risk to mammals. Furthermore, for mixture toxicity acceptable risk could be demonstrated
The risk assessment for secondary poisoning, required for zoxamide and its metabolites, showed that the risk for earthworm-eating and fish-eating mammals is acceptable following use of GLOB2007bF according to the proposed use pattern.
Furthermore, the risk assessment for exposure to zoxamide *via* drinking water also showed an acceptable risk.
Tests on other terrestrial vertebrate wildlife (reptiles and amphibians) are not required.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

An acceptable risk for the formulation GLOB2007bF in potato is acceptable with the following mitigation measures:

For the countries that accept the EU agreed endpoints (see part B8) and for the countries that do not accept the EU agreed endpoints but where R3 is not relevant:

- SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies.
- *OR in case VFSMOD is accepted: a 5 m no spray buffer zone including a 5 m vegetated filter strip.*

For the countries that do not accept the EU agreed endpoints and where R3 is relevant:

- SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 15 m including a 15 m vegetated filter strip to surface water bodies. Alternatively, apply up to 1.9L/ha and respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies.
- *OR in case VFSMOD is accepted: a 5 m no spray buffer zone including a 5 m vegetated filter strip.*

Concerned Member States must decide on the consideration of mitigation measures guidance on National level.

9.1.1.3 Effects on bees (KCP 10.3.1)

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

The risk for bees is acceptable when using GLOB2007bF according to the intended uses.
No risk mitigation measures are needed.

Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on National level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk for non-target arthropods is acceptable when using GLOB2007bF according to the intended uses.
No risk mitigation measures are needed.

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

The TER values indicate an acceptable risk for earthworms and other non-target soil organisms for the intended use of GLOB2007bF.
The EU review for zoxamide and propamocarb-HCl and the test on the formulation show that there are no effects on soil microbial activity at dose rates much higher than the corresponding PEC_{soil} of the intended use. Therefore, it is concluded that there is no unacceptable risk on soil microbial activity for GLOB2007bF.

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

First tier risk assessment indicates that there is no unacceptable risk from GLOB2007bF for non-target plants when applied according to the proposed use rates.

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

Tests on other non-target species are not required.

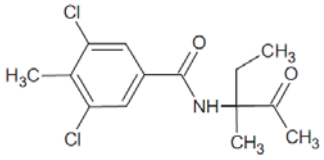
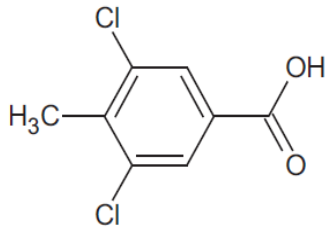
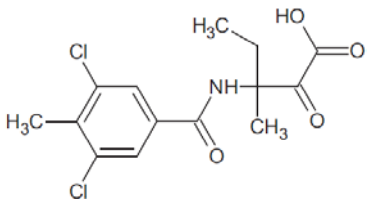
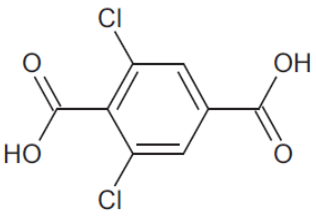
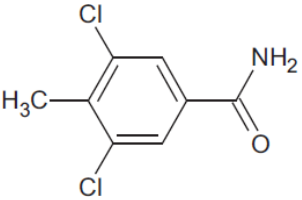
9.1.2 Grouping of intended uses for risk assessment

Not relevant, the GAP contains only one 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 GLOB2007bF is indicated in the table.

Table 9.1-2 Metabolites of zoxamide

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
RH-127450	302.15		Soil: 15.1% Water/Sediment: 39.3%	Yes, for soil and aquatic organisms
RH-24549	205		Soil: 33.8% Water/Sediment: 5%	Yes, for soil and aquatic organisms
RH-163353	332.15		Soil: 15% Water/Sediment: 20.6%	Yes, for soil and aquatic organisms
RH-141455	235.02		Soil: 8.4% Water/Sediment: 2.1%	Yes, for soil and aquatic organisms
RH-139432	204.06		Soil: 4.9% Water/Sediment: 42.4%	Yes, for aquatic organisms

There are no relevant metabolites of propamocarb-HCl to be considered.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with zoxamide and propamocarb-HCl. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide and propamocarb-HCl.
However, the provision of further data on the formulation is not considered essential, because the risk for birds from GLOB2007bF can be adequately assessed from the risk assessment of the active substances. 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)*	zoxamide	Acute	LD_{50} **>2000** mg/kg bw	EFSA Journal 2017;15(9):4980
Bobwhite quail *(Colinus virginianus)*	zoxamide	Short-term	LD_{50} = 1889.3 mg/kg bw/d	EFSA Journal 2017;15(9):4980
Mallard duck *(Anas plathyrhynchos)*	zoxamide	Short-term	LD_{50} = 1597.7 mg/kg bw/d	EFSA Journal 2017;15(9):4980
Mallard duck *(Anas plathyrhynchos)*	zoxamide	Reproduction	NOEC = **122.8** g a.s./kg bw/d	EFSA Journal 2017;15(9):4980
Bobwhite quail *(Colinus virginianus)*	zoxamide	Reproduction	NOEC = 170.9 g a.s./kg bw/d	EFSA Journal 2017;15(9):4980
Bobwhite quail	Propamocarb-HCl	Acute Oral	**>1842 mg a.s./kg b.w.**	EFSA Scientific Report (2006) 78, 1-80
Bobwhite quail	Propamocarb-HCl	Short-term dietary	>962 mg a.s./kg b.w./day*	EFSA Scientific Report (2006) 78, 1-80
Bobwhite quail	Propamocarb-HCl	Long-term dietary/reproductive	**105 mg a.s./kg b.w./day**	EFSA Scientific Report (2006) 78, 1-80

* No mortality or moribund effects were observed in the short-term dietary toxicity test providing an endpoint of >962 mg as/kg bw/day (corresponding to the maximal tested concentration of 5000 mg as/kg feed). Therefore, the LD_{50} >1842 mg a.s./kg b.w. can be used for the acute TER calculation for Propamocarb-HCl.
Values shown in **bold** used for risk assessment

9.2.1.1 Justification for new endpoints

Not relevant as there is no deviation from the EU agreed endpoints.

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 screening ~~and first-tier~~ risk assessments are summarised in the following tables.

Table 9.2-2: Screening ~~First-tier~~ assessment of the acute and long-term/reproductive risk for birds due to the use of GLOB2007bF in potato

Intended use	**Potato**				
Active substance/product	zoxamide				
Application rate (g/ha)	3 × 135				
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**
Screening step	Small omnivorous bird	158.8	1.6	34.3	58.3
Reprod. toxicity (mg/kg bw/d)	122.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small omnivorous bird	64.8	2.0 × 0.53	9.27	13.2
Intended use	**Potatoes**				
Active substance/product	Propamocarb-HCl				
Application rate (g/ha)	3 × 900 g a.i./ha				
Acute toxicity (mg/kg bw)	>1842				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
-	Small omnivorous bird	158.8	1.6	228.67	**8.2**
Reprod. toxicity (mg/kg bw/d)	105				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
-	Small omnivorous bird	64.8	2.0 x 0.53	61.82	**1.7**

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 the acute and chronic TER values of Propamocarb-HCl are below the thresholds, a first-tier assessment needs to be performed for this active only.

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of GLOB2007bF in potato

Intended use	**Potatoes**				
Active substance	Propamocarb-HCl				
Application rate (g/ha)	3 × 900 g a.i./ha				
Acute toxicity (mg/kg bw)	>1842				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
BBCH ≥ 20	Small insectivorous bird "wagtail"	25.2	1.6	36.288	50.8
BBCH ≥ 40	Small omnivorous bird "lark"	7.2	1.6	10.368	177.7
BBCH 10 - 39	Small omnivorous bird "lark"	24.0	1.6	34.56	53.3
Reprod. toxicity (mg/kg bw/d)	105				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 20	Small insectivorous bird "wagtail"	9.7	2.0 x 0.53	9.2538	11.3
BBCH ≥ 40	Small omnivorous bird "lark"	3.3	2.0 x 0.53	3.1482	33.4
BBCH 10 - 39	Small omnivorous bird "lark"	10.9	2.0 x 0.53	10.3986	10.1
Active substance/product	zoxamide				
Application rate (g/ha)	3 × 135 g a.i./ha				
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**
BBCH ≥ 20	Small insectivorous bird "wagtail"	25.2	1.6	5.4432	367.431
Reprod. toxicity (mg/kg bw/d)	122.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH 10 - 39	Small omnivorous bird "lark"	10.9	2.0 x 0.53	1.55979	78.729

Assessment of combined toxicity

Finney's equation approach (acute toxicity):

Step 1: Calculation of surrogate LD_{50} values for acute effects (mortality)

An often used model for estimating the toxicity of mixtures is the assumption of dose or concentration additivity of toxicity (Cf. 'Finney's equation': EFSA/2009/1438). There is evidence that such LD_{50} values predicted on the assumption of a similar mode-of-action should normally give a more conservative estimate of actual mixture toxicity than models based on the assumption of independent action. The

following equation can be used for deriving a surrogate LD50 for a mixture of active substances with known toxicity assuming dose 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]

Based on a.i. content in the formulation, according to the above equation, in GLOB2007bF the toxicity is:
$LD_{50(GLOB2007bF)}$ = 2000 mg/kg bw/d.

Step 2, Step 3: Not performed (not necessary since Step 1 was followed).

Step 4: Appropriate exposure estimates for a risk assessment based on calculated mixture toxicity

An LD_{50} for a mixture of active substances calculated assuming dose additivity can be conceived as an LD_{50} of a single virtual compound. It is thus deemed the most logical approach to also base the exposure side of the risk assessment on the same assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of this virtual compound.
In the case of GLOB2007bF, the total application rate will be 2×(65+450)=1030 g a.i. total/ha

For multiple applications, the default MAF values of Tier 1 can also be applied to the mixture as a single virtual compound.

Therefore, the risk assessment for the formulation GLOB2007bF is as follows:

Table 9.2-4: Screening step and Tier 1 assessment of the acute risk for birds due to the use of GLOB2007bF in potato (using Finney's equation approach)

Intended use	**Potato**				
Active substance/product	GLOB2007bF				
Application rate (kg/ha)	3 × 1.03				
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**
Screening step	Small omnivorous bird	158.8	1.6	261.7	7.6
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods	25.2	1.6	41.5	48.2
Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25%	7.2	1.6	11.9	168.6

	crop leaves 25% weed seeds 50% ground arthropods				
Potatoes BBCH 10 - 39	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	24.0	1.6	39.6	50.6

When a product contains more than one active substance, an additional assessment on combined toxicity risk has to be presented. It is considered that a quantitative toxicity risk assessment according to concentration addition is not needed if one of the following points applies:

- The risk assessment for all active substances in the product passes with a high margin of safety
- One active substance clearly drives the risk assessment

These conditions are assessed following a step-wise approach. Note that for the calculation only the scenario with the lowest TER values was considered (most critical scenario). This safely covers all other scenarios.

1st step: Margin of safety
Condition: all TER values are > Trigger × n (n = number active substances in the mixture; in this case 2). For this reason, the applicant has calculated the first-tier TER values for zoxamide with regard to the worst-case scenario for propamocarb-HCl at that level (Table 9.2-3).

2nd step: Risk per fraction
Condition: One a.s. contributes to ≥ 90% of the predicted combined toxicity of the product.
Assessment: The contribution of each individual a.s. to the combined toxicity (risk per fraction, rpf) is estimated based on the following equation:

$$rpf_{a.s.1} = \frac{1}{TER_{a.s.1}} / \left(\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} \ldots + \frac{1}{TER_{a.s.i}}\right)$$

The estimation is based on TER values from the same refinement level to assure comparability.

3rd step: TER_{MIX} calculation
Condition: The combined toxicity is acceptable if $TER_{MIX} \geq 10$ (acute) or 5 (long-term)
Assessment: The combined toxicity risk (TER_{MIX}) with concentration-addition is estimated based on the following equation:

$$TER_{mix} = 1 / \left(\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} \ldots + \frac{1}{TER_{a.s.i}}\right)$$

Table 9.2-5: Combined toxicity assessment – birds

Intended use	Potatoes, BBCH 21-79 ~~10-19~~				
Active substances	zoxamide, propamocarb-HCl				
Application rate	2 L/ha				
	TER values		**Step 1 All TER ≥ trigger x n**	**Step 2 Rpf_{max}**	**Step 3 TER_{MIX}**
	zoxamide	propamocarb-HCl			
~~Acute~~	~~267.474~~	~~50.9~~	~~Yes~~	~~Not needed~~	~~Not needed~~
Chronic	78.729	10.1	Yes	Not needed	Not needed

An acceptable acute and chronic risk for birds from the combined exposure to all active substances in the product can be concluded due to a high margin of safety (all TER values > trigger × 2).

9.2.2.2 Higher-tier risk assessment

Not needed.

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 GLOB2007bF is not a product for spray applications / not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a K(f)oc of 1207 mL/g, zoxamide belongs to the group of more sorptive substances.
With a K(f)oc of 263.65 mL/g, propamocarb-HCl belongs to the group of less sorptive substances.

To take into account the 3 applications, a MAF of 2 was used as a worst-case (effective application rate = application rate x 2).

zoxamide				
Effective application rate (g/ha)	=	270		
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.135
Reprod. toxicity (mg/kg bw/d)	=	122.8	quotient =	2.199
propamocarb-HCl				
Effective application rate (g/ha)	=	1800		
Acute toxicity (mg/kg bw)	=	1842	quotient =	0.977
Reprod. toxicity (mg/kg bw/d)	=	105	quotient =	17.143

~~To take into account the 3 applications, a MAF of 1.6 was used as a worst-case (effective application rate = application rate x 1.6).~~

~~zoxamide~~				
~~Effective application rate (g/ha)~~	~~=~~	~~216~~		
~~Acute toxicity (mg/kg bw)~~	~~=~~	~~2000~~	~~quotient =~~	~~0.108~~
~~Reprod. toxicity (mg/kg bw/d)~~	~~=~~	~~122.8~~	~~quotient =~~	~~1.759~~
~~propamocarb-HCl~~				
~~Effective application rate (g/ha)~~	~~=~~	~~1440~~		
~~Acute toxicity (mg/kg bw)~~	~~=~~	~~1842~~	~~quotient =~~	~~0.782~~
~~Reprod. toxicity (mg/kg bw/d)~~	~~=~~	~~105~~	~~quotient =~~	~~13.714~~

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of zoxamide 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 RH-127450 amounts to 3.5 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of RH-24549 amounts to 3.83 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of RH-163353 amounts to 1.43 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore not required.

The log P_{ow} of RH-141455 amounts to 1.94 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore not required.

The log P_{ow} of RH-139432 amounts to 2.7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore not required.

The log P_{ow} of Propamocarb-HCl amounts up to 0.67 at pH 9 (EFSA, 2006) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

Risk assessment for earthworm-eating birds via secondary poisoning

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

Table 9.2-6: Assessment of the risk for earthworm-eating birds due to exposure to zoxamide via bioaccumulation in earthworms (secondary poisoning) for the intended use in potato

Parameter	zoxamide	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.0618	See dRR Part B8.
log P_{ow} / P_{ow}	3.76 / 5754.4	
Koc	1207	EFSA Journal 2017;15(9):4980
Foc	0.02	Default
BCF_{worm}	2.895	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.179	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.188	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	122.8	EFSA Journal 2017;15(9):4980
TER_{lt}	653.6	TER criterion = $\geq$ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

Table 9.2-7: Assessment of the risk for earthworm-eating birds due to exposure to RH-127450 via bioaccumulation in earthworms (secondary poisoning) for the intended use in potato

Parameter	RH-127450	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.014	See dRR Part B8.
log P_{ow} / P_{ow}	3.5 / 3162	
Koc	669	EFSA Journal 2017;15(9):4980
Foc	0.02	Default
BCF_{worm}	2.899	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.041	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.043	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	12.28	EFSA Journal 2017;15(9):4980, parent endpoint divided by 10
TER_{lt}	288.2	TER criterion = ≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

Table 9.2-8: Assessment of the risk for earthworm-eating birds due to exposure to RH-24549 via bioaccumulation in earthworms (secondary poisoning) for the intended use in potato

Parameter	RH-24549	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.0133	See dRR Part B8.
log P_{ow} / P_{ow}	3.83 / 6761	
Koc	90.55	EFSA Journal 2017;15(9):4980
Foc	0.02	Default
BCF_{worm}	45.263	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.602	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.632	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	12.28	EFSA Journal 2017;15(9):4980, parent endpoint divided by 10
TER_{lt}	19.4	TER criterion = ≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

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

Parameter	zoxamide	comments
PEC_{sw} (twa = 21 d) (mg/L) Step 1	0.0210821	See dRR Part B8.
BCF_{fish}	136	
BMF	/	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	2.867	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.456	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	122.8	EFSA Journal 2017;15(9):4980 (parent endpoint devided by 10)
TER_{lt}	269.4	TER criterion = ≥ 5; acceptable risk

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 RH-127450 via bioaccumulation in fish (secondary poisoning) for the intended use in potato

Parameter	RH-127450	comments
PEC_{sw} (twa = 21 d) (mg/L) Step 1	0.0344781	See dRR Part B8.
BCF_{fish}	136	Parent value as a surrogate
BMF	/	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	4.6890	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.7456	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	12.28	EFSA Journal 2017;15(9):4980 (parent endpoint devided by 10)
TER_{lt}	16.471	TER criterion = ≥ 5; acceptable risk

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 RH-24549 via bioaccumulation in fish (secondary poisoning) for the intended use in potato

Parameter	RH-24549	comments
PEC_{sw} (twa = 21 d) (mg/L) Step 1	0.0283548	See dRR Part B8.
BCF_{fish}	136	Parent value as a surrogate
BMF	/	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	3.8563	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.6131	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	12.28	EFSA Journal 2017;15(9):4980 (parent endpoint devided by 10)
TER_{lt}	20.028	TER criterion = ≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

The TER_a value is greater than the Annex VI trigger of 10, indicating low acute risk to birds from zoxamide and metabolites following application of GLOB2007bF at the intended GAP. The TER_{lt} value for zoxamide and metabolites is greater than the Annex VI trigger of 5, indicating that GLOB2007bF presents no unacceptable long-term risk to birds when applied according to the proposed GAP.
The risk assessment for secondary poisoning, required for zoxamide and its metabolites, showed that the risk for earthworm-eating and fish-eating birds is acceptable following use of GLOB2007bF according to the proposed use pattern.
Furthermore, the risk assessment for exposure to zoxamide *via* drinking water also showed an acceptable risk.

Review comments:

The acute and long-term risk assessment for birds performed by the Applicant is agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.
According to the screening and first-tier assessment, the TERa and TERlt for both substances are greater than the Annex VI trigger of 5 and 10, respectively, indicating that the GLOB2007bF presents an acceptable acute and long-term risk to birds according to the intended uses on potato.
There were also no negative effects regarding to drinking water exposure and of secondary poisoning (zoxamide and its metabolites RH-127450, RH-24549). Furthermore, for mixture toxicity acceptable risk could be demonstrated.
Overall, acceptable acute and reproductive risk to birds may be concluded for application of GLOB2007bF in compliance with proposed GAP.

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

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with zoxamide and propamocarb-HCl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide. However, the provision of further data on the formulation GLOB2007bF is not considered essential, because the risk for mammals from GLOB2007bF can be adequately assessed from the risk assessment for the active substances.

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	zoxamide	Acute	LD_{50} >**5000** mg/kg bw	EFSA Journal 2017;15(9):4980
Rat	zoxamide	Long term (parental)	NOAEL = 360 mg/kg bw per day	EFSA Journal 2017;15(9):4980
Rat	zoxamide	Reproductive	NOAEL = 1474 mg/kg bw per day	EFSA Journal 2017;15(9):4980
Rat	zoxamide	Long term (offspring)	NOAEL = 360 mg/kg bw per day	EFSA Journal 2017;15(9):4980
Rabbit	zoxamide	Long term (development)	NOAEL = 1000 mg/kg bw per day	EFSA Journal 2017;15(9):4980
Rat	zoxamide	Long term (development)	NOAEL = 1000 mg/kg bw per day	EFSA Journal 2017;15(9):4980
Rat	zoxamide	* Value agreed in the Peer review meeting 160 by experts	**71*** mg/kg bw/d	EFSA Journal 2017;15(9):4980
Rat	Propamocarb-HCl	Oral Acute	LC_{50} = 1330 mg/kg bw	EFSA Scientific Report (2006) 78, 1-80
Rat	Propamocarb-HCl	Reproductive toxicity (long-term)	NOAEL = 104 mg/kg bw/day	EFSA Scientific Report (2006) 78, 1-80
* Conservative endpoint, provided according to Experts‘ consultation; see Peer Review Report page 622. Values shown in **bold** used for risk assessment				

9.3.1.1 Justification for new endpoints

Not relevant as there is no deviation from the EU agreed 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 screening and first-tier risk assessments are summarised in the following tables.

Table 9.3-2: Screening step of the acute and long-term/reproductive risk for mammals due to the use of GLOB2007bF in potato

Intended use	Potato

Active substance/product	zoxamide				
Application rate (g/ha)	3 × 135				
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**
Screening step	Small herbivorous mammal	118.4	1.6	25.57	195.5
Reprod. toxicity (mg/kg bw/d)	71				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small herbivorous mammal	48.3	2.0 × 0.53	6.91	10.27
Active substance/product	propamocarb-HCl				
Application rate (g/ha)	3 × 900				
Acute toxicity (mg/kg bw)	1330				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small herbivorous mammal	118.4	1.6	170.50	**7.8**
Reprod. toxicity (mg/kg bw/d)	104				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step	Small herbivorous mammal	48.3	2.0 × 0.53	46.08	**2.28**

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of GLOB2007bF in potato

Intended use	Potato				
Active substance/product	propamocarb-HCl				
Application rate (g/ha)	3 × 900				
Acute toxicity (mg/kg bw)	1330				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods	5.4	1.6	7.78	171.0
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	40.9	1.6	58.90	22.6
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	35.1	1.6	50.54	26.3

Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	10.5	1.6	15.12	88.0
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	17.2	1.6	24.77	53.7
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	5.2	1.6	7.49	177.6
Reprod. toxicity (mg/kg bw/d)	104				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods	1.9	1.06	0.25	413.1
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.06	20.70	5.0
Potatoes BBCH 10 - 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	14.3	1.06	1.89	54.9
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	4.3	1.06	4.10	25.4
Potatoes BBCH 10 - 39	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	7.8	1.06	7.44	14.0
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	1.06	2.19	47.4
Active substance/product	zoxamide				
Application rate (g/ha)	3 × 135				
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**
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	40.9	1.6	8.83	566.0

Reprod. toxicity (mg/kg bw/d)	71				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	1.9	1.06	3.11	22.9

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

Assessment of combined toxicity

Finney's equation approach (acute toxicity):

Step 1: Calculation of surrogate LD_{50} values for acute effects (mortality)

An often used model for estimating the toxicity of mixtures is the assumption of dose or concentration additivity of toxicity (Cf. 'Finney's equation': EFSA/2009/1438). There is evidence that such LD_{50} values predicted on the assumption of a similar mode-of-action should normally give a more conservative estimate of actual mixture toxicity than models based on the assumption of independent action. The following equation can be used for deriving a surrogate LD50 for a mixture of active substances with known toxicity assuming dose 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]

Based on a.i. content in the formulation, according to the above equation, in GLOB2007bF the toxicity is:
$LD_{50(\text{GLOB2007bF})}$ = 2163.9 mg/kg bw/d.

Step 2, Step 3: Not performed (not necessary since Step 1 was followed).

Step 4: Appropriate exposure estimates for a risk assessment based on calculated mixture toxicity

An LD_{50} for a mixture of active substances calculated assuming dose additivity can be conceived as an LD_{50} of a single virtual compound. It is thus deemed the most logical approach to also base the exposure side of the risk assessment on the same assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of this virtual compound.
In the case of GLOB2007bF, the total application rate will be 2×(65+450)=1030 g a.i. total/ha

For multiple applications, the default MAF values of Tier 1 can also be applied to the mixture as a single virtual compound.

Therefore, the risk assessment for the formulation GLOB2007bF is as follows:

Table 9.3-4: Screening step assessment of the acute risk for mammals due to the use of GLOB2007bF in potato (using Finney's equation approach)

Intended use	**Potato**				
Active substance/product	GLOB2007bF				
Application rate (kg/ha)	3 × 1.03				
Acute toxicity (mg/kg bw)	2163.9				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step	Small herbivorous mammal	121.95	1.6	195.12	11.1

When a product contains more than one active substance, an additional assessment on combined toxicity risk has to be presented. It is considered that a quantitative toxicity risk assessment according to concentration addition is not needed if one of the following points applies:

- The risk assessment for all active substances in the product passes with a high margin of safety
- One active substance clearly drives the risk assessment

These conditions are assessed following a step-wise approach. Note that for the calculation only the scenario with the lowest TER values was considered (most critical scenario). This safely covers all other scenarios.

1st step: Margin of safety
Condition: all TER values are > Trigger × n (n = number active substances in the mixture; in this case 2). For this reason, the applicant has calculated the first-tier TER values for zoxamide with regard to the worst-case scenario for propamocarb-HCl at that level (Table 9.3-3).

2nd step: Risk per fraction
Condition: One a.s. contributes to ≥ 90% of the predicted combined toxicity of the product.
Assessment: The contribution of each individual a.s. to the combined toxicity (risk per fraction, rpf) is estimated based on the following equation:

$$rpf_{a.s.1} = \frac{1}{TER_{a.s.1}} / \left(\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} \ldots + \frac{1}{TER_{a.s.i}}\right)$$

The estimation is based on TER values from the same refinement level to assure comparability.

3rd step: TER_{MIX} calculation
Condition: The combined toxicity is acceptable if $TER_{MIX} \geq 10$ (acute) or 5 (long-term)
Assessment: The combined toxicity risk (TER_{MIX}) with concentration-addition is estimated based on the following equation:

$$TER_{mix} = 1 / \left(\frac{1}{TER_{a.s.1}} + \frac{1}{TER_{a.s.2}} \ldots + \frac{1}{TER_{a.s.i}}\right)$$

Table 9.3-5: Combined toxicity assessment – mammals

Intended use	Potatoes, BBCH 21-79
Active substances	zoxamide, propamocarb-HCl
Application rate	2 L/ha

	TER values		Step 1 All TER ≥ trigger x n	Step 2 Rpf_{max}	Step 3 TER_{MIX}
	zoxamide	Propamocarb-HCl			
Acute – Small herbivorous mammal "vole" Crops & cereals (100% area)	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Chronic – small herbivorous mammal "vole"	22.9	5.0	No	0.817 (propamocarb-HCl)	**4.171**

For the chronic risk for mammals from combined exposure to all active substances in the product, further refinements are required as presented below. Note that for the calculation only the scenario with the lowest TER values was considered (one scenario with TER values below 10 for both actives *i.e. BBCH ≥ 40, Small herbivorous mammal "vole"*). For the other scenarios, the TER_{MIX} is higher than 10, showing an acceptable chronic risk for mammals from combined exposure to all active substances in the product.

9.3.2.2 Higher-tier risk assessment

Small herbivorous mammal "vole", BBCH ≥ 40

Deposition factor:
For the refinement, the deposition values as described in EFSA GD, Appendix E were used. Interception can only be taken be into account at later growth stages with high vegetation coverage. Interception values according FOCUS groundwater (EFSA Journal 2014;12(5):3662) were used.

For the uses in potatoes at BBCH 40-79, the interception is 85% leading to a deposition of 15% (used in the calculation of the refined SV, see Table 9.3-5).

Table 9.3-6: **Refined reproductive risk assessment for small herbivorous mammals exposed in potatoes at BBCH 40-89, interval between applications 7 days**

Intended use	Potatoes				
Active substance/product	Zoxamide				
Application rate (g/ha)	3 × 135				
Reprod. toxicity (mg/kg bw/d)	71				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**Refined SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 40	Small herbivorous mammal "vole"	FIRbw (1.33) x RUDmean (54.2) x deposition (0.15) = 10.8129	2.0 x 0.53	1.547	45.886
Intended use	Potatoes				
Active substance/product	Propamocarb-HCl				
Application rate (g/ha)	3 × 900				
Reprod. toxicity (mg/kg bw/d)	104				
TER criterion	5				

Crop scenario Growth stage	**Indicator/generic focal species**	**Refined SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
BBCH ≥ 40	Small herbivorous mammal "vole"	FIRbw (1.33) x RUDmean (54.2) x deposition (0.15) = 10.8129	2.0 x 0.53	10.316	10.081

After refinement, all TER values are > Trigger × n, showing an acceptable chronic risk for mammals from the combined exposure to all active substances in the product.

Review comments:

The Applicant proposed following refinement steps: to use a deposition factor of 0.15 considering 85% crop interception for BBCH 40-89 according to Appendix E of Guidance Document on Risk Assessment for Birds & Mammals (EFSA, 2009).
The Appendix E of the EFSA/2009/1438 provides interception / deposition factors for different crops and growth stages. In the context of a higher-tier assessment the more detailed interception values of the FOCUS Ground Water Assessments (Version 2.2, May 2014) can be used.
According the FOCUS Guidance for applications at BBCH 40-79 on potato, interception factor is considered 85%. Since Solanaceae plants would not be palatable itself, small herbivores would prefer and eat weeds and grasses potentially growing beneath the crop plants and crop interception applies.
Thus, zRMS considers relevant to use a deposition factor for the refinement.
In conclusion the refined long-term TER value for voles is greater than the respective trigger value, indicating an acceptable risk from proposed use of GLOB2007bF in potato.

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 1207 mL/g, zoxamide belongs to the group of more sorptive substances.
With a K(f)oc of 535.56 mL/g, propamocarb-HCl belongs to the group of more sorptive substances.

To take into account the 3 applications, a MAF of 2 was used as a worst-case (effective application rate = application rate x 2).

zoxamide				
Effective application rate (g/ha)	=	270		
Acute toxicity (mg/kg bw)	=	5000	quotient =	0.054
Reprod. toxicity (mg/kg bw/d)	=	71	quotient =	3.803
propamocarb-HCl				
Effective application rate (g/ha)	=	1800		
Acute toxicity (mg/kg bw)	=	1330	quotient =	1.353

Reprod. toxicity (mg/kg bw/d) =	104	quotient =	17.308

~~To take into account the 3 applications, a MAF of 1.6 was used as a worst-case (effective application rate = application rate x 1.6).~~

~~zoxamide~~			
~~Effective application rate (g/ha) =~~	~~216~~		
~~Acute toxicity (mg/kg bw) =~~	~~5000~~	~~quotient =~~	~~0.0432~~
~~Reprod. toxicity (mg/kg bw/d) =~~	~~71~~	~~quotient =~~	~~3.0423~~
~~propamocarb-HCl~~			
~~Effective application rate (g/ha) =~~	~~1440~~		
~~Acute toxicity (mg/kg bw) =~~	~~1330~~	~~quotient =~~	~~1.083~~
~~Reprod. toxicity (mg/kg bw/d) =~~	~~104~~	~~quotient =~~	~~13.846~~

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of zoxamide 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 RH-127450 amounts to 3.5 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of RH-24549 amounts to 3.83 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

The log P_{ow} of RH-163353 amounts to 1.43 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore not required.

The log P_{ow} of RH-141455 amounts to 1.94 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore not required.

The log P_{ow} of RH-139432 amounts to 2.7 and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore not required.

The log P_{ow} of Propamocarb-HCl amounts up to 0.67 at pH 9 (EFSA, 2006) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not 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 9.3-7: Assessment of the risk for earthworm-eating mammals due to exposure to zoxamide via bioaccumulation in earthworms (secondary poisoning) for the intended use in potato

Parameter	zoxamide	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.0618	See dRR Part B8.
log P_{ow} / P_{ow}	5754.4	
Koc	1207	EFSA Journal 2017;15(9):4980
foc	0.02	Default

Parameter	zoxamide	comments
BCF_{worm}	2.895	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.179	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.229	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	71	EFSA Journal 2017;15(9):4980
TER_{lt}	310.0	TER criterion = ≥ 5; acceptable risk

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 RH-127450 via bioaccumulation in earthworms (secondary poisoning) for the intended use in potato

Parameter	RH-127450	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.014	See dRR Part B8.
log P_{ow} / P_{ow}	3162	
Koc	669	EFSA Journal 2017;15(9):4980
foc	0.02	Default
BCF_{worm}	2.899	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.041	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.052	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	7.1	EFSA Journal 2017;15(9):4980, parent endpoint divided by 10
TER_{lt}	136.7	TER criterion = ≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

Table 9.3-9: Assessment of the risk for earthworm-eating mammals due to exposure to RH-24549 via bioaccumulation in earthworms (secondary poisoning) for the intended use in potato

Parameter	RH-24549	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.0133	See dRR Part B8.
log P_{ow} / P_{ow}	6761	
Koc	90.55	EFSA Journal 2017;15(9):4980
foc	0.02	Default
BCF_{worm}	45.263	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.602	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.771	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	7.1	EFSA Journal 2017;15(9):4980, parent endpoint divided by 10

Parameter	RH-24549	comments
TER_{lt}	9.2	TER criterion = ≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

Risk assessment for fish-eating mammals via secondary poisoning

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

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

Parameter	zoxamide	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0210821	See dRR Part B8.
BCF_{fish}	136	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	2.867	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.407	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	71	EFSA Journal 2017;15(9):4980
TER_{lt}	174.4	TER criterion = ≥ 5; acceptable risk

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 RH-127450 via bioaccumulation in fish (secondary poisoning) for the intended use in potato

Parameter	RH-127450	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0344781	See dRR Part B8.
BCF_{fish}	136	Parent endpoint as a surrogate
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	4.689	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.666	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	7.1	EFSA Journal 2017;15(9):4980 (parent endpoint devided by 10)
TER_{lt}	10.7	TER criterion = ≥ 5; acceptable risk

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 RH-24549 via bioaccumulation in fish (secondary poisoning) for the intended use in potato

Parameter	RH-24549	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0283548	See dRR Part B8.
BCF_{fish}	136	Parent endpoint as a surrogate

BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	3.856	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.548	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	7.1	EFSA Journal 2017;15(9):4980 (parent endpoint devided by 10)
TER_{lt}	13.0	TER criterion = ≥ 5; acceptable risk

TER values shown in bold fall below the relevant trigger.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

The risk assessments for mammals indicated that the TER_a and TER_{lt} values are greater than the Annex VI trigger of 10 or 5 respectively, indicating that the use of GLOB2007bF in potato according to the proposed GAP poses a low acute and long-term risk to mammals.

Review comments:

The acute and long-term risk assessment for mammals performed by the Applicant is agreed by the zRMS. It was performed in line with recommendations of the EFSA (2009) with assumption of EU agreed endpoints. No formulation study was required.
TER_A and TER_{LT} in the acute and long-term risk assessment indicated acceptable risk assessment for both active substances.
Provided risk assessment for the mixture indicated acceptable risk.
GLOB2007bF presents no unacceptable risk to mammals resulting from exposure via drinking water. Presented secondary poisoning for zoxamide and its metabolites RH-127450, RH-24549 presents no unacceptable risk to mammals.
Overall, acceptable acute and reproductive risk to mammals may be concluded for application of GLOB2007bF in compliance with proposed GAP.

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

Not required.

Review comments:

This issue is not assessed at the product level.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with zoxamide and its relevant metabolites and propamocarb-HCl. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide. 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 – zoxamide, propamocarb-HCl and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	zoxamide	Acute 96 hr (flow-through)	Mortality, LC_{50} **0.16 mg a.s./L (mm)**	EFSA Journal 2017;15(9):4980
Lepomis macrochirus	zoxamide	Acute 96 hr (flow-through)	Mortality, LC_{50} >0.79 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980
Pimephales promelas	zoxamide	Acute 96 hr (flow-through)	Mortality, LC_{50} >208 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980
Brachydanio rerio	zoxamide	Acute 96 hr (flow-through)	Mortality, LC_{50} >0.73 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980
Cyprinodon variegatus	zoxamide	Acute 96 hr (flow-through)	Mortality, LC_{50} >0.85 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980
Danio rerio	Preparation	Acute 96 hr (static)	Mortality, LC_{50} 0.865 mg prep./L (0.184 mg a.s./L)	EFSA Journal 2017;15(9):4980
Oncorhynchus mykiss	RH-139432	Acute 96 hr (flow-through)	Mortality, LC_{50} 2 mg/L (mm)	EFSA Journal 2017;15(9):4980
Oncorhynchus mykiss	RH-24549	48h-semi static	Mortality, LC_{50} 23 mg/L (mm)	EFSA Journal 2017;15(9):4980
Oncorhynchus mykiss	RH-141455	Acute 96 hr	Mortality, LC_{50} >100 mg/L (mm)	xxxxxxxx, 2020, 3202716 (Gowan, vertebrate study)
Oncorhynchus mykiss	RH-163353	Acute 96 hr	Mortality, LC_{50} >100 mg/L (mm)	xxxxxxxx, 2020, 3202385 (Gowan, vertebrate study)
Oncorhynchus mykiss	RH-127450	Acute 96 hr	Mortality, LC_{50} 4.17 mg/L	xxxxxxxxx, 2020, 3202373 (Gowan, vertebrate study)

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	zoxamide	95 d (flow- through, ELS)	NOEC **0.00348 mg a.s/L (mm)**	EFSA Journal 2017;15(9):4980
Pimephales promelas	zoxamide	202 d (flow- through, FLC)	NOEC 0.06 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980
Lepomis macrochirus	zoxamide	28 day (flow-through, bioaccumulation)	BCF 95-136 mg a.s./L	EFSA Journal 2017;15(9):4980
Danio rerio	zoxamide	30 days - post- hatch under flow-through conditions, ELS	NOEC ≥0.12 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980
Bluegill Sunfish (Lepomis macrochirus)	Propamocarb-HCl	96 hours	Mortality, LC_{50} > 92 mg/L	EFSA Scientific Report (2006) 78, 1-80
Bluegill sunfish (Lepomis macrochirus)	Propamocarb-HCl	32 days	NOEC >6.3 mg/L	EFSA Scientific Report (2006) 78, 1-80
Aquatic invertebrates				
Daphnia magna	zoxamide	48 h (flow- through)	Mortality, EC_{50} >0.78 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980
Mysidopsis bahia	zoxamide	96h (flow- through)	Mortality, LC_{50} **0.076 mg a.s./L (mm)**	EFSA Journal 2017;15(9):4980
Americamysis bahia	RH-163353	96h, s	Mortality, LC_{50} > **24 mg RH-163353/L** ~~a.s./L~~ (nominal)	Shaw, A., 2023, 14365.6102
Mysidopsis bahia	RH-139432	96h, ss	Mortality, LC_{50} **7.343 mg RH-139432 /L** (mm)	Mikulas, J., 2023, 25769-22
Mysidopsis bahia	RH-24549	96h, ss	Mortality, LC_{50} **35.766 mg RH-24549/L** (mm)	Doig, A., 2023, 25772-22
Mysidopsis bahia	RH-141455	96h, ss	Mortality, LC_{50} **> 100 mg RH-141455 /L** (nominal)	Mikulas, J., 2023, 25771-22
Mysidopsis bahia	RH-127450	96h, ss	Mortality, LC_{50} **0.364 mg RH-127450/L** (mm)	Mikulas, J., 2023, 25833-22
Daphnia magna	Preparation	48 h (static)	Mortality, EC_{50} >3.0 mg prep./L (>0.69 mg a.s./L)	EFSA Journal 2017;15(9):4980
Daphnia magna	zoxamide	21d (flow- through)	NOEC 0.039 mg a.s./L (mm)	EFSA Journal 2017;15(9):4980

Species	Substance	Exposure System	Results	Reference
Chironomus riparius	zoxamide	28d (flow- through)	NOEC 0.38 mg a.s./L (geomean) EC_{10} (development rate) **0.223mg a.s./L** EC_{10} (emergence rate) 0.318 mg a.s./L	EFSA Journal 2017;15(9):4980
Mysidopsis bahia	zoxamide	27 d (flow- through)	NOEC **0.0072 mg a.s./L (mm)**	EFSA Journal 2017;15(9):4980
Daphnia magna	RH-139432	48h (semi-static)	Mortality, EC_{50} 17 mg/L (mm)	EFSA Journal 2017;15(9):4980
Daphnia magna	RH-24549	48h - static	Mortality, EC_{50} 40 mg/L (mm)	EFSA Journal 2017;15(9):4980
Daphnia magna	Propamocarb-HCl	48 hours	48 hours Mortalities, EC_{50} >**100** mg/L	EFSA Scientific Report (2006) 78, 1-80
Daphnia magna	Propamocarb-HCl	21 days	NOEC=**12.3**mg/ L	EFSA Scientific Report (2006) 78, 1-80
Algae				
Selenastrum capricornutum	Preparation	96h (static)	Growth rate: E_rC_{50} (NOEC) **0.274 mg prep./L (0.0582 mg a.s./L)** Biomass: E_bC_{50} (NOEC) 0.24 mg prep./L (0.0514 mg a.s./L)	EFSA Journal 2017;15(9):4980
Pseudokirchneriella subcapitata	zoxamide	72h static	EC_{50} growth rate: 38.311 µg/L mm EC_{50} yield: 20.217 µg/L NOEC: 6.014 µg/L mm	Jarratt, N., 2023, FR/002786
Desmodesmus subspicatus	RH-24549	72h static	E_bC_{50} >60 mg/L (nom) Growth rate: E_rC_{50} **>60 mg/L (nom)**	EFSA Journal 2017;15(9):4980
Pseudokirchneriella subcapitata	RH-141455	72h static	EC_{50} **>100 mg/L (nom)** EC_{10} yield 96.3 mg/L (nom) EC_{10} growth rate >100 mg/L (nom)	EFSA Journal 2017;15(9):4980

Species	Substance	Exposure System	Results	Reference
Raphidocelis subcapitata	RH-163353	72h, s	EC_{50} yield > 100 mg/L (mm ~~nom~~) EC_{50} growth rate >**100 mg/L** (mm ~~nom~~)	Softcheck, K., 2023, 14365.6101
Pseudokirchneriella subcapitata	RH-127450	72h, s	EC_{50} yield > 1.32 mg/L (mm) EC_{50} growth rate **> 1.32** mg/L (mm)	Mikulas, J., 2023 25834-22
Pseudokirchneriella subcapitata	RH-139432	72h, s	EC_{50} yield 6.498 mg/L (mm) EC_{50} growth rate **> 9.987** mg/L (mm)	Mikulas, J., 2023, 25770-22
Pseudokirchneriella subcapitata	Propamocarb-HCl	72h, s	Growth Rate, EC_{50} >**85**mg/L	EFSA Scientific Report (2006) 78, 1-80
Aquatic plant				
Lemna gibba	zoxamide	14 d (static renewal)	7 d- EC_{50} >0.018 mg a.s./L(mm) 4 d- EC_{50} 0.017 mg a.s./L(mm) NOEC **0.009 mg a.s/L**	EFSA Journal 2017;15(9):4980
Lemna gibba	Propamocarb-HCl	14 days	Frond No., EC_{50} >18mg/L	EFSA Scientific Report (2006) 78, 1-80
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; im: based on initial measured concentrations

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB2007bF

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	GLOB2007bF	96 h, ss	LC_{50} > 9.45 mg/L $_{mm}$	xxxxxxxx, 2023 169561230
Daphnia magna	GLOB2007bF	48 h, ss	EC_{50} > 10.6 mg/L $_{mm}$	Thorpe, K., 2023 FR/002723
Pseudokirchneriella subcapitata	GLOB2007bF	72 h, s	E_rC_{50} = 1.137 mg/L $_{nom}$ E_yC_{50} = 0.531 mg/L $_{nom}$ 72-h ErC_{50} =86.2 µg zoxamide/L measured 72-h ErC_{50}=563 µg propamocarb hydrochloride /L measured	Wright, E., 2023 FR/002722

Species	Substance	Exposure System	Results	Reference
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

Not relevant as there is no deviation from the EU agreed endpoints.

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 zoxamide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB2007bF in potato (single application)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. Prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic Plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Mysidopsis bahia***	***Selenastrum capricornutum***	***Chironomus riparius***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{10} (development rate)	NOEC
(µg/L)		160	3.48	76	7.2	58.2	223	9
AF		100	10	100	10	10	10	10
RAC (µg/L)		1.6	0.348	0.76	0.72	5.82	22.3	0.9
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	18.4873	**11.5546**	**53.1244**	**24.3254**	**25.6768**	**3.1765**	0.8290	**20.5414**
Step 2								
NEU Mar-May	1.4308	0.8943	**4.1115**	**1.8826**	**1.9872**	0.2458	0.0642	**1.5898**
NEU Jun-Sep	1.4308	0.8943	**4.1115**	**1.8826**	**1.9872**	0.2458	0.0642	**1.5898**
NEU Oct-Feb	2.9934	**1.8709**	**8.6017**	**3.9387**	**4.1575**	0.5143	0.1342	**3.3260**
SEU Mar-May	2.4726	**1.5454**	**7.1052**	**3.2534**	**3.4342**	0.4248	0.1109	**2.7473**
SEU Jun-Sep	1.9517	**1.2198**	**5.6083**	**2.5680**	**2.7107**	0.3353	0.0875	**2.1686**
SEU Oct-Feb	2.4726	**1.5454**	**7.1052**	**3.2534**	**3.4342**	0.4248	0.1109	**2.7473**

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. Prolonged	Algae	Sed. dwell. prolonged	Aquatic Plants
Step 3 (set 1, early)								
D3/ditch	0.7069	0.4418	**2.0313**	0.9301	0.9818	0.1215	0.0317	0.7854
D4/pond	0.02855	0.0178	0.0820	0.0376	0.0397	0.0049	0.0013	0.0317
D4/stream	0.5522	0.3451	**1.5868**	0.7266	0.7669	0.0949	0.0248	0.6136
D6/ditch	0.699	0.4369	**2.0086**	0.9197	0.9708	0.1201	0.0313	0.7767
R1/pond	0.6936	0.4335	**1.9931**	0.9126	0.9633	0.1192	0.0311	0.7707
R1/stream	0.05828	0.0364	0.1675	0.0767	0.0809	0.0100	0.0026	0.0648
R2/stream	0.5313	0.3321	**1.5267**	0.6991	0.7379	0.0913	0.0238	0.5903
R3/stream	0.6475	0.4047	**1.8606**	0.8520	0.8993	0.1113	0.0290	0.7194
Step 3 (set 1, late)								
D3/ditch	0.7072	0.4420	**2.0322**	0.9305	0.9822	0.1215	0.0317	0.7858
D4/pond	0.02854	0.0178	0.0820	0.0376	0.0396	0.0049	0.0013	0.0317
D4/stream	0.5315	0.3322	**1.5273**	0.6993	0.7382	0.0913	0.0238	0.5906
D6/ditch	0.7025	0.4391	**2.0187**	0.9243	0.9757	0.1207	0.0315	0.7806
R1/pond	0.7072	0.4420	**2.0322**	0.9305	0.9822	0.1215	0.0317	0.7858
R1/stream	0.05129	0.0321	0.1474	0.0675	0.0712	0.0088	0.0023	0.0570
R2/stream	0.6144	0.3840	**1.7655**	0.8084	0.8533	0.1056	0.0276	0.6827
R3/stream	0.6577	0.4111	**1.8899**	0.8654	0.9135	0.1130	0.0295	0.7308
Step 3 (set 2, early)								
D3/ditch	0.7069	0.4418	**2.0313**	0.9301	0.9818	0.1215	0.0317	0.7854
D4/pond	0.02855	0.0178	0.0820	0.0376	0.0397	0.0049	0.0013	0.0317
D4/stream	0.5522	0.3451	**1.5868**	0.7266	0.7669	0.0949	0.0248	0.6136

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. Prolonged	Algae	Sed. dwell. prolonged	Aquatic Plants
D6/ditch	0.699	0.4369	**2.0086**	0.9197	0.9708	0.1201	0.0313	0.7767
R1/pond	0.6936	0.4335	**1.9931**	0.9126	0.9633	0.1192	0.0311	0.7707
R1/stream	0.04746	0.0297	0.1364	0.0624	0.0659	0.0082	0.0021	0.0527
R2/stream	0.5308	0.3318	**1.5253**	0.6984	0.7372	0.0912	0.0238	0.5898
R3/stream	0.6475	0.4047	**1.8606**	0.8520	0.8993	0.1113	0.0290	0.7194
Step 3 (set 2, late)								
D3/ditch	0.7072	0.4420	**2.0322**	0.9305	0.9822	0.1215	0.0317	0.7858
D4/pond	0.02854	0.0178	0.0820	0.0376	0.0396	0.0049	0.0013	0.0317
D4/stream	0.5315	0.3322	**1.5273**	0.6993	0.7382	0.0913	0.0238	0.5906
D6/ditch	0.7025	0.4391	**2.0187**	0.9243	0.9757	0.1207	0.0315	0.7806
R1/pond	0.7072	0.4420	**2.0322**	0.9305	0.9822	0.1215	0.0317	0.7858
R1/stream	0.04261	0.0266	0.1224	0.0561	0.0592	0.0073	0.0019	0.0473
R2/stream	0.6137	0.3836	**1.7635**	0.8075	0.8524	0.1054	0.0275	0.6819
R3/stream	0.6577	0.4111	**1.8899**	0.8654	0.9135	0.1130	0.0295	0.7308

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 zoxamide for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of GLOB2007bF in potato (multiple applications)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. Acute**	**Inverteb. Prolonged**	**Algae**	**Sed. dwell. prolonged**	**Aquatic Plants**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Mysidopsis bahia***	***Selenastrum capricornutum***	***Chironomus riparius***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{10} (development rate)	NOEC
(µg/L)		160	3.48	76	7.2	58.2	223	9
AF		100	10	100	10	10	10	10
RAC (µg/L)		1.6	0.348	0.76	0.72	5.82	22.3	0.9
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	55.4620	**34.6638**	**159.3736**	**72.9763**	**77.0306**	**9.5296**	**2.4871**	**61.6244**
Step 2								
NEU Mar-May	2.1328	**1.3330**	**6.1287**	**2.8063**	**2.9622**	0.3665	0.0956	**2.3698**
NEU Jun-Sep	2.1328	**1.3330**	**6.1287**	**2.8063**	**2.9622**	0.3665	0.0956	**2.3698**
NEU Oct-Feb	4.6098	**2.8811**	**13.2466**	**6.0655**	**6.4025**	0.7921	0.2067	**5.1220**
SEU Mar-May	3.7841	**2.3651**	**10.8739**	**4.9791**	**5.2557**	0.6502	0.1697	**4.2046**
SEU Jun-Sep	2.9585	**1.8491**	**8.5014**	**3.8928**	**4.1090**	0.5083	0.1327	**3.2872**
SEU Oct-Feb	3.7841	**2.3651**	**10.8739**	**4.9791**	**5.2557**	0.6502	0.1697	**4.2046**
Step 3 (set 1, early)								
D3/ditch	0.5139	0.3212	**1.4767**	0.6762	0.7138	0.0883	0.0230	0.5710
D4/pond	0.05267	0.0329	0.1514	0.0693	0.0732	0.0090	0.0024	0.0585

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. Prolonged	Algae	Sed. dwell. prolonged	Aquatic Plants
D4/stream	0.4128	0.2580	**1.1862**	0.5432	0.5733	0.0709	0.0185	0.4587
D6/ditch	0.5119	0.3199	**1.4710**	0.6736	0.7110	0.0880	0.0230	0.5688
R1/pond	0.5079	0.3174	**1.4595**	0.6683	0.7054	0.0873	0.0228	0.5643
R1/stream	0.09206	0.0575	0.2645	0.1211	0.1279	0.0158	0.0041	0.1023
R2/stream	0.7032	0.4395	**2.0207**	0.9253	0.9767	0.1208	0.0315	0.7813
R3/stream	0.4759	0.2974	**1.3675**	0.6262	0.6610	0.0818	0.0213	0.5288
Step 3 (set 1, late)								
D3/ditch	0.5146	0.3216	**1.4787**	0.6771	0.7147	0.0884	0.0231	0.5718
D4/pond	0.05265	0.0329	0.1513	0.0693	0.0731	0.0090	0.0024	0.0585
D4/stream	0.4265	0.2666	**1.2256**	0.5612	0.5924	0.0733	0.0191	0.4739
D6/ditch	0.5118	0.3199	**1.4707**	0.6734	0.7108	0.0879	0.0230	0.5687
R1/pond	0.5141	0.3213	**1.4773**	0.6764	0.7140	0.0883	0.0231	0.5712
R1/stream	0.1558	0.0974	0.4477	0.2050	0.2164	0.0268	0.0070	0.1731
R2/stream	0.7277	0.4548	**2.0911**	0.9575	**1.0107**	0.1250	0.0326	0.8086
R3/stream	0.4758	0.2974	**1.3672**	0.6261	0.6608	0.0818	0.0213	0.5287
Step 3 (set 2, early)								
D3/ditch	0.5142	0.3214	**1.4776**	0.6766	0.7142	0.0884	0.0231	0.5713
D4/pond	0.03696	0.0231	0.1062	0.0486	0.0513	0.0064	0.0017	0.0411
D4/stream	0.4128	0.2580	**1.1862**	0.5432	0.5733	0.0709	0.0185	0.4587
D6/ditch	0.5119	0.3199	**1.4710**	0.6736	0.7110	0.0880	0.0230	0.5688
R1/pond	0.5079	0.3174	**1.4595**	0.6683	0.7054	0.0873	0.0228	0.5643
R1/stream	0.06533	0.0408	0.1877	0.0860	0.0907	0.0112	0.0029	0.0726

Group		Fish acute	Fish prolonged	Inverteb. Acute	Inverteb. Prolonged	Algae	Sed. dwell. prolonged	Aquatic Plants
R2/stream	0.7026	0.4391	**2.0190**	0.9245	0.9758	0.1207	0.0315	0.7807
R3/stream	0.476	0.2975	**1.3678**	0.6263	0.6611	0.0818	0.0213	0.5289
Step 3 (set 2, late)								
D3/ditch	0.515	0.3219	**1.4799**	0.6776	0.7153	0.0885	0.0231	0.5722
D4/pond	0.03877	0.0242	0.1114	0.0510	0.0538	0.0067	0.0017	0.0431
D4/stream	0.4265	0.2666	**1.2256**	0.5612	0.5924	0.0733	0.0191	0.4739
D6/ditch	0.5118	0.3199	**1.4707**	0.6734	0.7108	0.0879	0.0230	0.5687
R1/pond	0.5145	0.3216	**1.4784**	0.6770	0.7146	0.0884	0.0231	0.5717
R1/stream	0.1152	0.0720	0.3310	0.1516	0.1600	0.0198	0.0052	0.1280
R2/stream	0.7269	0.4543	**2.0888**	0.9564	**1.0096**	0.1249	0.0326	0.8077
R3/stream	0.4758	0.2974	**1.3672**	0.6261	0.6608	0.0818	0.0213	0.5287

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

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-24549 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (single application)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Desmodesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		23000	35766	>60000
AF		100	100	10
RAC (µg/L)		230	357.66	6000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	9.5245	0.041	0.027	0.0016
Step 2				
NEU Mar-May	0.6033	0.003	0.002	0.0001
NEU Jun-Sep	0.6033	0.003	0.002	0.0001
NEU Oct-Feb	1.4559	0.006	0.004	0.0002
SEU Mar-May	1.1717	0.005	0.003	0.0002
SEU Jun-Sep	0.8875	0.004	0.002	0.0001
SEU Oct-Feb	1.1717	0.005	0.003	0.0002

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-24549 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (multiple applications)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Desmodesmus subspicatus***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		23000	35766	>60000
AF		100	100	10
RAC (µg/L)		230	357.66	6000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	28.5736	0.124	0.080	0.0048
Step 2				
NEU Mar-May	0.9714	0.004	0.003	0.0002
NEU Jun-Sep	0.9714	0.004	0.003	0.0002
NEU Oct-Feb	2.3139	0.010	0.006	0.0004
SEU Mar-May	1.8664	0.008	0.005	0.0003
SEU Jun-Sep	1.4189	0.006	0.004	0.0002
SEU Oct-Feb	1.8664	0.008	0.005	0.0003

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-127450 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (single application)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Pseudokirchneriella subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		4170	364	1320
AF		100	100	10
RAC (µg/L)		41.7	3.64	132
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	12.0507	0.289	**3.311**	0.0913
Step 2				
NEU Mar-May	0.9674	0.023	0.266	0.0073
NEU Jun-Sep	0.9674	0.023	0.266	0.0073
NEU Oct-Feb	2.0112	0.048	0.553	0.0152
SEU Mar-May	1.6633	0.040	0.457	0.0126
SEU Jun-Sep	1.3153	0.032	0.361	0.0100
SEU Oct-Feb	1.6633	0.040	0.457	0.0126

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-127450 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (multiple applications)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Pseudokirchneriella subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		4170	364	1320
AF		100	100	10
RAC (µg/L)		41.7	3.64	132
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	36.1520	0.867	**9.932**	0.2739
Step 2				
NEU Mar-May	1.6815	0.040	0.462	0.0127
NEU Jun-Sep	1.6815	0.040	0.462	0.0127
NEU Oct-Feb	3.3256	0.080	0.914	0.0252
SEU Mar-May	2.7775	0.067	0.763	0.0210
SEU Jun-Sep	2.2295	0.053	0.613	0.0169
SEU Oct-Feb	2.7775	0.067	0.763	0.0210

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-139432 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (single application)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Pseudokirchneriella subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		2000	7343	9987
AF		100	100	10
RAC (µg/L)		20	73.43	998.7
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	13.0512	0.653	0.1777	0.0131
Step 2				
NEU Mar-May	1.1363	0.057	0.0155	0.0011
NEU Jun-Sep	1.1363	0.057	0.0155	0.0011
NEU Oct-Feb	2.3677	0.118	0.0322	0.0024
SEU Mar-May	1.9573	0.098	0.0267	0.0020
SEU Jun-Sep	1.5468	0.077	0.0211	0.0015
SEU Oct-Feb	1.9573	0.098	0.0267	0.0020

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-139432 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (multiple applications)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Pseudokirchneriella subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		2000	7343	9987
AF		100	100	10
RAC (µg/L)		20	73.43	998.7
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	39.1536	**1.958**	0.5332	0.0392
Step 2				
NEU Mar-May	2.1763	0.109	0.0296	0.0022
NEU Jun-Sep	2.1763	0.109	0.0296	0.0022
NEU Oct-Feb	4.4046	0.220	0.0600	0.0044
SEU Mar-May	3.6618	0.183	0.0499	0.0037
SEU Jun-Sep	2.919	0.146	0.0398	0.0029
SEU Oct-Feb	3.6618	0.183	0.0499	0.0037

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-141455 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (single application)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Pseudokirchneriella subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		>100000	>100000	>100000
AF		100	100	10
RAC (µg/L)		1000	1000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	3.3045	0.003	0.003	0.0003
Step 2				
NEU Mar-May	0.286	0.00029	0.00029	0.00003
NEU Jun-Sep	0.286	0.00029	0.00029	0.00003
NEU Oct-Feb	0.6879	0.00069	0.00069	0.00007
SEU Mar-May	0.554	0.00055	0.00055	0.00006
SEU Jun-Sep	0.42	0.00042	0.00042	0.00004
SEU Oct-Feb	0.554	0.00055	0.00055	0.00006

Table 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-141455 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (multiple applications)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Pseudokirchneriella subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		>100000	>100000	>100000
AF		100	100	10
RAC (µg/L)		1000	1000	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	9.9136	0.010	0.010	0.0010
Step 2				
NEU Mar-May	0.6481	0.00065	0.00065	0.00006
NEU Jun-Sep	0.6481	0.00065	0.00065	0.00006
NEU Oct-Feb	1.5608	0.00156	0.00156	0.00016
SEU Mar-May	1.2565	0.00126	0.00126	0.00013
SEU Jun-Sep	0.9523	0.00095	0.00095	0.00010
SEU Oct-Feb	1.2565	0.00126	0.00126	0.00013

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-163353 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (single application)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Americamysis bahia***	***Raphidocelis subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		>100000	> 24000	>100000
AF		100	100	10
RAC (µg/L)		1000	240	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	14.7443	0.015	0.061	0.0015
Step 2				
NEU Mar-May	1.2162	0.0012	0.0051	0.00012
NEU Jun-Sep	1.2162	0.0012	0.0051	0.00012
NEU Oct-Feb	2.6845	0.0027	0.0112	0.00027
SEU Mar-May	2.1951	0.0022	0.0091	0.00022
SEU Jun-Sep	1.7056	0.0017	0.0071	0.00017
SEU Oct-Feb	2.1951	0.0022	0.0091	0.00022

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for RH-163353 for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (multiple applications)

Group		**Fish acute**	**Inverteb. Acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Americamysis bahia***	***Raphidocelis subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		>100000	> 24000	>100000
AF		100	100	10
RAC (µg/L)		1000	240	10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	44.2328	0.044	0.184	0.0044
Step 2				
NEU Mar-May	2.2888	0.0023	0.0095	0.00023
NEU Jun-Sep	2.2888	0.0023	0.0095	0.00023
NEU Oct-Feb	4.9424	0.0049	0.0206	0.00049
SEU Mar-May	4.0578	0.0041	0.0169	0.00041
SEU Jun-Sep	3.1733	0.0032	0.0132	0.00032
SEU Oct-Feb	4.0578	0.0041	0.0169	0.00041

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Propamocarb-HCl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (single application)

Group		~~**Fish acute**~~	**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic Plants**
Test species		~~***Oncorhynchus mykiss***~~	***Lepomis macrochirus***	***Lepomis macrochirus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	**Lemna gibba**
Endpoint		~~LC_{50}~~	LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{50}
(µg/L)		~~>99000~~	>92000	>6300	>100000	12300	>85000	>18000
AF		~~100~~	100	10	100	10	10	10
RAC (µg/L)		~~990~~	920	630	1000	1230	8500	1800
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	27.4947	~~0.0278~~	0.0299	0.0436	0.0275	0.0224	0.0032	0.015
Step 2								
NEU Mar-May	2.8739	~~0.0029~~	0.0031	0.0046	0.0029	0.0023	0.0003	0.0016
NEU Jun-Sep	2.8739	~~0.0029~~	0.0031	0.0046	0.0029	0.0023	0.0003	0.0016
NEU Oct-Feb	6.1002	~~0.0062~~	0.0066	0.0097	0.0061	0.0050	0.0007	0.0034
SEU Mar-May	5.0248	~~0.0051~~	0.0055	0.0080	0.0050	0.0041	0.0006	0.0028
SEU Jun-Sep	3.9493	~~0.0040~~	0.0043	0.0063	0.0039	0.0032	0.0005	0.0022
SEU Oct-Feb	5.0248	~~0.0051~~	0.0055	0.0080	0.0050	0.0041	0.0006	0.0028

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

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Propamocarb-HCl for each organism group based on FOCUS Steps 1 and 2 calculations for the use of GLOB2007bF in potato (multiple applications)

Group		~~**Fish acute**~~	**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic Plants**
Test species		~~***Oncorhynchus mykiss***~~	***Lepomis macrochirus***	***Lepomis macrochirus***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchneriella subcapitata***	**Lemna gibba**
Endpoint		~~LC_{50}~~	LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	EC_{50}
(µg/L)		~~>99000~~	>92000	>6300	>100000	12300	>85000	>18000
AF		~~100~~	100	10	100	10	10	10
RAC (µg/L)		~~990~~	920	630	1000	1230	8500	1800
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	82.4841	~~0.0833~~	0.0897	0.1309	0.0825	0.0671	0.0097	0.045
Step 2								
NEU Mar-May	5.9882	~~0.0060~~	0.0065	0.0095	0.0060	0.0049	0.0007	0.0033
NEU Jun-Sep	5.9882	~~0.0060~~	0.0065	0.0095	0.0060	0.0049	0.0007	0.0033
NEU Oct-Feb	13.0968	~~0.0132~~	0.0142	0.0208	0.0131	0.0106	0.0015	0.0072
SEU Mar-May	10.7272	~~0.0108~~	0.0117	0.0170	0.0107	0.0087	0.0013	0.0059
SEU Jun-Sep	8.3577	~~0.0084~~	0.0091	0.0133	0.0084	0.0068	0.0010	0.0046
SEU Oct-Feb	5.9882	~~0.0060~~	0.0065	0.0095	0.0060	0.0049	0.0007	0.0033

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 on potato, calculated PEC/RAC ratios for zoxamide did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish as characterised by a NOEC for *Oncorhynchus mykiss* of 3.48 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 9.5-17: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (early, single application)

Intended use		Potato					
Active substance		zoxamide					
Application rate (g/ha)		3 ×135					
Nozzle reduction	**No-spray buffer (m)**	1	5	10	5	10	5
	Vegetated filter strip (m)	None	None	None	5	10	5 (VFSMOD)
None	D3 ditch	-	0.2317	0.1229	-	-	-
50 %		**0.4274**	0.1158	0.06143	-	-	-
75 %		0.2137	0.05791	0.03084	-	-	-
90 %		0.08547	0.02691	0.01668	-	-	-
None	D4 pond	-	0.02741	0.01979	-	-	-
50 %		0.02479	0.01509	0.01094	-	-	-
75 %		0.01413	0.008933	0.006508	-	-	-
90 %		0.007735	0.005239	0.003853	-	-	-
None	D4 stream	-	0.2332	0.1239	-	-	-
50 %		**0.4297**	0.117	0.06219	-	-	-
75 %		0.2153	0.05886	0.03137	-	-	-
90 %		0.08667	0.02398	0.01288	-	-	-
None	D6 ditch	-	0.2291	0.1215	-	-	-
50 %		**0.4226**	0.1145	0.06074	-	-	-
75 %		0.2113	0.05726	0.03037	-	-	-
90 %		0.08451	0.02291	0.0125	-	-	-
None	D6 ditch	-	0.2291	0.1215	-	-	-
50 %		**0.4226**	0.1145	0.06074	-	-	-
75 %		0.2113	0.05726	0.03037	-	-	-
90 %		0.08451	0.02291	0.0125	-	-	-
None	R1 pond	-	0.05812	0.05157	0.04447	0.03102	-
50 %		0.05586	0.04752	0.04396	0.03382	0.02334	-
75 %		0.0467	0.04223	0.04016	0.02851	0.01951	-
90 %		0.0412	0.03906	0.03788	0.02532	0.01721	-
None	R1 stream	-	**0.5313**	**0.5313**	0.3465	0.2415	-

50 %		**0.5313**	**0.5313**	**0.5313**	0.3465	0.2415	-
75 %		**0.5313**	**0.5313**	**0.5313**	0.3465	0.2415	-
90 %		**0.5313**	**0.5313**	**0.5313**	0.3465	0.2415	-
None	R2 stream	-	0.2743	0.1459	0.2743	0.1459	-
50 %		**0.505**	0.1454	0.1454	0.138	0.07357	-
75 %		0.2536	0.1454	0.1454	0.09378	0.06495	-
90 %		0.1454	0.1454	0.1454	0.09378	0.06495	-
None	R3 stream	-	0.2909	0.1544	0.2909	0.1544	-
50 %		**0.5364**	0.1457	0.1303	0.1457	0.07762	-
75 %		0.2684	0.1303	0.1303	0.08452	0.05888	-
90 %		0.1303	0.1303	0.1303	0.08452	0.05888	-
RAC (µg/L) 0.348		**PEC/RAC ratio**					
None	D3 ditch	-	0.6658	0.3532	-	-	-
50 %		**1.2282**	0.3328	0.1765	-	-	-
75 %		0.6141	0.1664	0.0886	-	-	-
90 %		0.2456	0.0773	0.0479	-	-	-
None	D4 pond	-	0.0788	0.0569	-	-	-
50 %		0.0712	0.0434	0.0314	-	-	-
75 %		0.0406	0.0257	0.0187	-	-	-
90 %		0.0222	0.0151	0.0111	-	-	-
None	D4 stream	-	0.6701	0.3560	-	-	-
50 %		**1.2348**	0.3362	0.1787	-	-	-
75 %		0.6187	0.1691	0.0901	-	-	-
90 %		0.2491	0.0689	0.0370	-	-	-
None	D6 ditch	-	0.6583	0.3491	-	-	-
50 %		**1.2144**	0.3290	0.1745	-	-	-
75 %		0.6072	0.1645	0.0873	-	-	-
90 %		0.2428	0.0658	0.0359	-	-	-
None	D6 ditch	-	0.6583	0.3491	-	-	-
50 %		**1.2144**	0.3290	0.1745	-	-	-
75 %		0.6072	0.1645	0.0873	-	-	-
90 %		0.2428	0.0658	0.0359	-	-	-
None	R1 pond	-	0.1670	0.1482	0.1278	0.0891	-
50 %		0.1605	0.1366	0.1263	0.0972	0.0671	-
75 %		0.1342	0.1214	0.1154	0.0819	0.0561	-
90 %		0.1184	0.1122	0.1089	0.0728	0.0495	-
None	R1 stream	-	**1.5267**	**1.5267**	0.9957	0.6940	-
50 %		**1.5267**	**1.5267**	**1.5267**	0.9957	0.6940	-

75 %		**1.5267**	**1.5267**	**1.5267**	0.9957	0.6940	-
90 %		**1.5267**	**1.5267**	**1.5267**	0.9957	0.6940	-
None	R2 stream	-	0.7882	0.4193	0.7882	0.4193	-
50 %		**1.4511**	0.4178	0.4178	0.3966	0.2114	-
75 %		0.7287	0.4178	0.4178	0.2695	0.1866	-
90 %		0.4178	0.4178	0.4178	0.2695	0.1866	-
None	R3 stream	-	0.8359	0.4437	0.8359	0.4437	-
50 %		**1.5414**	0.4187	0.3744	0.4187	0.2230	-
75 %		0.7713	0.3744	0.3744	0.2429	0.1692	-
90 %		0.3744	0.3744	0.3744	0.2429	0.1692	-

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

Table 9.5-18: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (early, multiple applications)

Intended use		Potato					
Active substance		zoxamide					
Application rate (g/ha)		3 ×135					
Nozzle reduction	**No-spray buffer (m)**	1	5	10	5	10	5
	Vegetated filter strip (m)	None	None	None	5	10	5 (VFSMOD)
None	D3 ditch	-	0.165	0.08652	-	-	-
50 %		0.3118	0.08251	0.04326	-	-	-
75 %		0.1559	0.04157	0.02362	-	-	-
90 %		0.06236	0.02273	0.01504	-	-	-
None	D4 pond	-	0.05312	0.03817	-	-	-
50 %		0.04941	0.03021	0.02182	-	-	-
75 %		0.02927	0.01875	0.01365	-	-	-
90 %		0.01719	0.01189	0.008748	-	-	-
None	D4 stream	-	0.172	0.09037	-	-	-
50 %		0.3243	0.08645	0.04553	-	-	-
75 %		0.1628	0.04369	0.02312	-	-	-
90 %		0.06582	0.01803	0.009665	-	-	-
None	D6 ditch	-	0.1644	0.08617	-	-	-
50 %		0.3106	0.08218	0.04309	-	-	-
75 %		0.1553	0.04109	0.02204	-	-	-
90 %		0.06211	0.01937	0.01186	-	-	-
None	D6 ditch	-	0.1644	0.08617	-	-	-

50 %		0.3106	0.08218	0.04309	-	-	-
75 %		0.1553	0.04109	0.02204	-	-	-
90 %		0.06211	0.01937	0.01186	-	-	-
None	R1 pond	-	0.09177	0.07786	0.07505	0.05266	-
50 %		0.08831	0.07043	0.06262	0.05366	0.03737	-
75 %		0.06955	0.05977	0.05501	0.04297	0.02973	-
90 %		0.05831	0.05337	0.05118	0.03656	0.02515	-
None	R1 stream	-	**0.7032**	**0.7032**	**0.4587**	0.3197	0.148
50 %		**0.7032**	**0.7032**	**0.7032**	**0.4587**	0.3197	0.07495
75 %		**0.7032**	**0.7032**	**0.7032**	**0.4587**	0.3197	0.03864
90 %		**0.7032**	**0.7032**	**0.7032**	**0.4587**	0.3197	0.02072
None	R2 stream	-	**0.4198**	**0.4198**	0.2704	0.1871	-
50 %		**0.4198**	**0.4198**	**0.4198**	0.2704	0.1871	-
75 %		**0.4198**	**0.4198**	**0.4198**	0.2704	0.1871	-
90 %		**0.4198**	**0.4198**	**0.4198**	0.2704	0.1871	-
None	R3 stream	-	**0.7574**	**0.7574**	**0.4938**	0.3436	0.2075
50 %		**0.7574**	**0.7574**	**0.7574**	**0.4938**	0.3436	0.1043
75 %		**0.7574**	**0.7574**	**0.7574**	**0.4938**	0.3436	0.05345
90 %		**0.7574**	**0.7574**	**0.7574**	**0.4938**	0.3436	0.04508
RAC (µg/L) 0.348		**PEC/RAC ratio**					
None	D3 ditch	-	0.4741	0.2486	-	-	-
50 %		0.8960	0.2371	0.1243	-	-	-
75 %		0.4480	0.1195	0.0679	-	-	-
90 %		0.1792	0.0653	0.0432	-	-	-
None	D4 pond	-	0.1526	0.1097	-	-	-
50 %		0.1420	0.0868	0.0627	-	-	-
75 %		0.0841	0.0539	0.0392	-	-	-
90 %		0.0494	0.0342	0.0251	-	-	-
None	D4 stream	-	0.4943	0.2597	-	-	-
50 %		0.9319	0.2484	0.1308	-	-	-
75 %		0.4678	0.1255	0.0664	-	-	-
90 %		0.1891	0.0518	0.0278	-	-	-
None	D6 ditch	-	0.4724	0.2476	-	-	-
50 %		0.8925	0.2361	0.1238	-	-	-
75 %		0.4463	0.1181	0.0633	-	-	-
90 %		0.1785	0.0557	0.0341	-	-	-
None	D6 ditch	-	0.4724	0.2476	-	-	-
50 %		0.8925	0.2361	0.1238	-	-	-

75 %		0.4463	0.1181	0.0633	-	-	-
90 %		0.1785	0.0557	0.0341	-	-	-
None	R1 pond	-	0.2637	0.2237	0.2157	0.1513	-
50 %		0.2538	0.2024	0.1799	0.1542	0.1074	-
75 %		0.1999	0.1718	0.1581	0.1235	0.0854	-
90 %		0.1676	0.1534	0.1471	0.1051	0.0723	-
None	R1 stream	-	**2.0207**	**2.0207**	**1.3181**	0.9187	0.4253
50 %		**2.0207**	**2.0207**	**2.0207**	**1.3181**	0.9187	0.2154
75 %		**2.0207**	**2.0207**	**2.0207**	**1.3181**	0.9187	0.1110
90 %		**2.0207**	**2.0207**	**2.0207**	**1.3181**	0.9187	0.0595
None	R2 stream	-	**1.2063**	**1.2063**	0.7770	0.5376	-
50 %		**1.2063**	**1.2063**	**1.2063**	0.7770	0.5376	-
75 %		**1.2063**	**1.2063**	**1.2063**	0.7770	0.5376	-
90 %		**1.2063**	**1.2063**	**1.2063**	0.7770	0.5376	-
None	R3 stream	-	**2.1764**	**2.1764**	**1.4190**	0.9874	0.5963
50 %		**2.1764**	**2.1764**	**2.1764**	**1.4190**	0.9874	0.2997
75 %		**2.1764**	**2.1764**	**2.1764**	**1.4190**	0.9874	0.1536
90 %		**2.1764**	**2.1764**	**2.1764**	**1.4190**	0.9874	0.1295

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

Table 9.5-19: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (late, single application)

Intended use		Potato					
Active substance		zoxamide					
Application rate (g/ha)		3 ×135					
Nozzle reduction	**No-spray buffer (m)**	1	5	10	5	10	5
	Vegetated filter strip (m)	None	None	None	5	10	5 (VFSMOD)
None	D3 ditch	-	0.2318	0.1229	-	-	-
50 %		**0.4276**	0.1159	0.06145	-	-	-
75 %		0.2138	0.05859	0.03388	-	-	-
90 %		0.08551	0.03242	0.02189	-	-	-
None	D4 pond	-	0.02853	0.02069	-	-	-
50 %		0.02626	0.01622	0.01185	-	-	-
75 %		0.01562	0.01008	0.007425	-	-	-
90 %		0.009229	0.00639	0.004774	-	-	-
None	D4 stream	-	0.2246	0.1193	-	-	-
50 %		**0.4138**	0.1127	0.05996	-	-	-

75 %		0.2074	0.05677	0.0303	-	-	-
90 %		0.08359	0.0232	0.0125	-	-	-
None	D6 ditch	-	0.2302	0.1221	-	-	-
50 %		**0.4247**	0.1151	0.06104	-	-	-
75 %		0.2124	0.05755	0.03052	-	-	-
90 %		0.08494	0.02554	0.01545	-	-	-
None	D6 ditch	-	0.2302	0.1221	-	-	-
50 %		**0.4247**	0.1151	0.06104	-	-	-
75 %		0.2124	0.05755	0.03052	-	-	-
90 %		0.08494	0.02554	0.01545	-	-	-
None	R1 pond	-	0.05216	0.04503	0.0418	0.02942	-
50 %		0.05009	0.04095	0.03698	0.03055	0.02133	-
75 %		0.0404	0.03536	0.03295	0.02494	0.01729	-
90 %		0.03458	0.032	0.03054	0.02157	0.01486	-
None	R1 stream	-	**0.6144**	**0.6144**	**0.4003**	0.2788	0.2065
50 %		**0.6144**	**0.6144**	**0.6144**	**0.4003**	0.2788	0.1046
75 %		**0.6144**	**0.6144**	**0.6144**	**0.4003**	0.2788	0.05364
90 %		**0.6144**	**0.6144**	**0.6144**	**0.4003**	0.2788	0.02307
None	R2 stream	-	0.2797	0.149	0.2797	0.149	-
50 %		**0.5142**	0.1412	0.0819	0.1412	0.07553	-
75 %		0.2589	0.0819	0.0819	0.072	0.03883	-
90 %		0.1056	0.0819	0.0819	0.05346	0.03729	-
None	R3 stream	-	**0.4894**	**0.4894**	0.3197	0.2232	-
50 %		**0.5375**	**0.4894**	**0.4894**	0.3197	0.2232	-
75 %		**0.4894**	**0.4894**	**0.4894**	0.3197	0.2232	-
90 %		**0.4894**	**0.4894**	**0.4894**	0.3197	0.2232	-
RAC (µg/L) 0.348		**PEC/RAC ratio**					
None	D3 ditch	-	0.6661	0.3532	-	-	-
50 %		**1.2287**	0.3330	0.1766	-	-	-
75 %		0.6144	0.1684	0.0974	-	-	-
90 %		0.2457	0.0932	0.0629	-	-	-
None	D4 pond	-	0.0820	0.0595	-	-	-
50 %		0.0755	0.0466	0.0341	-	-	-
75 %		0.0449	0.0290	0.0213	-	-	-
90 %		0.0265	0.0184	0.0137	-	-	-
None	D4 stream	-	0.6454	0.3428	-	-	-
50 %		**1.1891**	0.3239	0.1723	-	-	-
75 %		0.5960	0.1631	0.0871	-	-	-

90 %		0.2402	0.0667	0.0359	-	-	-
None	D6 ditch	-	0.6615	0.3509	-	-	-
50 %		**1.2204**	0.3307	0.1754	-	-	-
75 %		0.6103	0.1654	0.0877	-	-	-
90 %		0.2441	0.0734	0.0444	-	-	-
None	D6 ditch	-	0.6615	0.3509	-	-	-
50 %		**1.2204**	0.3307	0.1754	-	-	-
75 %		0.6103	0.1654	0.0877	-	-	-
90 %		0.2441	0.0734	0.0444	-	-	-
None	R1 pond	-	0.1499	0.1294	0.1201	0.0845	-
50 %		0.1439	0.1177	0.1063	0.0878	0.0613	-
75 %		0.1161	0.1016	0.0947	0.0717	0.0497	-
90 %		0.0994	0.0920	0.0878	0.0620	0.0427	-
None	R1 stream	-	**1.7655**	**1.7655**	**1.1503**	0.8011	0.5934
50 %		**1.7655**	**1.7655**	**1.7655**	**1.1503**	0.8011	0.3006
75 %		**1.7655**	**1.7655**	**1.7655**	**1.1503**	0.8011	0.1541
90 %		**1.7655**	**1.7655**	**1.7655**	**1.1503**	0.8011	0.0663
None	R2 stream	-	0.8037	0.4282	0.8037	0.4282	-
50 %		**1.4776**	0.4057	0.2353	0.4057	0.2170	-
75 %		0.7440	0.2353	0.2353	0.2069	0.1116	-
90 %		0.3034	0.2353	0.2353	0.1536	0.1072	-
None	R3 stream	-	**1.4063**	**1.4063**	0.9187	0.6414	-
50 %		**1.5445**	**1.4063**	**1.4063**	0.9187	0.6414	-
75 %		**1.4063**	**1.4063**	**1.4063**	0.9187	0.6414	-
90 %		**1.4063**	**1.4063**	**1.4063**	0.9187	0.6414	-

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

Table 9.5-20: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (late, multiple applications)

Intended use		Potato					
Active substance		zoxamide					
Application rate (g/ha)		3 ×135					
Nozzle reduction	**No-spray buffer (m)**	1	5	10	5	10	5
	Vegetated filter strip (m)	None	None	None	5	10	5 (VFSMOD)
None	D3 ditch	-	0.1653	0.08665	-	-	-
50 %		0.3123	0.08263	0.04543	-	-	-

75 %		0.1561	0.04768	0.02976	-	-	-
90 %		0.06794	0.03159	0.02169	-	-	-
None	D4 pond	-	0.05632	0.04072	-	-	-
50 %		0.05351	0.03334	0.02432	-	-	-
75 %		0.03332	0.02186	0.01613	-	-	-
90 %		0.02121	0.01498	0.01122	-	-	-
None	D4 stream	-	0.1784	0.09392	-	-	-
50 %		0.336	0.09003	0.04759	-	-	-
75 %		0.1691	0.04585	0.02444	-	-	-
90 %		0.06891	0.01934	0.01464	-	-	-
None	D6 ditch	-	0.1643	0.08617	-	-	-
50 %		0.3106	0.08217	0.04308	-	-	-
75 %		0.1553	0.04198	0.0239	-	-	-
90 %		0.0621	0.02251	0.01484	-	-	-
None	D6 ditch	-	0.1643	0.08617	-	-	-
50 %		0.3106	0.08217	0.04308	-	-	-
75 %		0.1553	0.04198	0.0239	-	-	-
90 %		0.0621	0.02251	0.01484	-	-	-
None	R1 pond	-	0.1586	0.1494	0.1127	0.07818	-
50 %		0.157	0.1451	0.1397	0.09595	0.06552	-
75 %		0.145	0.1383	0.1349	0.08909	0.0606	-
90 %		0.1379	0.1342	0.132	0.08498	0.05765	-
None	R1 stream	-	**0.7277**	**0.7277**	**0.474**	0.3303	0.1488
50 %		**0.7277**	**0.7277**	**0.7277**	**0.474**	0.3303	0.1263
75 %		**0.7277**	**0.7277**	**0.7277**	**0.474**	0.3303	0.1263
90 %		**0.7277**	**0.7277**	**0.7277**	**0.474**	0.3303	0.1263
None	R2 stream	-	0.3092	0.3092	0.1999	0.1378	-
50 %		**0.376**	0.3092	0.3092	0.1992	0.1378	-
75 %		0.3092	0.3092	0.3092	0.1992	0.1378	-
90 %		0.3092	0.3092	0.3092	0.1992	0.1378	-
None	R3 stream	-	**0.6643**	**0.6643**	**0.4341**	0.3028	0.2079
50 %		**0.6643**	**0.6643**	**0.6643**	**0.4341**	0.3028	0.1056
75 %		**0.6643**	**0.6643**	**0.6643**	**0.4341**	0.3028	0.1044
90 %		**0.6643**	**0.6643**	**0.6643**	**0.4341**	0.3028	0.1044
RAC (µg/L) 0.348		**PEC/RAC ratio**					
None	D3 ditch	-	0.4750	0.2490	-	-	-
50 %		0.8974	0.2374	0.1305	-	-	-
75 %		0.4486	0.1370	0.0855	-	-	-

90 %		0.1952	0.0908	0.0623	-	-	-
None	D4 pond	-	0.1618	0.1170	-	-	-
50 %		0.1538	0.0958	0.0699	-	-	-
75 %		0.0957	0.0628	0.0464	-	-	-
90 %		0.0609	0.0430	0.0322	-	-	-
None	D4 stream	-	0.5126	0.2699	-	-	-
50 %		0.9655	0.2587	0.1368	-	-	-
75 %		0.4859	0.1318	0.0702	-	-	-
90 %		0.1980	0.0556	0.0421	-	-	-
None	D6 ditch	-	0.4721	0.2476	-	-	-
50 %		0.8925	0.2361	0.1238	-	-	-
75 %		0.4463	0.1206	0.0687	-	-	-
90 %		0.1784	0.0647	0.0426	-	-	-
None	D6 ditch	-	0.4721	0.2476	-	-	-
50 %		0.8925	0.2361	0.1238	-	-	-
75 %		0.4463	0.1206	0.0687	-	-	-
90 %		0.1784	0.0647	0.0426	-	-	-
None	R1 pond	-	0.4557	0.4293	0.3239	0.2247	-
50 %		0.4511	0.4170	0.4014	0.2757	0.1883	-
75 %		0.4167	0.3974	0.3876	0.2560	0.1741	-
90 %		0.3963	0.3856	0.3793	0.2442	0.1657	-
None	R1 stream	-	**2.0911**	**2.0911**	**1.3621**	0.9491	0.4276
50 %		**2.0911**	**2.0911**	**2.0911**	**1.3621**	0.9491	0.3629
75 %		**2.0911**	**2.0911**	**2.0911**	**1.3621**	0.9491	0.3629
90 %		**2.0911**	**2.0911**	**2.0911**	**1.3621**	0.9491	0.3629
None	R2 stream	-	0.8885	0.8885	0.5744	0.3960	-
50 %		**1.0805**	0.8885	0.8885	0.5724	0.3960	-
75 %		0.8885	0.8885	0.8885	0.5724	0.3960	-
90 %		0.8885	0.8885	0.8885	0.5724	0.3960	-
None	R3 stream	-	**1.9089**	**1.9089**	**1.2474**	0.8701	0.5974
50 %		**1.9089**	**1.9089**	**1.9089**	**1.2474**	0.8701	0.3034
75 %		**1.9089**	**1.9089**	**1.9089**	**1.2474**	0.8701	0.3000
90 %		**1.9089**	**1.9089**	**1.9089**	**1.2474**	0.8701	0.3000

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

Additional aquatic risk assessment using geomean Koc

An additional aquatic risk assessment is provided here for zoxamide parent at step 4, using the geomean Koc (see part B8).

As the zoxamide metabolites and propamocarb-HCl are passing the risk assessment at step 1 at the vast majority, and only in a few cases in step 2 with such a wide margin that it would also cover a tenfold PECsw, it is not considered necessary to repeat the risk assessment with the recalculated PECsw with geomean Koc here.
For the same reason, no additional risk assessment has been provided in the cases of secondary poisoning in birds and mammals, as the margin is very wide.

Therefore, the risk assessment is provided here for zoxamide only at step 4.

Table 9.5-21: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (early, single application) - using geomean Koc

Intended use		Potato		
Active substance		zoxamide		
Application rate (g/ha)		3 ×135		
Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD
	No spray buffer (m)	10	15	5
None	D3 ditch	0.1229	-	0.2317
None	D4 pond	0.01979	-	0.02741
None	D4 stream	0.1239	-	0.2332
None	D6 ditch	0.1215	-	0.2291
None	D6 ditch	0.1206	-	0.2273
None	R1 pond	0.03134	-	0.02747
None	R1 stream	0.2467	-	0.2073
None	R2 stream	0.1459	-	0.2743
None	R3 stream	0.1544	-	0.2909
RAC (µg/L)				
0.348		**PEC/RAC ratio**		
Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD
	No spray buffer (m)	10	15	5
None	D3 ditch	0.353	-	0.666
None	D4 pond	0.057	-	0.079
None	D4 stream	0.356	-	0.670
None	D6 ditch	0.349	-	0.658
None	D6 ditch	0.347	-	0.653

None	R1 pond	0.090	-	0.079
None	R1 stream	0.709	-	0.596
None	R2 stream	0.419	-	0.788
None	R3 stream	0.444	-	0.836

Table 9.5-22: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (early, multiple applications) - using geomean Koc

Intended use		Potato			
Active substance		zoxamide			
Application rate (g/ha)		3 ×135			3 ×130*
Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD	10
	No spray buffer (m)	10	15	5	10
None	D3 ditch	0.08652	0.05868	0.165	0.0833
None	D4 pond	0.03821	0.02959	0.05317	0.03698
None	D4 stream	0.09037	0.06129	0.172	0.08705
None	D6 ditch	0.08618	0.05844	0.1644	0.08297
None	D6 ditch	0.0855	0.05799	0.1631	0.08297
None	R1 pond	0.05307	0.04071	0.05084	0.05126
None	R1 stream	0.3264	0.2505	0.148	0.314
None	R2 stream	0.1912	0.1462	0.199	0.184
None	R3 stream	0.3512	0.2693	0.2075	0.3377
RAC (µg/L)		**PEC/RAC ratio**			
0.348					
Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD	10
	No spray buffer (m)	10	15	5	10
None	D3 ditch	0.249	0.169	0.474	0.239
None	D4 pond	0.110	0.085	0.153	0.106
None	D4 stream	0.260	0.176	0.494	0.250
None	D6 ditch	0.248	0.168	0.472	0.238
None	D6 ditch	0.246	0.167	0.469	0.238
None	R1 pond	0.153	0.117	0.146	0.147
None	R1 stream	0.938	0.720	0.425	0.902
None	R2 stream	0.549	0.420	0.572	0.529
None	R3 stream	**1.009**	0.774	0.596	0.970

*additionally run in order to demonstrate a safe use at 10m VFS when R3 scenario is relevant

Table 9.5-23: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (late, single application) - using geomean Koc

Intended use		Potato		
Active substance		zoxamide		
Application rate (g/ha)		3 ×135		
Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD
	No spray buffer (m)	10	15	5
None	D3 ditch	0.1229	-	0.2318
None	D4 pond	0.02069	-	0.02853
None	D4 stream	0.1193	-	0.2246
None	D6 ditch	0.1221	-	0.2303
None	D6 ditch	0.1229	-	0.2318
None	R1 pond	0.02967	-	0.02862
None	R1 stream	0.2848	-	0.2065
None	R2 stream	0.149	-	0.2797
None	R3 stream	0.228	-	0.2915
RAC (µg/L)				
0.348		**PEC/RAC ratio**		
Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD
	No spray buffer (m)	10	15	5
None	D3 ditch	0.353	-	0.666
None	D4 pond	0.059	-	0.082
None	D4 stream	0.343	-	0.645
None	D6 ditch	0.351	-	0.662
None	D6 ditch	0.353	-	0.666
None	R1 pond	0.085	-	0.082
None	R1 stream	0.818	-	0.593
None	R2 stream	0.428	-	0.804
None	R3 stream	0.655	-	0.838

Table 9.5-24: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for zoxamide based on FOCUS Step 4 calculations and toxicity data for fish with mitigation of spray drift and run-off for the use of GLOB2007bF in potato (late, multiple applications) - using geomean Koc

Intended use	Potato
Active substance	zoxamide
Application rate (g/ha)	3 ×135

Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD
	No spray buffer (m)	10	15	5
None	D3 ditch	0.08665	-	0.1653
None	D4 pond	0.04076	-	0.05637
None	D4 stream	0.09392	-	0.1784
None	D6 ditch	0.08617	-	0.1644
None	D6 ditch	0.1852	-	0.1852
None	R1 pond	0.0791	-	0.06745
None	R1 stream	0.3368	-	0.1488
None	R2 stream	0.1409	-	0.1999
None	R3 stream	0.3092	-	0.2079
RAC (µg/L) 0.348		**PEC/RAC ratio**		
Nozzle reduction	Vegetative strip (m) (R scenarios only)	10	15	5 VFSMOD
	No spray buffer (m)	10	15	5
None	D3 ditch	0.249	-	0.475
None	D4 pond	0.117	-	0.162
None	D4 stream	0.270	-	0.513
None	D6 ditch	0.248	-	0.472
None	D6 ditch	0.532	-	0.532
None	R1 pond	0.227	-	0.194
None	R1 stream	0.968	-	0.428
None	R2 stream	0.405	-	0.574
None	R3 stream	0.889	-	0.597

In addition, the risk of the formulation using SWASH drift calculator has been added.

Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB2007bF for each organism group based on the PECsw of the formulation

Group			**Fish acute**	**Inverteb. Acute**	**Algae**
Test species			***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint			LC_{50}	EC_{50}	E_rC_{50}
(µg/L)			9450	10600	1137
AF			100	100	10
RAC (µg/L)			94.5	100.6	113.7
Cropping scenario	**FOCUS scenario**	**Max. PECsw (µg/L)**			
Potato, 1 x 2.1976 g/ha	Ditch	11.6734	0.124	0.116	0.103
	Pond	0.4662	0.005	0.005	0.004
	Stream	9.0925	0.096	0.090	0.080
		10.911*	0.115	0.108	0.096
Potato, 3 x 2.1976 g/ha	Ditch	8.4813	0.090	0.084	0.075
	Pond	0.3276	0.003	0.003	0.003
	Stream	6.5755	0.070	0.065	0.058
		7.8906*	0.083	0.078	0.069

*taking into account the 20% contribution from the upstream catchment

Combined exposure of GLOB2007bF on potato

Decision scheme for mixture toxcitiy risk assessment for GLOB2106cF

Step 1. Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?

Only for the a.s. (ECxa.s.): Go to 7
For both formulation (ECxPPP) and a.s. (ECxa.s.): Go to 2

Answer: Measured toxicity data for the formulation and the a.s. are available for fish, daphnia, algae..→ Go to 2

STEP 2. Check the plausibility of the measured formulation toxicity (EC_xPPP) against the calculated mixture toxicity EC_{xmix}-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (EC_xPPP) by means of the model deviation ratio (MDR = EC_{xmix}-CA/EC_xPPP).

If MDR = 0.2–5 (CA approximately holds for the mixture)
If MDR > 5 (mixture more toxic than CA)
If MDR < 0.2 (mixture less toxic than CA)

Equation 13:

$$EC_{X_{mix-CA}} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{X_i}} \right)^{-1}$$

Equation 15:

$$MDR = \frac{EC_{X_{mix-CA}}\ (calculated\ mixture\ toxicity)}{EC_{X_{PPP}}\ (measured\ mixture\ toxicity)}$$

Calculation of the acute mixture toxicity of the formulation

Composition			
Name/code of the product	**GLOB2007bF**		
Name of the active substance A	Propamocarb-HCl	Fraction considering density [%]	$p_{i\ mix}$ = Fraction of active substance i in the mixture with $\sum p_{i\ mix} = 100$ [%]
Name of the active substance B	Zoxamide		
Density [g product/cm^3]	1.0988		
	Nominal [g a.s./kg or L product]		
Concentrations of the active substance A in the product	450	41.0%	87.0%
Concentrations of the active substance B in the product	67.5	6.1%	13.0%

Comparison of the toxicity of GLOB2007bF to the predicted one based on Propamocarb-HCl and zoxamide

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x\ PPP}$) [mg a.s./L]	Toxicity of the a.s. propamocarb ($EC_{x\ A}$) [mg a.s./L]	Toxicity of the a.s. zoxamide ($EC_{x\ B}$) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
Fish	9.45	4,451	92	0.16	0,01
Daphnia	10.6	4,992	100	0.78	0,01
Algae	1.137	0,535	85	0.0582	0,1

Calculation of toxicity exposure in

Toxicity per fraction of the propamocarb ($1/TU_A$) [mg a.s./L]	Toxicity per fraction of the zoxamide ($1/TU_B$) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA} = 1/\sum (TU_i)$) [mg a.s./L]	Model deviation ratio (MDR = $EC_{x\ mix\text{-}CA}/EC_{x\ PPP}$)	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) (at lower exposure tier)
105.8	1.223	1,213	0,272	2,710
115	5.98	5,684	1,139	2,635
97.75	0.46	0,444	0,829	2,724

Answer: MDR for fish daphnia and algae is between 0.2 -5. Therefore, go to Step 3.

Answer:Yes. → Go to step 3

Step 3. Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECx PPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECx PPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECx mix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).

Results of compareECmix-CA(a.s. in PPP) to ECmix-CA (a.s. in PECmix)

Endpoint/Test species		Triggers	
	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix)	0.8-1.2	<0.8 or >1.2

fish	2,710		**Yes**
daphnia	2,635		**Yes**
algae	2,724		**yes**

Answer: Calculated factors gives results outside 0.8-1.2 Therefore, go to step 5.

STEP 5. Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECx PPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation (≥ 90 %) comes from a single a.s. (TUi)?

Since GLOB2007bF contains two active ingredients the formulation toxicity should also be considered.

The check of a single driver for the toxicity was done and is presented below. The check is made with the calculation which is done according to the following formula:

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

in which TU is the ratio between the concentration (i.e. ci) of a mixture component and its toxicological acute (e.g. EC_{50}) 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))
Contribution to toxicity = TOXsum(ai) * TU(ai) * 100

Table 9.5-25: Contribution to toxicity of GLOB2007bF by zoxamide and propamocarb-HCl

Organism	Active substance	EC_{50} (mg/L)	Fraction in mixture	Toxic unit	Tox of the sum ai	Contribution to toxicity (%)
Fish	zoxamide	0.16	0.13	1.231	0.0093	1.150
	propamocarb-HCl	92	0.87	105.747		98.850
Fish (*Lepomis*)	zoxamide	0.79	0.13	6.077	0.0089	5.434
	propamocarb-HCl	92	0.87	105.747		94.566
Daphnia	zoxamide	0.78	0.13	6.000	0.0083	4.961
	propamocarb-HCl	100	0.87	114.943		95.039
Algae	zoxamide	0.0582	0.13	0.448	0.0102	0.456
	propamocarb-HCl	85	0.87	97.701		99.544

Answer: The toxicity drivers were found for fish, daphnia and algae. Therefore, got to Step 6.
Conduct a RA based on single-substance toxicity data (ECx a.s.) for the identified ‘driver‘ of mixture toxicity, with the exposure-toxicity ratio (ETRa.s.) being defined as the PECa.s. divided by the measured ECx a.s. and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.

For aquatic organisms the toxicity is driven by zoxamide (contribution ≥90%). Therefore, the risk assessment can be based on single-substance toxicity data (ECx a.s.) for the identified ‘driver’ of mixture toxicity, which is in this case zoxamide.

9.5.3 Overall conclusions

For the countries that accept the EU agreed endpoints (see part B8) and for the countries that do not accept the EU agreed endpoints but where R3 is not relevant:

- SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies.
- *OR in case VFSMOD is accepted: a 5 m no spray buffer zone including a 5 m vegetated filter strip.*

For the countries that do not accept the EU agreed endpoints and where R3 is relevant:

- SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 15 m including a 15 m vegetated filter strip to surface water bodies. Alternatively, apply up to 1.9L/ha and respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies.
- *OR in case VFSMOD is accepted: a 5 m no spray buffer zone including a 5 m vegetated filter strip.*

Conclusion for the whole Central zone

Group	Mitigation (covers worst-case among single and multiple application)	Scenario
Potato 3 x 0.3 L prod./ha BBCH21-79 (worst case mitigation among early and late applications)	5 m buffer zone or 75%DRN	D3/ditch D4/stream D6/ditch
	10 m vegetated filter strip (Landscape Mitigation) or 5 m vegetated filter strip (VFSmod)	R1/stream R3/stream
	For countries that only accept geomean endpoints: 15 m vegetated filter strip (Landscape Mitigation) or 5 m vegetated filter strip (VFSmod)	R3/stream
	5 m vegetated filter strip (Landscape Mitigation)	R2/stream
Potato 3 x 0.29 L prod./ha BBCH21-79 (worst case mitigation among early and late applications)	5 m buffer zone or 75%DRN	D3/ditch D4/stream D6/ditch
	10 m vegetated filter strip (Landscape Mitigation) or 5 m vegetated filter strip (VFSmod)	R1/stream R3/stream
	5 m vegetated filter strip (Landscape Mitigation)	R2/stream

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). An additional aquatic risk assessment is provided for zoxamide parent at step 4, using the geomean Koc (see part B8).

Regarding potato, calculated PEC/RAC ratios for propamocarb-HCl are passing the risk assessment at step 1 indicate an acceptable risk in FOCUS step 2.
For the intended use on potato, calculated PEC/RAC ratios for zoxamide did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish as characterised by a NOEC for Oncorhynchus mykiss of 3.48 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 PECSW considering reduced exposure of surface water bodies. Based on the mixture toxicity, the toxicity is driven by zoxamide (contribution ≥90%). Therefore, the risk assessment is be based on single-substance toxicity data for the identified ‘driver’ of mixture toxicity, which is in this case zoxamide.

The PEC/RAC ratios for zoxamide and its metabolites and for propamocarb-HCl are less than the trigger

value of 1, indicating that the risk to aquatic organisms is acceptable following use of GLOB2007bF according to the proposed use pattern when considering the following mitigation measures:

For the countries that accept the EU agreed endpoints (see part B8) and for the countries that do not accept the EU agreed endpoints (where risk assessment is provided for zoxamide parent at step 4, using the geomean Koc (see part B8)) but where R3 is not relevant:

-
- SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies.
- or in case VFSMOD is accepted: a 5 m no spray buffer zone including a 5 m vegetated filter strip.

For the countries that do not accept the EU agreed endpoints (where risk assessment is provided for zoxamide parent at step 4, using the geomean Koc (see part B8)) and where R3 is relevant:

• SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 15 m including a 15 m vegetated filter strip to surface water bodies. Alternatively, apply up to 1.9L/ha and respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies.
• or in case VFSMOD is accepted: a 5 m no spray buffer zone including a 5 m vegetated filter strip.

The zRMS add the table for clarity, with mitigation proposals by each scenario, for the MS who wish to use them according to their local conditions.
In addition, the risk of the formulation using SWASH drift calculator has been added.

The acceptability of proposed risk mitigation measures used in refined risk assessment should be considered on National level.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with zoxamide and propamocarb-HCl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide and propamocarb-HCl. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	RH-117,281 2F	Acute, oral	Oral toxicity (LD_{50}) >147 µg formulation/bee (corresponding to >33 µg a.s./bee)	EFSA Journal 2017;15(9):4980
Apis mellifera	zoxamide	Acute, contact	Contact toxicity (LD_{50}) > 100 µg a.s./bee	EFSA Journal 2017;15(9):4980
Apis mellifera	RH-117,281 2F	Acute, contact	Contact toxicity (LD_{50}) >200 µg formulation/bee (corresponding to >43.2 µg a.s./bee)	EFSA Journal 2017;15(9):4980
Apis mellifera	Zoxium 240 SC	Chronic	10 d- LC_{50} >5000 mg a.s./kg feed 174.8 µg a.s./bee/day	EFSA Journal 2017;15(9):4980

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Zoxium 240 SC	Semi-field bee brood test	No effects on bee brood development up to 3.47 g Zoxium 240 SC/L feeding solution corresponding to 0.75 g a.s./L feeding solution	EFSA Journal 2017;15(9):4980
Apis mellifera	zoxamide	Larvae, chronic	NOEDD > 71.51 µg a.s./larva	Aguilar-Alberola, J., 2023
Apis mellifera	Propamocarb-HCl	Acute, oral	LD_{50} >84 µg a.s./bee	EFSA Scientific Report (2006) 78, 1-80
Apis mellifera	Propamocarb-HCl	Acute, contact	LD_{50} >100 µg a.s./bee	EFSA Scientific Report (2006) 78, 1-80
Apis mellifera	GLOB2007bF	Oral, acute, 48 h	LD_{50} > 221 ~~200~~ µg product/bee	Knautz, 2022
Apis mellifera	GLOB2007bF	Contact, acute, 48 h	LD_{50} > 200 ~~221~~ µg product/bee	Knautz, 2022
Bombus terrestris	GLOB2007bF	Oral, acute	LD_{50} > 203.5 µg product/bumblebee	Chwiesko, 2023
Bombus terrestris	GLOB2007bF	Contact, acute	LD_{50} > 200 µg product/bumblebee	Chwiesko, 2023
Apis mellifera	GLOB2007bF	Adult, chronic	LDD_{50} > 111.6 µg product/bee/day NOEDD ≥ 111.6 µg product/bee/day	Schabio, 2023
Apis mellifera	GLOB2007bF	Larvae, chronic, 22 d	NOED > 100 µg product/larva	Colli, 2022
Higher-tier studies (tunnel test, field studies)				
Not performed.				

Review comments:
Endpoints relevant for the risk assessment for bees and presented in table 9.6-2 are in line with EU agreed endpoints reported in EFSA Journal 2017;15(9):4980 for zoxamide and EFSA Scientific Report (2006) 78, 1-80 for Propamocarb- HCl.
Concerned member states must decide on the applicability of the new studies in RA for bees.

9.6.1.1 Justification for new endpoints

Not relevant as there is no deviation from the EU agreed 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 GLOB2007bF in potato

Intended use	Potato		
Active substance	zoxamide		
Application rate (g/ha)	3 × 135		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 33	135	< 4.09
Contact toxicity	> 100		< 1.35
Intended use	Potato		
Active substance	propamocarb-HCl		
Application rate (g/ha)	3 × 900		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 84	900	< 10.71
Contact toxicity	> 100		< 9
Product	GLOB2007bF		
Application rate (g/ha)	3 × 2197.6*		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>221 ~~200~~	2197.6	< 9.94 ~~< 10.988~~
Contact toxicity	> 200 ~~221~~		< 10.988 ~~< 9.94~~

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*based on product density of 1.0988 g/mL

All the hazard quotients are below 50, indicating that the application of GLOB2007bF poses a low acute risk to bees.

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

Not relevant.

Review Comments:

Since acceptable acute risk have been concluded for bees exposed to GLOB2007bF at the Tier 1 level, a higher-tier risk assessment is not required for the proposed uses of GLOB2007bF.

9.6.3 Effects on bumble bees

Table 9.6-3: First-tier assessment of the risk for bumble bees due to the use of GLOB2007bF

Intended use	Potato

Product	GLOB2007bF
Application rate (g/ha)	3 × 2197.6*

Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)***	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 203.5	2197.6	< 10.799
Contact toxicity	> 200		< 10.988

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*based on product density of 1.0988 g/mL

All the hazard quotients are below 50, indicating that the application of GLOB2007bF poses a low acute risk to bumble bees.

Review Comments:

The evaluation of the risk for bumblebees 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 available toxicity data for GLOB2007bF to bumblebees indicate comparable toxicity to honeybees. The submitted risk assessment, according to the guidance currently in force, has been accepted. It can therefore, be concluded that there will be negligible risk associated with the exposure of bumblebees to GLOB2007bF.

9.6.4 Effects on solitary bees

No data/information available.

Review Comments:

According to SANCO/10329/2002 rev 2 final, the risk assessment for solitary bees is not required.

9.6.5 Effects on honey bee development and other honey bee life stages

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 and 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 plant material as proposed in the EPPO 2010 scheme (see Note 6 therein), 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[1], as proposed in the EPPO scheme, have been used to estimate the consumption by bee larvae:

[1] 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 for 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. With an application rate of 2L GLOB2007bF /ha (corresponding with 135 g zoxamide/ha and 2.1976 g GLOB2007bF/ha) this results into an exposure of nectar and pollen as follows:

Zoxamide
Nectar dose: 0.135 x 2.9 x 198/1000 = 0.077517 µg a.s./larva
Pollen dose: 0.135 x 6.1 x 2/1000 = 0.001647 µg a.s./larva

GLOB2007bF
Nectar dose: 2.1976 x 2.9 x 198/1000 = 1.26186192 µg formulation/larva
Pollen dose: 2.1976 x 6.1 x 2/1000 = 0.02681072 µg formulation/larva

Total exposure ETE = 0.079164 µg zoxamide/larva and 1.28867264 µg GLOB2007bF/larva.
This can be compared to the larval NOEDD of 71.51 µg zoxamide/larva and 100 µg GLOB2007bF/larva.

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:

GLOB2007bF
Daily dose (µg product/bee) = A.R. x [128 mg/(1000 x 0.3)] x RUD = 2.1976 x [128/(1000x0.3)] x 2.9 = 2.719 µg/bee

A.R. = application rate in kg product/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 = 111.6/2.719 = 41.045

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 41.045, the proposed use of GLOB2007bF poses an acceptable risk to adult bees.

Review comments:
The hazard quotients are below the trigger value of 1 considering the modified EPPO 2010 approach according to the ECPA proposal of 9 June 2017 (POS/17/LO/28028) indicating that the active substances and formulation pose an acceptable chronic risk to honey bee larvae and adult honey bees.

Screening step EFSA 2013

Application rate:

kg/ha	2.1976
g/ha	2197.6
mg/seed	

Toxicity endpoints in µg/bee for contact assessments:

	HB	BB	SB
Acute contact - LD_{50}	200	200	

……..

Toxicity endpoints in µg/bee, µg/bee/day or µg/larva/developmental period for oral assessments:

	HB	BB	SB
Acute oral - LD_{50}	221	202.5	
Adult Chronic - LDD_{50}	111.6		
Larva - NOEL	100		
HPG - NOEL			

Contact route of exposure			
	"calculation factor" (linked with dust)	HQ*	Trigger
HB	1	11.0	42
BB	1	**11.0**	7

*values in **bold** are above the trigger value

Oral route of exposure (pollen and nectar)			
	"calculation factor" (Ef x SV)	ETR	Trigger
HB - acute	7.6	0.08	0.2
HB - chronic	7.6	**0.150**	0.03
HB - larvae	4.4	0.10	0.2
BB - acute	11.2	**0.12**	0.036

*values in **bold** are above the trigger value

Tier 1 acute contact for potato (bumblebee) EFSA 2013

scenario	BBCH	fdep	Bumble bee	
			HQ	trigger
treated crop	< 40	0	0.0	7
treated crop	≥ 40	0	0.0	7
field margin	< 40	0.028	0.3	7
field margin	≥ 40	0.028	0.3	7

Tier 1 acute oral for potato (bumblebee) EFSA 2013

category	scenario	BBCH	Ef	SV	TWA	ETR	trigger
chronic	treated crop	10 - 39	1	2.3	1	0.0250	0.036
chronic	treated crop	40 - 69	1	2.3	1	0.0250	0.036
chronic	treated crop	≥ 70	1	0	1	0.0000	0.036
chronic	field margin	10 - 39	0.0092	6.5	1	0.0006	0.036
chronic	field margin	40 - 69	0.0092	6.5	1	0.0006	0.036
chronic	field margin	≥ 70	0.0092	6.5	1	0.0006	0.036

chronic	adjacent crop	10 - 39	0.0033	11.2	1	0.0004	0.036
chronic	adjacent crop	40 - 69	0.0033	11.2	1	0.0004	0.036
chronic	adjacent crop	≥ 70	0.0033	11.2	1	0.0004	0.036
chronic	next crop	10 - 39	1	0.9	1	0.0098	0.036
chronic	next crop	40 - 69	1	0.9	1	0.0098	0.036
chronic	next crop	≥ 70	1	0.9	1	0.0098	0.036

Tier 1 chronic oral for potato (honeybee adult) EFSA 2013

category	scenario	BBCH	Ef	SV	TWA	ETR	trigger
chronic	treated crop	10 - 39	1	0.92	0.72	0.013	0.03
chronic	treated crop	40 - 69	1	0.92	0.72	0.013	0.03
chronic	treated crop	≥ 70	1	0	0.72	0.000	0.03
chronic	field margin	10 - 39	0.0092	2.9	0.72	0.000	0.03
chronic	field margin	40 - 69	0.0092	2.9	0.72	0.000	0.03
chronic	field margin	≥ 70	0.0092	2.9	0.72	0.000	0.03
chronic	adjacent crop	10 - 39	0.0033	5.8	0.72	0.000	0.03
chronic	adjacent crop	40 - 69	0.0033	5.8	0.72	0.000	0.03
chronic	adjacent crop	≥ 70	0.0033	5.8	0.72	0.000	0.03
chronic	next crop	10 - 39	1	0.54	0.72	0.008	0.03
chronic	next crop	40 - 69	1	0.54	0.72	0.008	0.03
chronic	next crop	≥ 70	1	0.54	0.72	0.008	0.03

Larval and adult chronic risk assessment according to EFSA, 2013

The chronic risk to bees has been also assessed according to the EFSA Guidance Document on bees (EFSA Journal 2013;11(7):3295, revision of 4 July 2014) considering an application rate of 2L GLOB2007bF /ha (corresponding with 2.1976 g GLOB2007bF/ha).

BBCH stages < 10 and weed scenario have not been included as not relevant to GLOB2007bF use on potato.

Non-relevance of weeds in potato fields has been further confirmed by the most recent EFSA guidance (EFSA Journal 2023;21(5):7989, 11 May 2023) that considers this scenario as not existing (see table 5 and 6 in the guidance, point 4.3.2 ‘Weeds in treated fields', page 26-27) for all potato growth stages, based on available scientific evidence. Even if it is still an un-noted guidance, the regulatory consensus is that this scenario is not relevant for potato and therefore, risk assessment or other refinements on this particular scenario are not required.

Results of Tier 1 assessment are presented in the Table below.

Table 9.6-4: First-tier assessment of the chronic risk for bees due to the use of GLOB2007bF according to EFSA, 2013

category	scenario	BBCH	Honeybee	
			ETR	trigger
chronic	treated crop	10 - 39	0.01	0.03
chronic	treated crop	40 - 69	0.01	0.03
chronic	treated crop	≥ 70	0.00	0.03
chronic	field margin	10 - 39	0.00	0.03
chronic	field margin	40 - 69	0.00	0.03
chronic	field margin	≥ 70	0.00	0.03

chronic	adjacent crop	10 - 39	0.00	0.03
chronic	adjacent crop	40 - 69	0.00	0.03
chronic	adjacent crop	≥ 70	0.00	0.03
chronic	next crop	10 - 39	0.01	0.03
chronic	next crop	40 - 69	0.01	0.03
chronic	next crop	≥ 70	0.01	0.03
larva	treated crop	10 - 39	0.00	0.2
larva	treated crop	40 - 69	0.00	0.2
larva	treated crop	≥ 70	0.00	0.2
larva	field margin	10 - 39	0.00	0.2
larva	field margin	40 - 69	0.00	0.2
larva	field margin	≥ 70	0.00	0.2
larva	adjacent crop	10 - 39	0.00	0.2
larva	adjacent crop	40 - 69	0.00	0.2
larva	adjacent crop	≥ 70	0.00	0.2
larva	next crop	10 - 39	0.01	0.2
larva	next crop	40 - 69	0.01	0.2
larva	next crop	≥ 70	0.01	0.2

ETR values are below the triggers of 0.03 for adult and 0.2 for larvae chronic risk respectively, indicating a low risk to bees in the field.

Review comments:

zRMS did not evaluate Larval and adult chronic risk assessment according to EFSA, 2013 since the risk assessment was performed according to SANCO/10329/2002 rev 2.
Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on national level.

9.6.6 Overall conclusions

HQ values for oral and contact exposure are below the relevant trigger. Therefore, it can be assumed that the intended uses of GLOB2007bF represent low risk exposure to honeybees.

The chronic TERs for honey bee adults and larvae are higher than the EPPO 2010 trigger of 1 and below the EFSA 2013 triggers of 0.03 for adult and 0.2 for larvae chronic risk respectively, indicating that the proposed use according to the intended GAP of GLOB2007bF poses an acceptable chronic risk to honey bee larvae and adults.

Review comments:

The evaluation has been performed in line with SANCO/10329/2002 rev 2 final. The risk assessment performed for active substances and the formulated product GLOB2007bF is agreed by the zRMS.
All hazard quotients calculated are lower than 50, indicating that the acute oral and contact risk to bees is acceptable following the use according to the proposed use pattern GLOB2007bF
According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): Applicant provided chronic test on bees and evaluation of effects on honey bee development with formulated product. Nevertheless, such studies were deemed not necessary to finalize the risk assessment.
zRMS did not evaluate Larval and adult chronic risk assessment according to EFSA, 2013 since the risk assessment was performed according to SANCO/10329/2002 rev 2.

Concerned Member States must decide on the consideration of EPPO 2010 approach since there is no harmonized approach for the chronic risk assessment for bees.
Concerned Member States must decide on the consideration of data requirements of the EFSA Bee guidance (2013) on national level.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

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

Effects on non-target arthropods of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Test Substance	Exposure System	Results	Reference
Typhlodromus pyri	GLOB2007bF	Mortality, LR_{50} Reproduction, ER_{50}	LR_{50} = 5536 mL formulation/ha ER_{50} = 2768 mL formulation/ha	Leopold, J., 2022
Aphidius rhopalosiphi	GLOB2007bF	Mortality, LR_{50} Reproduction, ER_{50}	LR_{50} > 6750 mL formulation/ha ER_{50} > 6750 mL formulation/ha	Leopold, J., 2022
Field or semi-field tests				
-				

9.7.1.1 Justification for new endpoints

Not relevant as there is no deviation from the EU agreed 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 GLOB2007bF in potato

Intended use	potato		
Active substance/product	GLOB2007bF		
Application rate (mL/ha)	3 × 2000		
MAF	2.3		
Test species Tier I	**LR_{50} (lab.) (mL/ha)**	**$PER_{in\text{-}field}$ (mL/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	5536	4600	0.831
Aphidius rhopalosiphi	> 6750		< 0.681

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 GLOB2007bF in potato

Intended use	Potato				
Active substance/product	GLOB2007bF				
Application rate (mL/ha)	3 × 2000				
MAF	2.3				
vdf	5*				
Test species Tier I	**LR_{50} (lab.) (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	5536	0.0277	25.484	10	0.046
Aphidius rhopalosiphi	> 6750				< 0.038

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).

Review comments:
Risk assessment presented by the Applicant is considered acceptable since it is worst case.

However, **f**or some countries vdf of 10 is used, thus zRMS also calculated the off-field exposure with this parameter.

Intended use	potato
Product	GLOB2013F
Application rate (mL formulation/ha)	3 x 2000
MAF	2.3
vdf	10

Test species	**Tier I**				
	LR_{50} (lab.) [mL formulation/ha]	**Drift rate (%)**	**$PER_{off\text{-}field}$ [L/ha]**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	5536	0.0277	12.74	10	0.0023
Aphidius rhopalosiphi	> 6750				0.0018

Concerned MS should decide of use of vdf 5 or 10 on the National Level.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The risk for non-target arthropods is acceptable when using GLOB2007bF according to the intended uses. No risk mitigation measures are needed.

Review comments:

Acceptable risk for in-field and off-field habitats may be concluded with no need for risk mitigation measures.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with zoxamide and its relevant metabolites and propamocarb-HCl. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide and propamocarb-HCl. 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	zoxamide	Overspray 56 d, chronic Artificial soil**	NOEC = 1 mg/kg dw $NOEC_{corr}$ = 0.5 mg/kg dw*	Nienstedt, K. (1999), RAR of zoxamide, Volume 3 CA B9****
Eisenia fetida	zoxamide	Mixed into substrate 56 d, chronic Natural soil***	NOEC = 7 mg/kg dw $NOEC_{corr}$ = 3.5 mg/kg dw*	Nienstedt, K. M. (2001), RAR of zoxamide, Volume 3 CA B9****
Eisenia fetida	RH-141455	56d – artificial soil	NOEC = 5 mg/kg dw	EFSA Journal 2017;15(9):4980
Eisenia fetida	Propamocarb- HCl	Reproductive toxicity	NOEC = 362 mg a.s./kg dry soil	EFSA Scientific Report (2006) 78, 1-80
Eisenia andrei	GLOB2007bF	Mixed into substrate 56 d, chronic Artificial soil**	NOEC ≥ 10 mg/kg dw $NOEC_{corr}$ = 5 mg/kg dw*	Straube, D., 2022
Eisenia andrei	GLOB2007bF	Mixed into substrate 56 d, chronic Artificial soil**	NOEC = 5.72 mg/kg dw $NOEC_{corr}$ = 2.86 mg/kg dw*	Straube, D., 2022
Eisenia andrei	GLOB2007bF	Mixed into substrate 56 d, chronic Natural soil (Lufa 2.2)***	NOEC = 14.8 mg/kg dw $NOEC_{corr}$ = 7.4 mg/kg dw* EC_{10}=22.7 mg/kg dw EC_{10}corr=11.35 mg/kg dw	Straube, D., 2023*****
Eisenia andrei	RH-127450	Mixed into substrate 56 d, chronic Artificial soil	NOEC = 13.9 mg/kg dw $NOEC_{corr}$ = 6.95 mg/kg dw*	Straube, D., 2023
Eisenia andrei	RH-24549	Mixed into substrate 56 d, chronic Artificial soil	NOEC = 13.9 mg/kg dw $NOEC_{corr}$ = 6.95 mg/kg dw*	Straube, D., 2023
Eisenia andrei	RH-163353	Mixed into substrate 56 d, chronic Artificial soil	NOEC ≥ 25 mg/kg dw	Straube, D., 2023
Folsomia candida	GLOB2007bF	28 d, chronic Artificial soil	NOEC ≥ 1000 mg/kg dw $NOEC_{corr}$ = 500 mg/kg dw* EC_{10} > 1000 mg formulation/kg dw	Straube, D., 2022

Species	Substance	Exposure System	Results	Reference
Hypoaspis aculeifer	GLOB2007bF	14 d, chronic Artificial soil	NOEC ≥ 1000 mg/kg dw $NOEC_{corr}$ = 500 mg/kg dw* NOECreproduction= 556 mg formulation/kg soil dry weight $NOEC_{reoroductioncorr}$=278 mg formulation/kg soil dry weight	Straube, D., 2022
Field studies				
-				
Litter bag test				
-				

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002 (for substances with log P_{OW} > 2).
** Studies with artificial soil
*** Studies with natural soil LUFA 2.2 as refinement
**** SANTE/10052/2018 Rev 2 (23 March 2018): The two studies available for assessing the chronic risk to earthworms were considered not valid by EFSA. However, the validity threshold for the "coefficient of variation in control" (30%) is not exceeded when results are compared to solvent controls rather than untreated controls. The studies can therefore be considered as valid. The type of soil used in the natural soil study is a recognised standard agricultural soil and represents a worst case due to its low carbon content: it is therefore appropriate to use this study to assess the chronic risk to earthworms from zoxamide.
***** Study generated with LUFA 2.2 following Nienstedt, K. M. (2001) approach to achieve a refined endpoint for the formulation.

Review comments:
According to EFSA A Journal 2017;15(9):4980 further data are needed for the chronic risk assessment to earthworm of the active substance and the metabolites RH-127450, RH-24549, RH-16335. The applicant presented new studies for metabolites which were assessed in Annex 2.

9.8.1.1 Justification for new endpoints

Not relevant as there is no deviation from the EU agreed endpoints.

9.8.2 Risk assessment

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

9.8.2.1 First-tier risk assessment

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

Risk assessment on the chronic effects to earthworms did not show an acceptable result when artificial soil was used in the 56d study, neither for zoxamide a.s. nor for GLOB2007bF.
Following the approach of Nienstedt, K. M. (2001) in the RAR of zoxamide, accepted by SANTE/10052/2018 Rev 2 (23 March 2018), the applicant generated a study on the formulation (Straube, D., 2023) using the same LUFA 2.2 soil as a refinement in order to obtain an acceptable risk assessment. All results, including artificial soil ones, are displayed in the table below for the sake of completeness.

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 GLOB2007bF in potato

Intended use	Potato		
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
zoxamide	0.5*	0.1481	**3.376**
zoxamide	3.5**	0.1481	23.633
propamocarb-HCl	362	1.3901	260.413
GLOB2007bF	5*	1.1721	**4.266**
GLOB2007bF	2.86*	1.1721	**2.440**
GLOB2007bF	7.4**	1.1721	6.313
RH-127450	6.95	0.0219	317.352
RH-24549	6.95	0.0279	249.104
RH-163353	25	0.0291	859.107
RH-141455	5	0.0120	416.667
Chronic effects on other soil macro- and mesofauna: *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
GLOB2007bF	500	1.1721	426.585
Chronic effects on other soil macro- and mesofauna : *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
GLOB2007bF	278 ~~500~~	1.1721	237.18 ~~426.585~~

TER values shown in bold fall below the relevant trigger.
* Endpoints obtained with artificial soil
** Refined endpoints obtained with natural soil LUFA 2.2

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The TER values indicate an acceptable risk for earthworms and other non-target soil organisms for the intended use of GLOB2007bF.

Review comments:

Applicant presented three new laboratory studies for the formulation GLOB2007bF. PECsoil values for active substances and their metabolites were agreed in Section 8.

Based on the provided Risk Assessment zRMS notes that the TER_{LT} for the chronic effects on earthworms for zoxamide and formulation were less than the trigger of 5 indicating that chronic risk is unacceptable. Since first-tier assessment of chronic risk for earthworms due to the use of GLOB2007bF in potato is considered unacceptable further refinement should be provided.
The Applicant presented and follow the approach of Nienstedt, K. M. (2001) provided in the RAR of zoxamide, accepted by SANTE/10052/2018 Rev 2 (23 March 2018). Reproductive effects were seen in a 56-day laboratory study of chronic toxicity conducted with an artificial soil and TER values were below the trigger value of 5. However, TERs calculated using the NOEC of 7 mg/kg taken from a long-term study conducted in natural soil are above the trigger value.

The Applicant generated a study on the formulation (Straube, D., 2023) using the same LUFA 2.2 soil as a refinement in order to obtain an acceptable risk assessment. In the study all study validity criteria were met according to OECD 222.
No mortality was observed in any treatment group.
The body weight changes of the earthworms after 28 days exposure to GLOB2007bF were not statistically significantly different compared to the control up to and including the highest test concentration of 279.9 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).
The reproduction rates were not statistically significantly different compared to the control up to and the test concentration of 14.8 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 26.7 and above, the reproduction was statistically significantly reduced compared to the control.
No behavioural abnormalities were observed in any of the treatment groups.

Additionally, GLOB2007bF is not expected to cause any significant effects on soil microbial populations when applied at label recommended doses. On the basis of the study results, it was concluded that GLOB2007bF at the concentrations corresponding to the PEC: 8.79 mg of the test item/kg dry weight of soil and 5xPEC: 44.0 mg of the test item/kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils (please, refer to commenting table in point 9.9).
Taking in to consideration that DT_{50}s of zoxamide and metabolites which is < 60 days, field studies are not necessary. Maximum DT_{50}s from laboratory studies of zoxamide is 13.6 days it means that in the field predicted values would be even lower.
Taking in to consideration available data the risk to soil non-target macro-organisms is considered to be acceptable.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with zoxamide and its relevant metabolites and propamocarb-HCl. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide and propamocarb-HCl. 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	zoxamide	42 d, aerobic	<25% effect at 2 mg a.s./kg soil	EFSA Journal 2017;15(9):4980
C-mineralisation	zoxamide	14 d, aerobic	<25% effect at 2 mg a.s./kg soil	EFSA Journal 2017;15(9):4980
N-mineralisation	RH-141455	28 d, aerobic	<25% effect at 0.2-1 mg/kg soil	EFSA Journal 2017;15(9):4980
N-mineralisation	RH-127450		<25% effect at 0.2 mg met./kg soil dw	Parent endpoint divided by 10
N-mineralisation	RH-24549		<25% effect at 0.2 mg met./kg soil dw	Parent endpoint divided by 10
N-mineralisation	RH-163353		<25% effect at 0.2 mg met./kg soil dw	Parent endpoint divided by 10
N-mineralisation	Propamocarb-HCl	Study duration 28 d	≥ 28.9 mg a.s./kg dw	EFSA Scientific Report (2006) 78, 1-80
C-mineralisation	Propamocarb-HCl	Study duration 28 d	≥ 28.9 mg a.s./kg dw	EFSA Scientific Report (2006) 78, 1-80
N-mineralisation	GLOB2007bF	28 d, aerobic	On the basis of the results, it was concluded that GLOB2007bF at the concentrations corresponding to the PEC: 8.79 mg of the test item/kg dry weight of soil and 5xPEC: 44.0 mg of the test item/kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils. < 25% effect at 8.79-44 mg test item/kg soil dw	Hammesfahr, U., 2022

9.9.1.1 Justification for new endpoints

Not relevant as there is no deviation from the EU agreed endpoints.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of GLOB2007bF in potato

Intended use	Potato		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
zoxamide	2 (at 48 d)	0.1481	Yes
RH-127450	0.2	0.0219	Yes
RH-24549	0.2	0.0279	Yes
RH-163353	0.2	0.0291	Yes
RH-141455	1 (at 28d)	0.0120	Yes
propamocarb-HCl	28.9 (at 28 d)	1.3901	Yes
GLOB2007bF	44	1.1721	Yes

9.9.3 Overall conclusions

The EU review for zoxamide and propamocarb-HCl and the test on the formulation show that there are no effects on soil microbial activity at dose rates much higher than the corresponding PEC_{soil} of the intended use. Therefore, it is concluded that there is no unacceptable risk on soil microbial activity for GLOB2007bF.

Review comments:

The risk assessment for soil micro-organisms exposed to GLOB2007bF, following the proposed uses of the formulation, was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002).
The risk assessment presented in Table 9.9-2 is agreed by the zRMS. The relevant PECsoil for risk assessments is taken from Section 8 (Environmental Fate), for details please, refer to Section 8.
Based on the obtained results, soil nitrate formation rates were below the 25% trigger value. Thus, it is concluded that GLOB2007bF had no significant impact on soil microorganisms when applied at test item concentrations up to 44 mg formulation/kg soil dry weight.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with zoxamide and its relevant

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

Effects on non-target terrestrial plants of GLOB2007bF were not evaluated as part of the EU assessment of zoxamide and propamocarb-HCl. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.
Vegetative vigour and seedling emergence studies have been conducted with GLOB2007bF. New data submitted with this application are summarized in Appendix 2 - Effects on terrestrial non-target higher plants.
An overview of the endpoints and effects values of the formulation GLOB2007bF is provided in Table 9.10-1 below.

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

Species	Substance	Exposure System	Results	Reference
Avena sativa (oats)$_m$ *Allium cepa* (onion)$_m$ *Zea mays* (corn)$_m$ *Lolium perenne* (ryegrass)$_m$ *Beta vulgaris* (sugar beet)$_d$ *Helianthus annuus* (sunflower)$_d$ *Glycine max* (soybean)$_d$ *Solanum lycopersicum* (tomato)$_d$ *Brassica napus* (oilseed rape)$_d$ *Cucumis sativus* (cucumber)$_d$	GLOB2007bF	21 d Seedling emergence	ER_{50} emergence > 8 L/ha ER_{50} percentage survival > 8 L/ha ER_{50} shoot fresh weight > 8 L/ha ER_{50} visual injury > 8 L/ha	Stead, A., 2023, STC/22/E1556
Avena sativa (oats)$_m$ *Allium cepa* (onion)$_m$ *Zea mays* (corn)$_m$ *Lolium perenne* (ryegrass)$_m$ *Beta vulgaris* (sugar beet)$_d$ *Helianthus annuus* (sunflower)$_d$ *Glycine max* (soybean)$_d$ *Solanum lycopersicum* (tomato)$_d$ *Brassica napus* (oilseed rape)$_d$ *Cucumis sativus* (cucumber)$_d$	GLOB2007bF	21 d Vegetative vigour	ER_{50} visual injury > 8 L/ha ER_{50} plant survival > 8 L/ha ER_{50} shoot fresh weight > 8 L/ha	Davies, C., 2023, STC/22/E1555

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

Not relevant as there is no deviation from the EU agreed 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.

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is derived by the *BBA (2000)*[2] from the spray-drift predictions of *Ganzelmeier & Rautmann (2000)*[3] as recommended by the "Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC, SANCO/10329/2002.

Consequently, the initial exposure assessment was based on air deposition following the application of GLOB2007bF to areas adjacent to the field without and (if appropriate) with consideration of drift mitigation measures. Predicted exposure rates were calculated with the following formula:

$PER_{off\text{-}field}$ = (Appl. rate x Spray drift)

where PER = Predicted Environmental Rate (kg/ha or L/ha)
Appl. rate = rate of a single application expressed in the same units as the PER
Spray drift = % of the applied rate deposited to the off-field area by spray drift

Table 9.10-2a: Assessment of the risk for non-target plants due to the use of GLOB2007bF in potato

Intended use	Potato			
Product	GLOB2007bF			
Application rate (L formulation/ha)	2			
Test species	**ER_{50} (g formulation L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Avena sativa (oats)$_m$ *Allium cepa* (onion)$_m$ *Zea mays* (corn)$_m$ *Lolium perenne* (ryegrass)$_m$ *Beta vulgaris* (sugar beet)$_d$ *Helianthus annuus* (sunflower)$_d$ *Glycine max* (soybean)$_d$ *Solanum lycopersicum* (tomato)$_d$ *Brassica napus* (oilseed rape)$_d$ *Cucumis sativus* (cucumber)$_d$	> 8	2.77%	0.0554	144.4

m: monocotyledonous; d: dicotyledonous
PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

For countries that require MAF value in the Risk assessment the additional table was added below.

Table 9.10-3b: Assessment of the risk for non-target plants due to the use of GLOB2007bF in potato

Intended use	Potato

[2] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.
[3] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

Product	GLOB2007bF			
Application rate (L formulation/ha)	3 x 2			
MAF	2.3			
Test species	**ER_{50} (formulation L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Avena sativa (oats)$_m$ *Allium cepa* (onion)$_m$ *Zea mays* (corn)$_m$ *Lolium perenne* (ryegrass)$_m$ *Beta vulgaris* (sugar beet)$_d$ *Helianthus annuus* (sunflower)$_d$ *Glycine max* (soybean)$_d$ *Solanum lycopersicum* (tomato)$_d$ *Brassica napus* (oilseed rape)$_d$ *Cucumis sativus* (cucumber)$_d$	> 8	2.77%	0.12742	62.78

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

First tier risk assessment indicates that there is no unacceptable risk from GLOB2007bF for non-target plants when applied according to the proposed use rates.

Review comments:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).
Based on the risk assessment it can be concluded that the proposed use of GLOB2007bF poses no unacceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from GLOB2007bF applications are not required for the protection of terrestrial non-target plants.
In Table 9.10 2b, zRMS provided the current risk assessment with MAF values.

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

Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

Implications for labelling resulting from ecotoxicological assessment (justification is provided in Part C):

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to toxicological data is proposed for the preparation:

Table 9.13-1: Justified proposals for classification and labelling for GLOB2007bF according to Regulation (EC) No 1272/2008

Hazard class(es), categories	~~Aquatic Acute 1~~ Aquatic Chronic 1
Hazard pictograms or Code(s) for hazard pictogram(s)	GHS09
Signal word	Warning
Hazard statement(s)	~~H400~~ H410
Precautionary statement(s)	~~P273~~ P391 P501
Additional labelling phrases	To avoid risks to man and the environment, comply with the instructions for use. [EUH401] SPe3: To protect aquatic organisms respect an unsprayed buffer zone of 10 m including a 10 m vegetated filter strip to surface water bodies. *OR in case VFSMOD is accepted: a 5 m no spray buffer zone including a 5 m vegetated buffer strip.*

Review comments:

Since endpoints from acute studies with the formulation are above 1.0 mg/L, classification for acute hazard is not necessary.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1	xxxxxx	2023	GLOB2007bF: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-hour Semi-Static Test, xxxxxxxxxxxx, Report No.: 169561230, GLP, Unpublished	Y	Globachem NV
KCP 10.2.1	Thorpe, K.	2023	GLOB2007bF: *Daphnia magna* Acute Immobilisation Test, Fera Science Ltd, Report No.: FR/002723, GLP, Unpublished	N	Globachem NV
KCP 10.2.1	Wright, E.	2023	GLOB2007bF: *Pseudokirchneriella subcapitata* Growth Inhibition Test, Fera Science Ltd, Report No.: FR/002722, GLP, Unpublished	N	Globachem NV
KCP 10.3.1.1	Knautz, T.	2022	GLOB2007bF: Effects (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory, Ibacon Gmbh, Report No.: 169561035, GLP, Unpublished	N	Globachem NV
KCP 10.3.1.1	Chwiesko, D.	2023	GLOB2007bF: Acute Contact and Oral Toxicity to Bumblebees (*Bombus terrestris* L.) in the Laboratory, Ibacon Gmbh, Report No.: 169561105, GLP, Unpublished	N	Globachem NV
KCP 10.3.1.2	Schabio, S.	2023	GLOB2007bF: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera* L.) in the Laboratory, Ibacon Gmbh, Report No.: 169561136, GLP, Unpublished	N	Globachem NV
KCP 10.3.1.3	Colli, M.	2022	Effects of GLOB2007bF on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Biotecnologie Bt S.R.L., Report No.: BT127/22, GLP, Unpublished	N	Globachem NV
KCP 10.3.2.1	Leopold, J.	2022	GLOB2007bF: Effects on the Parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates, Ibacon Gmbh, Report No.: 169561001, GLP, Unpublished	N	Globachem NV
KCP 10.3.2.1	Leopold, J.	2022	GLOB2007bF: Effects on the Predatory Mite *Typhlodromus pyri* (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates, Ibacon Gmbh, Report No.: 169561063, GLP, Unpublished	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.1	Straube, D.	2022	GLOB2007bF (Zoxamide 67.5 g/L + propamocarb HCL 450 g/L SC): Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Ibacon Gmbh, Report No.: 169561022, GLP, Unpublished	N	Globachem NV
KCP 10.4.1.1	Straube, D.	2023	GLOB2007bF (Zoxamide 67.5 g/L + propamocarb HCL 450 g/L SC): Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Ibacon Gmbh, Report No.: 169562022, GLP, Unpublished	N	Globachem NV
KCP 10.4.1.1	Straube, D.	2023	GLOB2007bF: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Natural Soil, Ibacon Gmbh, Report No.: 169563022, GLP, Unpublished	N	Globachem NV
KCP 10.4.2.1	Straube, D.	2022	GLOB2007bF (Zoxamide 67.5 g/L + propamocarb HCL 450 g/L SC): Effects on Reproduction of Collembola (*Folsomia candida*) in Artificial Soil, Ibacon Gmbh, Report No.: 169561016, GLP, Unpublished	N	Globachem NV
KCP 10.4.2.1	Straube, D.	2022	Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil, Ibacon Gmbh, Report No.: 169561089, GLP, Unpublished	N	Globachem NV
KCP 10.5	Hammesfahr, U.	2022	GLOB2007bF: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation), Ibacon Gmbh, Report No.: 169561080, GLP, Unpublished	N	Globachem NV
KCP 10.6.2	Davies, C.	2023	GLOB2007bF: OECD Terrestrial Plant Test - Vegetative Vigour Test, Stockbridge Technology Centre Ltd., Report No.: STC/22/E1555, GLP, Unpublished	N	Globachem NV
KCP 10.6.2	Stead, A.	2023	GLOB2007bF: OECD Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test, Stockbridge Technology Centre Ltd., Report No.: STC/22/E1556, GLP, Unpublished	N	Globachem NV
KCA 8.2.1	xxxxxx	2020	RH-163353: Fish, acute toxicity test - Amended final report 1, xxxxxxxxxx, Report No.: 3202385, GLP, Unpublished	Y	Gowan*
KCA 8.2.1	xxxxxxxx	2020	RH-141455: Fish, acute toxicity test, xxxxxxxxxxxx, Report No.: 3202716, GLP, Unpublished	Y	Gowan*
KCA 8.2.1	xxxxxxxx	2020	RH-127450: Fish, acute toxicity test, xxxxxxxxxxxx, Report No.: 3202373, GLP, Unpublished	Y	Gowan*
KCA 8.2.4.2	Mikulas, J.	2023	RH-139432 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Stillmeadow Inc, Report No.: 25769-22, 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 8.2.4.2	Doig, A.	2023	RH-24549 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Stillmeadow Inc, Report No.: 25772-22, GLP, Unpublished	N	Globachem NV
KCA 8.2.4.2	Mikulas, J.	2023	RH-127450 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Stillmeadow Inc, Report No.: 25833-22, GLP, Unpublished	N	Globachem NV
KCA 8.2.4.2	Mikulas, J.	2023	RH-141455 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Stillmeadow Inc, Report No.: 25771-22, GLP, Unpublished	N	Globachem NV
KCA 8.2.4.2	Shaw, A.	2023	RH-163353 - Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions, Smithers Ers Ltd, Report No.: 14365.6102, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Jarratt, N.	2023	Zoxamide Technical: *Pseudokirchneriella subcapitata* Growth Inhibition Test, Fera Science Ltd, Report No.: FR/002786, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Softcheck, K.	2023	RH-163353 - 72-Hour Toxicity Test with the Freshwater Green Alga, *Raphidocelis subcapitata*, Smithers Ers Ltd, Report No.: 14365.6101, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Mikulas, J.	2023	RH-139432 72-Hour Algal Inhibition Test with *Pseudokirchneriella subcapitata*, Stillmeadow Inc, Report No.: 25770-22, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Mikulas, J.	2023	RH-127450 72-Hour Algal Inhibition Test with *Pseudokirchneriella subcapitata*, Stillmeadow Inc, Report No.: 25834-22, GLP, Unpublished	N	Globachem NV
KCA 8.3.1.3	Aguilar-Alberola, J.	2023	Zoxamide technical: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions, Eurofins Trialcamp S.L.U., Report No.: S23-106642, GLP, Unpublished	N	Globachem NV
KCA 8.4.1	Straube, D.	2023	RH-24549: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Ibacon Gmbh, Report No.: 166191022, GLP, Unpublished	N	Globachem NV
KCA 8.4.1	Straube, D.	2023	RH-127450: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Ibacon Gmbh, Report No.: 175161022, GLP, Unpublished	N	Globachem NV
KCA 8.4.1	Straube, D.	2023	RH-163353: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Ibacon Gmbh, Report No.: 175171022, GLP, Unpublished	N	Globachem NV

*Vertebrate data access is currently negotiated with the Notifier. The negotiation e-mail exchange was already sent to all MSs with the data matching package.

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

*Studies in the table below were generated to data match the AIR protected studies from the main notifier. The data matching package has been evaluated by the RMS Latvia and a copy was already sent to all MS.

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 8.2.4.1	Siche, O.	2022	RH-24549: Acute Toxicity to *Daphnia magna* in a Static 48-hour Immobilisation Test, Ibacon Gmbh, Report No.: 166191220, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Siche, O.	2023	(R)-Zoxamide: Toxicity to *Desmodesmus subspicatus* in an Algal Growth Inhibition Test, Ibacon Gmbh, Report No.: 168331210, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Siche, O.	2023	(S)-Zoxamide: Toxicity to *Desmodesmus subspicatus* in an Algal Growth Inhibition Test, Ibacon Gmbh, Report No.: 168321210, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Siche, O.	2022	Algae Growth Inhibition Study Green Algae (*Desmodesmus subspicatus*), Ibacon Gmbh, Report No.: 166191210, GLP, Unpublished	N	Globachem NV
KCA 8.2.6.1	Siche, O.	2022	RH-141455: Toxicity to *Pseudokirchneriella subcapitata* in an Algal Growth Inhibition Test, Ibacon Gmbh, Report No.: 166221210, GLP, Unpublished	N	Globachem NV
KCA 8.4.1	Straube, D.	2022	RH-141455: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Ibacon Gmbh, Report No.: 166221022, GLP, Unpublished	N	Globachem NV
KCA 8.5	Bauer, J.	2022	RH-141455: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation), Ibacon Gmbh, Report No.: 166221080, GLP, Unpublished	N	Globachem NV
KCP 10.2.1	Wright, E.	2023	GLOB2013F: *Pseudokirchneriella subcapitata* Growth Inhibition Test, Fera Science Ltd, Report No.: FR/002720, GLP, Unpublished	N	Globachem NV
KCP 10.3.1.2	Konieczna, A.	2021	Honey Bee, chronic oral toxicity test of the test item Zoxamide 450 SC according to OECD 245 Guideline, Sorbolab Research Laboratory Llc, Report No.: 0064/0015/E, GLP, Unpublished	N	Globachem NV
KCP 10.3.1.2	Konieczna, A.	2021	Honey Bee Larval Toxicity Test following Repeated Exposure to the test item Zoxamide 450 SC according to OECD GD 239 ENV/JM/MONO(2016)34, Sorbolab Research Laboratory Llc, Report No.: 0064/0012/E, GLP, Unpublished	N	Globachem NV

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

Review Comment:
In order to provide sufficient detail, where appropriate, the following studies summaries have been adapted by the zRMS. Details were taken directly from the full studies reports provided in the dossier. zRMS text is highlighted in grey. The comments on individual studies are provided in grey comment boxes.

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

No data submitted.

Summarised in Section 6 (Mammalian Toxicology)

A 2.2 KCP 10.2 Effects on aquatic organisms

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

Comments of zRMS:	The study was conducted to OECD guideline 203 (1992) and according to the principles of GLP. In the definitive test all validity criteria were met. The mortality of fish in the control was 0%. The dissolved oxygen concentration was ≥ 90% of air saturation value. Samples of each test item concentration and the control collected at exposure initiation and termination were chemically analysed. The analytical measurements demonstrated that the test item concentration throughout the test for Propamocarb-HCl was within 80-120% of nominal but for zoxamide was outside this range. For this reason all results refer to measured concentrations. The study is reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1
Report	GLOB2007bF: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-hour Semi-Static Test, xxxxx, 2023, xxxxxxxxx, Report No.: 169561230
Guideline(s):	OECD 203
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / supplementary~~
Duplication (if vertebrate study)	No

Materials and methods

Test Item: GLOB2007bF; batch no.: LCM22012601;

	Zoxamide (sum of both stereoisomers): 67.5 g/L (nominal), 5.846 ± 0.007 % w/w or 58.46 ± 0.07 g/kg or 64.23 ± 0.07 g/L (analytical) Propamocarb-HCl: 450 g/L (nominal), 40.57 ± 0.12 % w/w or 405.7 ± 1.2 g/kg or 445.8 ± 1.3 g/L (analytical), according to certificate of analysis
Test Species:	Juvenile rainbow trout (*Oncorhynchus mykiss)*, mean length: 4.60 cm ± 0.3 cm; source: Mohnen aquaculture, Teichanlage Schevenhütte, Nideggener Strasse 124-128 and 150, 52224 Stolberg, Germany
Test Design:	This study encompassed 6 treatment groups (5 dose rates of the test item and a control) each containing 7 individuals. The acute toxicity to unfed juvenile rainbow trout was determined in an aerated, semi-static, 96-hour test. The test fish were observed at test start and after approximately 2, 5, 24, 29, 48, 54, 72, 76 and 96 hours test duration for sublethal effects and mortality.
Endpoints:	NOEC after 96 h, LOEC after 96 h; LC_{50}: lethal concentration producing 50 % mortality after 96 h of exposure.
Test Concentrations:	12, 6, 3, 1.5 and 0.75 mg test item/L (spacing factor 2) and a control The test concentration corresponded to time weighted average concentrations based on measured concentrations of Zoxamide of 0.583, 1.04, 2.25, 4.98 and 9.45 mg test item/L.
Test Conditions:	Water temperature: 12.6 to 13.4 °C (target: 10 – 14 °C); pH value: 7.3 to 7.7 (target: 6.0 – 8.5); dissolved oxygen concentration: 90 to 100 % of the air saturation value (target: ≥ 60 %); photoperiod: 12 h light - 12 h dark; light intensity: 640 to 840 lux (target: 540 – 1000 lux). Thus, test conditions were within the ranges requested by guideline OECD 203.

Results and discussions

Validity Criteria:	In the control no fish died. The dissolved oxygen concentration in the test media did not fall below 90 % of air saturation value. The test concentration was analysed. Thus, all validity criteria were met.
Biological test results:	In the control and up to and including the time weighted average concentration of 9.45 mg test item/L, all fish survived until the end of the experiment. The following symptoms were observed for the surviving fish: loss of equilibrium, abnormal swimming behaviour, abnormal skin pigmentations and aggressive behaviour. The mortality observed is summarised in Table 1, details on symptoms are shown in Table 5 to Table 8.

Analytical results

Zoxamide:
In the freshly prepared test media at the start of the test and at the renewal of the test media, 92 % of the nominal test concentrations were found (average of all test concentrations). In the aged test media after 24 hours test duration, 65 % of the nominal value was determined (average of all test concentrations). During the test, the test organism were exposed to a mean of 79 % of nominal.

The determined recovery values correspond to time-weighted average concentrations of 0.583, 1.04, 2.25, 4.98 and 9.45 mg test item/L (0.0341, 0.0608, 0.131, 0.291, 0.552 mg Zoxamide/L) for the nominal test concentrations of 0.75, 1.5, 3, 6 and 12 mg test item/L, respectively.

Propamocarb-HCL

In the freshly prepared test media at the start of the test and at the renewal of the test media, 108 % of the nominal test concentrations were found (average of all test concentrations). In the aged test media after 24 hours test duration, 108 % of the nominal value was determined (average of all test concentrations). During the test, the test organism were exposed to a mean of 108 % of nominal.

Table 3. Summary of Analytical Results for Zoxamide

Nominal concentration [mg test item/L]	fresh (0h) % of nominal[1]	fresh (0h) RSD [%]	fresh (0h) n	aged (24h) % of nominal[1]	aged (24h) RSD [%]	aged (24h) n	Time Weighted Average[2] [%]	Time Weighted Average[2] concentration [mg test item/L]
Control	n.a.	n.a.	4	n.a.	n.a.	4	n.a.	n.a.
0.75	88	6	4	68	4	4	78	0.583
1.5	86	5	4	64	3	4	69	1.04
3	90	5	4	61	4	4	75	2.25
6	98	3	4	70	9	4	83	4.98
12	96	9	4	64	19	4	79	9.45

[1] mean value of all measured samples per treatment group
[2] Time weighted average calculated according to OECD Guidance Document No. 23, Annex 2
RSD: relative standard deviation per treatment group; n: number of analysed samples; n.a.: not applicable

Table 4. Summary of Analytical Results for Propamocarb-HCL

Nominal concentration [mg test item/L]	fresh (0h) % of nominal[1]	fresh (0h) RSD [%]	fresh (0h) n	aged (24h) % of nominal[1]	aged (24h) RSD [%]	aged (24h) n	Time Weighted Average[2] [%]	Time Weighted Average[2] concentration [mg test item/L]
Control	n.a.	n.a.	4	n.a.	n.a.	4	n.a.	n.a.
0.75	108	4	4	107	3	4	108	0.806
1.5	107	4	4	105	5	4	106	1.60
3	107	3	4	107	3	4	107	3.22
6	110	2	4	110	4	4	110	6.60
12	109	4	4	109	3	4	109	13.0

[1] mean value of all measured samples per treatment group
[2] Time weighted average calculated according to OECD Guidance Document No. 23, Annex 2
RSD: relative standard deviation per treatment group; n: number of analysed samples; n.a.: not applicable

Table 2. Observed Mortality of unfed Rainbow Trout (*Oncorhynchus mykiss*) exposed to GLOB2007bF for 96 hours

Time weighted average concentration	Mortality (No. of dead fish)				
[mg test item/L]	0 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
0.583	0	0	0	0	0
1.04	0	0	0	0	0
2.25	0	0	0	0	0
4.98	0	0	0	0	0

9.45	0	0	0	0	0
LC_{50} [mg test item/L]	-	> 9.45	> 9.45	> 9.45	> 9.45
95% CI.	-	n.d.	n.d.	n.d.	n.d.

n.d.: not determinable
- not relevant
CI.: Confidence interval
Values refer to time weighted average test concentrations.

Conclusion

The study is valid since all required validity criteria were met.
The acute effect of the test item GLOB2007bF to rainbow trout (*Oncorhynchus mykiss)* was assessed in a semi-static concentration-response test with daily test medium renewal. Based on the test results, the 96-hour LC_{50} was determined to be > 9.45 mg test item/L based on time weighted average concentrations.
The NOEC based on mortality was determined to be > 9.45 mg test item/L also based on time weighted average concentrations.
The initial concentrations and the maintenance of the exposure concentrations during the test were determined in the analytical part. All reported results refer to time-weighted average concentrations based on Zoxamide since the Zoxamide concentrations were not within ± 20 % of the nominal concentrations during the measured water renewal periods.

Comments of zRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test the validity criteria were met. The study is considered to be reliable and suitable for the risk assessment. Results are reported using the geometric mean measured concentrations of the active ingredients zoxamide and propamocarb HCl and a calculated geometric mean measured concentration of the formulation that is adjusted for the recovery of the lowest component (zoxamide).

Reference:	KCP 10.2.1
Report	GLOB2007bF: *Daphnia magna* Acute Immobilisation Test, Thorpe, K., Fera Science Ltd, Report No.: FR/002723
Guideline(s):	OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / supplementary~~

Materials and methods

Test Item:	GLOB2007bF
Test Species:	*Daphnia magna* (Clone 5), an aquatic Cladoceran
Source:	CCSS (Centre for Chemical Stewardship and Safety), Fera

Test Media: Elendt M4 medium

Test System: *Daphnia magna* were exposed to a test concentration series of the test item diluted with Elendt M4 medium, compared with a negative control group (M4 medium only). The test was performed under semi-static conditions with renewal of the test media after 24 hours.

Test Item Concentrations: Nominal concentrations of GLOB2007bF were 3.175, 6.35, 12.7, 25.4 and 50.8 mg/L (as formulation), with a negative control of untreated test media.

Replicates: There were four replicates per treatment. Five test organisms were added to each treatment replicate.

Observations: Immobility – Neonates that were unable to swim within 15 seconds after gentle agitation of the test vessel were recorded as immobile, even where the antennae were still moving.

Test Endpoints: The test endpoint was the proportion of immobile and mobile test organisms at 24 hours and 48 hours after the start of the exposure.

All samples were analysed by LCMS-MS. Triplicate samples were analysed from fresh media at the start of each 24-h renewal period (0 and 24-h) and from pooled replicate media at the end of each (24 and 48-h); a summary of the results for zoxamide and propamocarb HCl are presented in **Table 1 and Table 2,** respectively.

Mean measured concentrations at 0, 24 and 48 hours indicated that zoxamide was not stable within the test system, with the percent recoveries at the highest test concentration decreasing from between 36 and 44% of nominal in the fresh media to between 10 and 12% of nominal in the aged media. Propamocarb HCl was stable within the test system.

As the test item, GLOB2007bF, is a mixture, of two active ingredients (zoxamide and propamocarb HCl) and co-formulants, which was tested as a whole substance, results are reported using nominal concentrations of the test item. Additionally, due to the low recoveries of zoxamide, results are also reported using the geometric mean measured concentrations of the active ingredients zoxamide and propamocarb HCl.

Results and discussions

Table 1: Summary of analytical measurement results of zoxamide in the test solutions

Nominal Concentration of GLOB2007bF (mg/L)	Nominal Concentration of Zoxamide (µg/L)	Mean Measured Concentration of Zoxamide (µg/L)				Geometric Mean Measured Concentration of Zoxamide (µg/L)
		0-h (Fresh)	24-h (Aged)	24-h (Fresh)	48-h (Aged)	
Control	-	n.d.	n.d.	n.d.	n.d.	n/a
3.18	185.6	89.4*	120.7	89.6*	101.2*	99.4

6.35	371.2	167.8	159.6	174.9	157.8	164.9
12.7	742.4	324.5	174.6	315.8	172.3	235.6
25.4	1484.7	445.6	221.1	609.8	228.8	342.4
50.8	2969.5	1071.6	352.9	1313.1	295.0	618.7

n.d. = not determined; n/a = not applicable; LOQ = limit of quantification = 110 µg/L

*For the lowest exposure concentration, the measured concentrations of zoxamide were found to be below the validated limit of quantification for the analytical method. As there was no evidence of an effect of exposure to the formulation within the test, and statistical analysis was not required, this deviation from the analytical method is not considered to have an impact on study outcomes.

Table 2: Summary of analytical measurement results of propamocarb HCl in the test solutions

Nominal Concentration of GLOB2007bF (mg/L)	Nominal Concentration of Propamocarb HCl (mg/L)	Mean Measured Concentration of Propamocarb HCl (mg/L)				Geometric Mean Measured Concentration of Propamocarb HCl (mg/L)
		0-h (Fresh)	24-h (Aged)	24-h (Fresh)	48-h (Aged)	
Control	-	n.d.	n.d.	n.d.	n.d.	n/a
3.18	1.288	1.177	1.247	1.214	1.218	1.214
6.35	2.576	2.448	2.523	2.461	2.556	2.497
12.7	5.153	4.939	5.071	4.884	4.954	4.961
25.4	10.31	9.993	10.016	9.730	10.202	9.984
50.8	20.61	20.222	20.976	19.814	20.841	20.458

n.d. = not determined; n/a = not applicable; LOQ = limit of quantification = 0.14 mg/L

Table 3: Calculated concentrations of GLOB2007bF in the test solutions

Nominal Concentration of GLOB2007bF (mg/L)	% Recovery of Zoxamide	% Recovery of Propamocarb HCl	Concentration of GLOB2007bF (mg/L) adjusted based on the recovery of Zoxamide.
Control	-	-	n/a
3.18	53.6	94.3	1.70
6.35	44.4	96.9	2.82
12.7	31.7	96.3	4.03
25.4	23.1	96.8	5.86

50.8	20.8	99.3	10.6

Statistical analysis was not performed at 24 or 48 hours due to lack of immobilization within the test. The EC_{50} and NOEC are therefore defined as greater than the highest test concentration.

The following validity criteria were met in the test:

- *In the control not more than 10% of the daphnids should have been immobilised.* In this test, 0% immobility was observed in the control treatment.
- *The dissolved oxygen concentration at the end of the test should be greater than 3 mg/L in control and test vessels.* In this test the minimum dissolved oxygen recorded in an aged solution of the test was 9.17 mg/L.

Conclusion

The immobilization EC_{50} for GLOB2007bF was greater than **10.6 mg/L** based on the adjusted formulation concentrations. The EC_{50} and for zoxamide and propamocarb HCl were greater than **0.619 mg/L** and **20.5 mg/L** based on mean measured concentrations, respectively.

EFSA (2017) requested in its Peer Review Conclusion; "*Further data or refinement on aquatic invertebrates (Mysidopsis bahia) are needed to cover the risk for the metabolites RH-127450, RH-24549, RH-163353, RH-141455 and RH-139432. The Applicant provided the studies for mentioned metabolites.*

Comments of zRMS: The study on the mysid was conducted following the guideline OCSPP 850.1035 (2016), under GLP certification for RH-163353.

Validity criteria were met as follows:

Acceptability Criteria	Study Results	Criterion Met (Yes/No)
Exposure vessels will be identical.	Exposure vessels were identical	yes
Treatments will be indiscriminately assigned to individual exposure vessel locations and individual test organisms will be indiscriminately assigned to exposure vessels.	Treatments were indiscriminately assigned to individual exposure vessel locations and individual test organisms were indiscriminately assigned to each exposure vessel	yes
A dilution water control, and solvent control, if necessary, will be included in the test	A dilution water control was included. No solvent was used during this exposure	yes
No more than 10% of organisms in the control, or solvent control, if applicable, can show signs of disease, stress (e.g.,	Mortality of 5% was observed in the control; no adverse effects were observed among control organisms	yes

	discoloration, unusual behavior, immobilization), and/or death.		
	A surfactant or dispersant was not used in preparation of a stock or exposure solution	Neither a surfactant nor dispersant was used during preparation of solutions for this study	yes
	The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. All results refer to nominal concentrations.		

Reference: KCA 8.2.4.2

Report: RH-163353 - Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions, Shaw, A., 2023, Smithers Ers Ltd, Report No.: 14365.6102

Guideline(s): OCSPP 850.1035

Deviations: No

GLP: Yes

Acceptability: Yes ~~/ No / Supplementary~~

Materials and methods

The purpose of this study was to estimate the 96-hour acute toxicity (LC_{50}) of RH-163353 to mysids (*Americamysis bahia*) under static conditions. The LC_{50} is defined as the concentration of the test substance in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of potential acute hazards resulting from release of the test substance into aquatic environments. Results of this study are presented based on nominal concentrations of RH-163353.

Test Substance	
Name:	RH-163353
Synonym:	3-(3,5-dichloro-4-methylbenzamido)-3-methyl-2-oxopentanoic acid (RH-163353)
Batch No.:	GD-003454-03
CAS No.:	401520-47-6
Purity:	100%
Storage Conditions:	Stored at room temperature in a dark, ventilated cabinet in the original container
Expiry Date:	9 March 2024
Date Received:	16 March 2023
Received From:	Globachem Discovery Ltd., Macclesfield, United Kingdom

Test Concentrations	
Nominal Concentrations:	1.0, 2.3, 5.0, 11, and 24 mg/L
Mean Measured Concentrations:	0.99, 2.1, 4.7, 10, and 24 mg/L
Co-Solvent:	None
Co-Solvent Load:	Not applicable
Interval(s) of Analytical Verification:	0 and 96 hours

Results and discussions/Conclusion

The results of the analysis of the exposure solutions for test substance concentration are presented in table below.

Table 2. 96-Hour Static Exposure of Mysids (*Americamysis bahia*) to RH-163353 - Concentrations Measured in the Exposure Solutions

Nominal Concentration (mg/L)	Measured Concentration (mg/L)			Percent of Nominal[a]
	0-Hour	96-Hour	Mean[a]	
Control	<0.10[b]	<0.10	NA[c]	NA
1.0	0.99	0.99	0.99	99
2.3	2.1	2.2	2.1	93
5.0	4.5	4.9	4.7	94
11	8.7	12	10	92
24	24	25	24	100
QC[d]#1 0.500	0.500 (99.9)	0.427 (85.4)		
QC#2 5.00	4.31 (86.1)	4.28 (85.7)		
QC#3 25.0	25.8 (103)	24.8 (99.4)		

[a] Mean measured concentration and percent of nominal values were calculated using the actual analytical (unrounded) results and not the rounded values presented in this table.
[b] Concentrations expressed as less than values were below the limit of detection (LOD). The LOD is dependent upon the lowest concentration calibration standard and the dilution factor of the controls (i.e., 0.0000300 mg/L × 3330 = 0.10 mg/L).
[c] NA = Not Applicable
[d] QC = Quality Control sample. Percent recovery for each QC sample is presented in parentheses.

The biological endpoint results are based on nominal concentrations. The nominal concentrations tested, the corresponding cumulative percent and number of mortalities, and the observations made during the definitive exposure are presented in table below.

Table 3. 96-Hour Static Exposure of Mysids (*Americamysis bahia*) to RH-163353 - Nominal Concentrations Tested, Corresponding Cumulative Percent and Number of Mortalities, and Observations

Nominal Concentration (mg/L)	Number of mysids added	Cumulative Percent Mortality[a]							
		24 Hour		48 Hour		72 Hour		96 Hour	
		Mortality	Sublethal	Mortality	Sublethal	Mortality	Sublethal	Mortality	Sublethal
Control	20	5 (1)	--	5 (1)	--	5 (1)	--	5 (1)	--
1.0	20	0 (0)	--	0 (0)	--	0 (0)	--	0 (0)	--
2.3	20	0 (0)	--	0 (0)	--	0 (0)	--	0 (0)	--
5.0	20	5 (1)	--	5 (1)	--	5 (1)	--	5 (1)	--
11	20	10 (2)	--	10 (2)	--	10 (2)	--	10 (2)	--
24	20	15 (3)	--	15 (3)	--	15 (3)	--	15 (3)	--

[a] The actual number of mortalities is presented in parentheses and any sublethal effects are presented below.
'--' = no sublethal effects were observed

LC_{50} values and corresponding 95% confidence intervals and the No-Observed-Effect Concentration (NOEC) through 96 hours are presented in table below.

Results Based on Nominal Concentrations:	
96-Hour LC_{50} Value:	>24 mg/L
No-Observed-Effect Concentration (NOEC):	24 mg/L
Highest Concentration Producing 0% Toxicant-Related Mortality:	2.3 mg/L
Lowest Concentration Producing 100% Mortality:	>24 mg/L
Rationale for No Further Testing:	Although an LC_{50} value was not achieved, per discussion with the Study Sponsor, there is no need to test at higher concentrations.

Comments of zRMS: The study on the mysid was conducted following the guideline OCSPP 850.1035 (2016), under GLP certification for RH-139432.

Validity criteria were met as follows:

Acceptability Criteria	Study Results	Criterion Met (Yes/No)
Exposure vessels will be identical.	Exposure vessels were identical	yes
Treatments will be indiscriminately assigned to individual exposure vessel locations and individual test organisms will be indiscriminately assigned to exposure vessels.	Treatments were indiscriminately assigned to individual exposure vessel locations and individual test organisms were indiscriminately assigned to each exposure vessel	yes
A dilution water control, and solvent control, if necessary, will be included in the test	A control solution was made using only dilution water	yes
No more than 10% of organisms in the control, or solvent control, if applicable, can show signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death.	Mortality of 3.33 % was observed in the control; no adverse effects were observed among control organisms	yes
A surfactant or dispersant was not used in preparation of a stock or exposure solution	Neither a surfactant nor dispersant was used during preparation of solutions for this study	yes

The 96-hour (LC_{50}) was 19.203 mg/L (7.343 mg/L) with 95% confidence limits of 15.889 - 25.914 mg/L (6.2379 mg/L - 9.6840 mg/L). LC_{50} used for the risk

	assessment purpose would be provided based on the measured concentration of 7.343 mg RH-139432 /L.

Reference:	KCA 8.2.4.2
Report	RH-139432 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Mikulas, J., 2023, Stillmeadow Inc, Report No.: 25769-22
Guideline(s):	OCSPP 850.1035
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

This study was conducted to assess the toxicity of the test item, RH-139432, to the mysid shrimp, *Mysidopsis bahia,* in a 96-hour test with daily renewals.

Test concentrations were determined by a preliminary range-finding test. The test item concentrations of 1.5625 mg/L, 3.125 mg/L, 6.25 mg/L, 12.5 mg/L, and 25 mg/L were administered to the test system, *Mysidopsis bahia*, in synthetic seawater with daily renewals. Three replicates of ten organisms were treated with each concentration of the test item. Each of the three control containers contained 10 organisms in synthetic seawater and no test item. Dissolved oxygen, temperature, salinity and pH measurements were recorded at dosing and daily through study termination. Observations for mortality were made at 0, 24, 48, 72 and 96 hours after treatment. The test was terminated after 96 ± 2 hours of exposure.

Results and discussions/Conclusion

Dose Verification
The concentration of the active ingredient, 3,5-dichloro-4-methylbenzamide, in the test item was determined in each new test solution at 0, 24, 48 and 72 hours and in each old test solution at 24, 48, 72 and 96 hours by validated standard analytical methods.

Table 2 - Dose Verification Results

Nominal Concentration (mg/L)	Control	1.5625	3.125	6.25	12.5	25
0 Hour New (mg/L)	0	2.175	1.487	2.845	5.053	8.950
24 Hour New (mg/L)	0	1.774	1.992	2.746	3.877	7.939
24 Hour Old (mg/L)	0	2.469	1.983	3.484	5.412	9.417
48 Hour New (mg/L)	0	3.383	4.466	3.782	5.420	9.088
48 Hour Old (mg/L)	0	2.788	2.845	3.104	4.320	9.702
72 Hour New (mg/L)	0	4.079	5.659	4.565	6.356	9.458
72 Hour Old (mg/L)	0	4.728	4.508	5.017	5.552	10.339
96 Hour Old (mg/L)	0	4.032	5.220	4.065	4.765	9.714
Average (mg/L)	**0**	**3.02**	**3.16**	**3.62**	**5.04**	**9.30**

At 48 hours, the No Observed Effect Concentration (NOEC) of RH-139432 was determined to be 12.5 mg/L (5.04 mg/L), the 48-hour Median Lethal Concentration (LC_{50}) was > 25 mg/L (> 9.3 mg/L), and the 48-hour Lowest Observed Effect Concentration (LOEC) was 25 mg/L (9.3 mg/L).

At 96 hours, the (NOEC) of RH-139432 was determined to be 12.5 mg/L (5.04 mg/L), the 96-hour (LC_{50}) was > 19.203 mg/L (> 7.343 mg/L) with 95% confidence limits of 15.889 - 25.914 mg/L (6.2379 mg/L - 9.6840 mg/L), and the 96-hour (LOEC) was 25 mg/L (9.3 mg/L).
Parenthetical values are dose verified concentrations.

Table 3 - Definitive Tests Mortality Results

Nominal Conc. (mg/L)	Replicate	Number of Surviving Organisms					Mortality
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	
Control	A	10	10	10	10	10	3.33%
	B	10	9[A]	9	9	9	
	C	10	10	10	10	10	
1.5625	A	10	10	9[C]	9	9[F]	3.33%
	B	10	10	10	10	10[F]	
	C	10	10	10	10	10[F]	
3.125	A	10	10	10	10	10[F]	0.00%
	B	10	10	10	10	10[F]	
	C	10	10	10	10	10[F]	
6.25	A	10	10	10	10	10[F]	3.33%
	B	10	10	10	10	10[F]	
	C	10	10	10	9[A]	9[F]	
12.5	A	10	10	10	10	10[F]	10.00%
	B	10	10	9[C]	8[A]	8[F]	
	C	10	10	10	9[A]	9[F]	
25	A	10	10[B]	8[D]	6[E]	4[G]	86.67%
	B	10	10[B]	9[D]	5[E]	0[G]	
	C	10	10[B]	5[D]	0[E]	-	

Note: All organisms had no observable abnormalities (NOA) unless otherwise indicated.
(-) - Not Applicable, all organisms dead; Conc. - Concentration
[A] - One mysid missing; Rest NOA; Water NOA.
[B] - Shrimp not swimming normal; Most moving lethargically; Water NOA
[C] - One mysid dead; Other replicates NOA; Water NOA.
[D] - Two mysid dead in replicate A; 5 dead in replicate C; Mysid lethargic and twitching on bottom; Water NOA.
[E] - Two mysid dead in replicate A; 4 dead in replicate B, 1 twitching when prodded; 5 dead in replicate C; Surviving organisms swimming lethargically.
[F] - Organisms appear not to have grown at same rate as control; Water NOA.
[G] - Two mysid dead in replicate A; 5 dead in replicate B; Organisms appear not to have grown at same rate as control; Water NOA.

Comments of zRMS: The study on the mysid was conducted following the guideline OCSPP 850.1035 (2016), under GLP certification for RH-24549.

Validity criteria were met as follows:

Acceptability Criteria	Study Results	Criterion Met (Yes/No)
Exposure vessels will be identical.	Exposure vessels were identical	yes
Treatments will be indiscriminately assigned to individual exposure vessel locations and individual test organisms will be indiscriminately assigned to exposure vessels.	Treatments were indiscriminately assigned to individual exposure vessel locations and individual test organisms were indiscriminately assigned to each exposure vessel	yes
A dilution water control, and solvent control, if necessary, will be included in	A control solution was made using only dilution water	yes

	the test		
	No more than 10% of organisms in the control, or solvent control, if applicable, can show signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death.	Mortality of 10 % was observed in the control; no adverse effects were observed among control organisms	yes
	A surfactant or dispersant was not used in preparation of a stock or exposure solution	Neither a surfactant nor dispersant was used during preparation of solutions for this study	yes
	The 96-hour LC50 was determined to be 88.506 mg/L (35.766 mg/L) LC_{50} used for the risk assessment purpose would be provided based on the measured concentration of 35.766 mg RH-24549./L.		

Reference:	KCA 8.2.4.2
Report	RH-24549 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Doig, A., 2023, Stillmeadow Inc, Report No.: 25772-22
Guideline(s):	OCSPP 850.1035
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

This study was conducted to assess the toxicity of the test item, RH-24549, to the mysid shrimp, *Mysidopsis bahia,* in a 96-hour test with daily renewals.

Test concentrations were determined by a preliminary range-finding test. The test item concentrations of 6.25 mg/L, 12.5 mg/L, 25.0 mg/L, 50.0 mg/L, and 100 mg/L were administered to the test system, *Mysidopsis bahia*, in synthetic seawater with daily renewals. Three replicates of ten organisms were treated with each concentration of the test item. Each of the three control containers contained 10 organisms in synthetic seawater and no test item. Dissolved oxygen, temperature, salinity and pH measurements were recorded at dosing and daily through study termination. Observations for mortality were made at 0, 24, 48, 72 and 96 hours after treatment. The test was terminated after 96 ± 1 hours of exposure.

Results and discussions/Conclusion

Dose verification resulted in dose verified concentrations of 2.65, 6.68, 12.74, 22.12 and 39.84 mg/L for the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L, respectively.

Table 2 - Dose Verification Results

Nominal Concentration (mg/L)	Control	6.25	12.5	25	50	100
0 Hour New (mg/L)	0	2.713	6.398	11.273	20.647	39.524
24 Hour New (mg/L)	0	2.391	4.737	10.284	18.118	34.545
24 Hour Old (mg/L)	0	2.694	8.813	13.656	20.855	39.829
48 Hour New (mg/L)	0	2.583	5.958	10.242	20.818	38.889
48 Hour Old (mg/L)	0	2.458	6.002	12.808	19.932	37.728
72 Hour New (mg/L)	**0**	2.908	6.197	12.906	23.822	42.556
72 Hour Old (mg/L)	**0**	2.583	8.109	13.777	24.113	41.754
96 Hour Old (mg/L)	**0**	2.886	8.292	18.851	30.818	44.772
Geometric Mean (mg/L)	**0**	**2.65**	**6.68**	**12.74**	**22.12**	**39.84**

Definitive Test A mortality of 10.00% was observed in the control group with 3 organisms missing from replica C at 72 hours. A 0.00% mortality was observed in mysid treated with 6.25 mg/L of the test item. A mortality of 3.33%, 16.66%, 13.33%, and 63.33% was observed in mysid treated with 12.5 mg/L, 25 mg/L, 50 mg/L 100 mg/L of the test item, respectively. There were no observable abnormalities, besides mortality, in all groups treated with the test item and the control. Results presented are based on nominal concentrations and are presented in the following table.

Table 3 - Definitive Test and Mortality Results

Nominal Conc. (mg/L)	Replicate	Number of Surviving Organisms					Mortality
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	
Control	A	10	10	10	10	10	**10.00%**
	B	10	10	10	10	10	
	C	10	10	10	7^{F}	7	
6.25	A	10	10	10	10	10	**0.00%**
	B	10	10	10	10	10	
	C	10	10	10	10	10	
12.5	A	10	10	10	10	10	**3.33%**
	B	10	10	10	9^{G}	9	
	C	10	10	10	10	10	
25	A	10	10	10	9^{G}	8^{H}	**16.66%**
	B	10	10	10	10	10	
	C	10	10	10	10	7^{F}	
50	A	10	10	10	9^{G}	6^{F}	**13.33%**
	B	10	10	10	10	10	
	C	10	10	10	10	10	
100	A	10^{A}	10^{A}	7^{DE}	6^{BE}	2^{I}	**63.33%**
	B	10^{A}	10^{A}	8^{CE}	5^{DE}	4^{H}	
	C	10^{A}	10^{A}	9^{BE}	6^{DE}	5^{H}	

Note: All organisms had no observable abnormalities (NOA) unless otherwise indicated; Conc. - Concentration
[A] - Water clear with some undissolved test material on top.
[B] - One organism dead on bottom.
[C] - Two organisms dead on bottom.
[D] - Three organisms dead on bottom.
[E] - Water clear with some undissolved test material stuck on sides of beakers and floating on top.
[F] - Three organisms missing.
[G] - One organism missing.
[H] - One organism dead.
[I] - Four organisms dead.

At 48 hours, the No Observed Effect Concentration (NOEC) of RH-24549 was determined to be 50 mg/L (22.12 mg/L). The 48-hour Median Lethal Concentration (LC_{50}) was determined to be > 100 mg/L (> 39.84 mg/L) and the 48-hour Lowest Observed Effect Concentration (LOEC) was 100 mg/L (39.84 mg/L).
At 96 hours, the NOEC of RH-24549 was determined to be 50 mg/L (22.12 mg/L). The 96-hour LC_{50} was determined to be 88.506 mg/L (35.766 mg/L) and the 96-hour LOEC was 100 mg/L (39.84 mg/L). Parenthetical values are dose verified concentrations.

Comments of zRMS:

The study on the mysid was conducted following the guideline OCSPP 850.1035 (2016), under GLP certification for RH-127450.
According to the method. in some situations, it is only necessary to ascertain that the 96-h LC_{50} is above a certain limit concentration (i.e., 96-h LC_{50} greater than (>) limit concentration). And the of limit test design can be performed.
In a limit test, at least 20 mysids, divided equally into a minimum of 2 replicates, are exposed to a single "limit concentration," with the same number of organisms in appropriate controls. In current study three replicates of 10 organisms were treated witch the concentration.
Except for the number of test concentrations, limit tests should follow the same test procedures, have the same duration
as the multiple-concentration definitive test and have both a dilution water control and a vehicle (solvent) control, if a vehicle is used).
In current study only one concentration was measured and design of the study was limit test. The control should demonstrate good condition of the population while the mortality in control (diluted water only) was twice higher than at the measured concentration. Since mortality in limit test appeared a multiple-concentration 96-hour test should be conducted according to OCSPP 850.1035 method.

Validity criteria were met as follows:

Acceptability Criteria	Study Results	Criterion Met (Yes/No)
Exposure vessels will be identical.	There was only one vessel	Comparison not possible
Treatments will be indiscriminately assigned to individual exposure vessel locations and individual test organisms will be indiscriminately assigned to exposure vessels.	There was only one treatment	Yes
A dilution water control, and solvent control, if necessary, will be included in the test	A dilution water control was included. No solvent was used during this exposure	yes
No more than 10% of organisms in the control, or solvent control, if applicable, can show signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death.	Mortality of 6.67 % was observed in the control; no adverse effects were observed among control organisms	yes
A surfactant or dispersant was not used in preparation of a stock or exposure solution	Neither a surfactant nor dispersant was used during preparation of solutions for this study	yes

The 96-hour LC_{50} was determined to be 1 mg/L (0.364 mg/L)
LC_{50} used for the risk assessment purpose would be provided based on the measured concentration of 0.364 mg RH-127450/L.

Reference:	KCA 8.2.4.2
Report	RH-127450 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Mikulas, J., 2023, Stillmeadow Inc, Report No.: 25833-22
Guideline(s):	OCSPP 850.1035
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

This study was conducted to assess the toxicity of the test item, RH-127450, to the mysid shrimp, *Mysidopsis bahia*, in a 96-hour test with daily renewals.

Test concentrations were determined by a preliminary range-finding test. The test item concentration of 1.0 mg/L was administered to the test system, *Mysidopsis bahia*, in synthetic seawater with daily renewals. Three replicates of ten organisms were treated with ~~each~~ one concentration of the test item. Each of the three control containers contained 10 organisms in synthetic seawater and no test item. Dissolved oxygen, temperature, salinity and pH measurements were recorded at dosing and daily through study termination. Observations for mortality were made at 0, 24, 48, 72 and 96 hours after treatment. The test was terminated after 96 ± 2 hours of exposure.

Dose Verification
Dose verification resulted in a dose verified concentration of 0.364 mg/L for the nominal concentration of 1.0 mg/L

Table 1 - Dose Verification Results

Nominal Concentration (mg/L)	Control	1.0
0 Hour New (mg/L)	0	0.509
24 Hour New (mg/L)	0	0.295
24 Hour Old (mg/L)	0	0.502
48 Hour New (mg/L)	0	0.458
48 Hour Old (mg/L)	0	0.358
72 Hour New (mg/L)	0	0.179
72 Hour Old (mg/L)	0	0.533
96 Hour Old (mg/L)	0	0.264
Geometric Mean (mg/L)	0	0.364

Results and discussions/Conclusion

Table 2 - Limit Test Mortality Results

Nominal Conc. (mg/L)	Replicate	Number of Surviving Organisms					Mortality
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	
Control	A	10	10	10	10	10	6.67%
	B	10	10	8[A]	8	8	
	C	10	10	10	10	10	
1.0	A	10	10	10	10	10	3.33%
	B	10	10	10	10	10	
	C	10	10	9[B]	9	9	

Note: All organisms had no observable abnormalities (NOA) unless otherwise indicated; Conc. - Concentration

[A] – Two organisms missing; Rest NOA.
[B] – One organism dead on bottom; Rest NOA.

At 48 and 96 hours, the No Observed Effect Concentration (NOEC) of RH-127450 was determined to be 1.0 mg/L (0.364 mg/L). The Median Lethal Concentration (LC_{50}) and the Lowest Observed Effect Concentration (LOEC) were > 1.0 mg/L (> 0.364 mg/L).
Parenthetical value~~s~~ is ~~are~~ dose verified concentration~~s.~~

Comments of zRMS: The study on the mysid was conducted following the guideline OCSPP 850.1035 (2016), under GLP certification for RH-141455.

Validity criteria were met as follows:

Acceptability Criteria	Study Results	Criterion Met (Yes/No)
Exposure vessels will be identical.	Exposure vessels were identical	yes
Treatments will be indiscriminately assigned to individual exposure vessel locations and individual test organisms will be indiscriminately assigned to exposure vessels.	Treatments were indiscriminately assigned to individual exposure vessel locations and individual test organisms were indiscriminately assigned to each exposure vessel	yes
A dilution water control, and solvent control, if necessary, will be included in the test	A control solution was made using only dilution water	yes
No more than 10% of organisms in the control, or solvent control, if applicable, can show signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death.	No mortality was observed in the control; no adverse effects were observed among control organisms	yes
A surfactant or dispersant	Neither a surfactant nor	yes

	was not used in preparation of a stock or exposure solution	dispersant was used during preparation of solutions for this study	
	The analytical measurements demonstrated that the test item concentrations throughout the test was within 80-120% of nominal and for this reason endpoints are expressed as nominal concentrations. The study is reliable and suitable for the risk assessment. All results refer to nominal concentrations.		

Reference: KCA 8.2.4.2

Report: RH-141455 Mysid Shrimp (*Mysidopsis bahia*) 96-Hour Acute Toxicity Test, Mikulas, J., 2023, Stillmeadow Inc, Report No.: 25771-22

Guideline(s): OCSPP 850.1035

Deviations: No

GLP: Yes

Acceptability: Yes ~~/ No /Supplementary~~

Materials and methods

This study was conducted to assess the toxicity of the test item, RH-141455, to the mysid shrimp, *Mysidopsis bahia,* in a 96-hour test with daily renewals.

Dose Verification
Dose verification resulted in a dose verified concentration of 6.44, 12.37, 25.07, 49.91 and 100.9 mg/L for the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L, respectively.

Table 2 - Dose Verification Results

Nominal Concentration (mg/L)	Control	6.25	12.5	25	50	100
0 Hour New (mg/L)	0	6.815	12.420	25.377	51.666	101.427
24 Hour New (mg/L)	0	6.339	12.546	24.234	48.477	96.469
24 Hour Old (mg/L)	0	6.442	11.863	23.997	48.913	97.317
48 Hour New (mg/L)	0	6.461	12.748	25.307	49.476	101.729
48 Hour Old (mg/L)	0	6.364	12.312	24.781	49.933	100.816
72 Hour New (mg/L)	0	6.279	12.102	25.224	50.129	101.201
72 Hour Old (mg/L)	0	6.475	12.559	25.870	49.846	102.456
96 Hour Old (mg/L)	0	6.377	12.393	25.820	50.885	106.137
Geometric Mean (mg/L)	0	6.44	12.37	25.07	49.91	100.90

Test concentrations were determined by a preliminary range-finding test. The test item concentrations of 6.25 mg/L, 12.5 mg/L, 25.0 mg/L, 50.0 mg/L, and 100 mg/L were administered to the test system, *Mysidopsis bahia,* in synthetic seawater with daily renewals. Three replicates of ten organisms were treated with each concentration of the test item. Each of the three control containers contained 10 organisms in synthetic seawater and no test item. Dissolved oxygen, temperature, salinity and pH measurements were recorded at dosing and daily through study termination. Observations for mortality were made at 0, 24, 48, 72 and 96 hours after treatment. The test was terminated after 96 ± 2 hours of exposure.

Results and discussions/Conclusion

A 0.00% mortality was observed in control mysid and mysid treated with 6.25 mg/L, 12.5 mg/L, 25 mg/L and 50 mg/L of the test item. A mortality of 6.67% was observed in mysid treated with 100 mg/L of the test item. Observable abnormalities, besides mortality, included organisms' appearing to be bigger in size beginning at 72 hours in all groups treated with the test item and the control. Results presented are based on nominal concentrations and are presented in the following table.

Table 3 - Definitive Test Mortality Results

Nominal Conc. (mg/L)	Replicate	Number of Surviving Organisms					Mortality
		0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	
Control	A	10	10	10	10[A]	10[A]	0.00%
	B	10	10	10	10[A]	10[A]	
	C	10	10	10	10[A]	10[A]	
6.25	A	10	10	10	10[A]	10[A]	0.00%
	B	10	10	10	10[A]	10[A]	
	C	10	10	10	10[A]	10[A]	
12.5	A	10	10	10	10[A]	10[A]	0.00%
	B	10	10	10	10[A]	10[A]	
	C	10	10	10	10[A]	10[A]	
25	A	10	10	10	10[A]	10[A]	0.00%
	B	10	10	10	10[A]	10[A]	
	C	10	10	10	10[A]	10[A]	
50	A	10	10	10	10[A]	10[A]	0.00%
	B	10	10	10	10[A]	10[A]	
	C	10	10	10	10[A]	10[A]	
100	A	10	9[B]	9	10[AC]	10[A]	6.67%
	B	10	8[B]	8	8[A]	8[A]	
	C	10	10[B]	10	10[A]	10[A]	

Note: All organisms had no observable abnormalities (NOA) unless otherwise indicated.
[A] - Organisms look bigger in size compared to Day 1; Water NOA.
[B] - One organism missing from replicate A and B; One organism eaten in replicate B; Rest NOA.
[C] - Missing organism in replicate A found.

At 48 and 96 hours, the No Observed Effect Concentration (NOEC) of RH-141455 was determined to be 100 mg/L (100.9 mg/L), the Median Lethal Concentration (LC_{50}) and the Lowest Observed Effect Concentration (LOEC) was > 100 mg/L (> 100.9 mg/L).
Parenthetical values are dose verified concentrations.

Comments of zRMS:	The study was conducted according to OECD guideline 201 and principles of GLP. No deviations were noted. Validity criteria were met: - the biomass in the control increased by a factor of 131 within the 72-hour test period (criterion: at least a 16-fold growth) - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 2.4 % (criterion: it must not exceed 7%). - the mean coefficient of variation for the section-by-section growth rate in the control culture was 8.9 % (criterion: it must not exceed 35%). In the study report in point 7.Conclusion, there is a mistake of active substance name: „*Pseudokirchneriella subcapitata* was exposed to GLOB2007bF, a suspension concentrate formulation with the active ingredients flufenacet and diflufenican, over a 72-hour period''. It should be corrected.

Reference:	KCP 10.2.1
Report	GLOB2007bF: *Pseudokirchneriella subcapitata* Growth Inhibition Test, Wright, E., Fera Science Ltd, Report No.: FR/002722
Guideline(s):	OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF
Test Species:	*Pseudokirchneriella subcapitata*
Source and Strain:	CCAP (Culture Collection of Algae and Protozoa), Oban, Scotland. Strain CCAP 278/4
Test Media:	OECD medium (OECD Test Guideline 201, 2011, Annex 3)
Test System:	The test system is defined as a test vessel (glass conical flask) containing approximately 100 mL of test medium and the test organisms (algal cells). The flasks were closed with a gas permeable plug during incubation.
Test Item Concentrations:	Nominal concentrations of GLOB2007bF were 0.15625, 0.3125, 0.625, 1.25, 2.5 and 5 mg/L, with a negative control of untreated test media.
Reference Item:	The performance of the test system was assessed using 3,5-dicholorophenol (3,5-DCP) as the reference item treatment.

Nominal test concentration of 3,5-DCP was 2 mg/L, prepared with acetone as a solvent carrier to increase solubility.

Replicates: There were six replicate control vessels and three for each test item treatment. The positive control had four replicate vessels.

Toxic Endpoints: Two endpoints were calculated, the growth rate and yield; these are expressed as per cent inhibition relative to the control.

For dose confirmation, all samples were analysed by LC-MS-MS. Triplicate samples were analysed from fresh media at the start of the test and from pooled media from replicates at the end of the test; a summary of the results for zoxamide are presented in Table 3 and for propamocarb in Table 4 (Report section 6.2).

Results and discussions

Mean measured concentrations at 0 and 72 hours indicated that zoxamide was not within 20% of the expected nominal concentration; the mean recoveries ranged between 72 and 167% of expected. The results also varied between 0 and 72 hours, although some dose rates increased in concentration while others decreased. The RSDs were also high (above 20%) suggesting that Zoxamide was not homogenous in the test media. Therefore, results are reported as geometric mean measured concentrations of zoxamide.

Mean measured concentrations at 0 and 72 hours indicated that propamocarb hydrochloride was not within 20% of the expected nominal concentration, as the mean recoveries ranged between 113 and 127% of expected. However, the measured concentrations were stable between test start and termination (as 72 hour mean results within +/- 4% of the 0-hour results) Therefore, analysis is reported as the 0-h mean measured concentrations of propamocarb hydrochloride.

As the test item, GLOB2007bF, is a mixture of two active ingredients, zoxamide and propamocarb HCl, and co-formulants which was tested as a whole substance, nominal concentrations of the test item were used for data analysis and reporting.

The results of the definitive bioassay after 72-h of exposure indicated little to no inhibition of growth at 0.15625 and 0.3125 mg formulation/L, and slight inhibition at 0.625 mg/L nominal concentration of GLOB2007bF. Clear inhibition of cell number increase were observed in the 1.250, 2.500 and 5.000 mg/L test groups.

Table 3: Summary of mean measured concentrations of zoxamide in the test solutions

Nominal Concentration of Zoxamide (µg/L)	Mean Measured Concentrations (µg/L)		Geometric Mean Measured Concentration (µg/L)
	0-h	72-h	
Negative Control	n.d.	n.d.	n/a
9.1	14.53	13.32	13.91
18.3	30.49	25.36	27.81
36.5	51.72	52.23	51.97
73.1	87.71	98.40	92.90
146.1	157.0	182.4	169.2
292.2	267.0	210.4	237.0

n.d. = not detected; n/a = not applicable; LOQ = limit of quantification

LOQ = 2.7 µg/L; Fortified sample recovery was 118% for sample analysis.

Table 4: Summary of mean measured concentrations of propamocarb hydrochloride in the test solutions

Nominal Concentration of Propamocarb HCl (µg/L)	Mean Measured Concentrations (µg/L)	
	0-h	72-h
Negative Control	n.d.	n.d.
63.4	75.32	74.88
126.8	150.0	155.1
253.5	310.8	321.8
507.1	619.8	637.3
1014	1229	1255
2028	2397	2286

n.d. = not detected; n/a = not applicable; LOQ = limit of quantification

LOQ = 19 µg/L; Fortified sample recovery was 112% for sample analysis.

Conclusion

Table 6. Mean average specific growth rate and yield for the test groups as geometric mean measured concentrations, and the corresponding percent inhibition, relative to the control

Nominal Concentration of GLOB2007bF (mg/L)	Average Specific Growth Rate (0 – 72h)		Yield (72h)	
	Growth Rate (1/d)	Inhibition Relative to Control (%)	Yield (cells x10^4/mL)	Inhibition Relative to Control (%)
Negative Control	1.623	n/a	130.0	n/a
0.15625	1.606	1.0	126.2	2.9
0.3125	1.560	3.9	107.2	17.5
0.625	1.332	18.0	53.6	58.8
1.250	0.472	70.9	3.2	97.6
2.500	0.369	77.3	2.0	98.4
5.000	0.397	75.5	2.3	98.2
Positive Control	0.903	44.3	14.1	89.2

Pseudokirchneriella subcapitata was exposed to GLOB2007bF, a suspension concentrate formulation with the active ingredients zoxamide and propamocarb HCl, over a 72-hour period.

- Based on nominal concentrations of GLOB2007bF, the 72-h EC_{50} was 1137 µg/L for growth rate and 531 µg/L for yield; the NOEC for both growth rate and yield was 156.25 µg/L.

- Based on geometric mean measured concentrations of zoxamide, the 72-h EC_{50} was 86.2 µg/L for growth rate and 44.8 µg/L for yield; the NOEC for both growth rate and yield was 13.912 µg/L.

- Based on the 0-hr mean measured concentrations of propamocarb hydrochloride, the 72-h EC_{50} was 562.9 µg/L for growth rate and 260.5 µg/L for yield; the NOEC for both growth rate and yield was 75.32 µg/L.

Comments of zRMS:	The study was conducted according to OECD guideline 201 and principles of GLP. No deviations were noted. Validity criteria were met: - the biomass in the control increased by a factor of 234 within the 72-hour test period (criterion: at least a 16-fold growth) - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 1.1 % (criterion: it must not exceed 7%). - the mean coefficient of variation for the section-by-section growth rate in the control culture was 7.3 % (criterion: it must not exceed 35%). The analytical measurements demonstrated that the test item concentrations throughout the test was outside the range 80-120% of nominal and for this reason endpoints are expressed as geometric mean measured concentrations of zoxamide.

Reference:	KCA 8.2.6.1
Report	Zoxamide Technical: *Pseudokirchneriella subcapitata* Growth Inhibition Test, Jarratt, N., Fera Science Ltd, Report No.: FR/002786
Guideline(s):	OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	Zoxamide Technical
Test Species:	*Pseudokirchneriella subcapitata*
Source and Strain:	CCAP (Culture Collection of Algae and Protozoa), Oban, Scotland. Strain CCAP 278/4
Test Media:	OECD medium (OECD Test Guideline 201, 2011, Annex 3)
Test System:	The test system is algal medium (to which test item is added); *Pseudokirchneriella subcapitata* in an exponential phase of growth was added to the algal medium.

Test Item Concentrations: Nominal concentrations of Zoxamide Technical were 6.25, 12.5, 25, 50 and 100 µg/L, with a negative control of untreated test media and a solvent control of acetone and test media (0 mg/L).

Reference Item: The performance of the test system was assessed using 3,5-dicholorophenol (3,5-DCP) as the reference item treatment.

Nominal test concentration of 3,5-DCP was 2 mg/L, prepared with acetone as a solvent carrier to increase solubility.

Replicates: There were six replicate vessels for each control and three for each test item treatment. The positive control had four replicate vessels.

Toxic Endpoints: Two endpoints were calculated, the growth rate and yield following 72 hours of exposure; these are expressed as per cent inhibition relative to the control.

All samples were analysed by LC-MS. Triplicate samples were analysed from fresh media at the start of the test and from pooled media from replicates at the end of the test for the test item, zoxamide.

Results and discussions

Mean measured concentrations at 0 and 72 hours indicated that zoxamide was not stable over the exposure period (e.g. variation greater than 20%); the range was from 57 to 126%. Therefore, analysis is reported as geometric mean measured concentrations (i.e. geo mean concentration) of zoxamide.

Table 6. Average specific growth rate and yield for the test groups as geometric mean measured concentrations relative to the solvent control

Geometric Mean Measured Concentration of Zoxamide (µg/L)	Average Specific Growth Rate (0 – 72-h)		Yield	
	Growth Rate (1/d)	Inhibition Relative to Solvent Control (%)	Yield (cells/mL, x10^{-4})	Inhibition Relative to Solvent Control (%)
Negative Control	1.82	n/a	233.29	n/a
Solvent Control	1.80	n/a	222.42	n/a
6.01	1.80	-0.04	222.40	0.01
11.62	1.61	10.51 [a]	130.93	41.13 [a]
17.43	1.74	3.24 [a]	185.99	16.38 [a]
35.97	0.82	54.72 [a]	10.56	95.25 [a]
79.52	0.39	78.34 [a]	2.23	99.00 [a]
Positive Control	0.60	66.44 [a]	5.17	97.67 [a]

[a] Statistically significant effect ($p<0.05$)

The results of the definitive bioassay after 72-h of exposure indicated no effect on cell numbers at 6.01, 11.62 or 17.43 µg/L geometric mean measured concentration of zoxamide. Clear inhibition for algal cell number increase were observed in the 35.97 and 79.52 µg/L groups.

	EC_x with 95% Lower and Upper Confidence Limits (µg/L)			**NOEC (µg/L)**	**LOEC (µg/L)**
	EC_{50}	**EC_{20}**	**EC_{10}**		
Growth Rate	38.311 (33 – 45)	20.714 (16 – 25)	15.020 (11 – 19)	6.014	11.62
Yield	20.217 (15 – 31)	12.003 (5.1 – 16)	9.140 (2.5 – 13)	6.014	11.62

Conclusion

Pseudokirchneriella subcapitata was exposed to zoxamide technical, a technical grade of the chemical zoxamide, over a 72-hour period. Based on geometric mean measured concentrations of zoxamide, the 72-h EC_{50} was 38.311 µg/L for growth rate and 20.217 µg/L for yield; the NOEC for both growth rate and yield was 6.014 µg/L.

Comments of zRMS:	The study was conducted according to OECD guideline 201 and principles of GLP. No deviations were noted. Validity criteria were met: - the biomass in the control increased by a factor of 104 within the 72-hour test period (criterion: at least a 16-fold growth) - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 4.51 % (criterion: it must not exceed 7%). - the mean coefficient of variation for the section-by-section growth rate in the control culture was 11.18 % (criterion: it must not exceed 35%). The analytical measurements demonstrated that the test item concentrations throughout the test was outside the range 80-120% of nominal and for this reason endpoints are expressed as geometric mean measured concentrations of RH-163353.

Reference:	KCA 8.2.6.1
Report	RH-163353 - 72-Hour Toxicity Test with the Freshwater Green Alga, *Raphidocelis subcapitata*, Softcheck, K., Smithers Ers Ltd, Report No.: 14365.6101
Guideline(s):	OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/~~No/Supplementary~~

Materials and methods

The purpose of this study was to determine the effect of RH-163353 on the growth of the freshwater green alga, *Raphidocelis subcapitata*, under static conditions. The results of this study are presented based on mean measured concentrations of RH-163353 and are reported as the 72-hour EC_{10}, EC_{20}, and EC_{50} values for average specific growth rate and yield, denoted as E_rC_{10}, E_rC_{20}, E_rC_{50}, and E_yC_{10}, E_yC_{20}, E_yC_{50}, respectively. If possible, the 72-hour No-Observed Effect Concentration (NOEC) and Lowest-Observed Effect Concentration (LOEC) values were also determined.

Test Substance	
Name:	RH-163353
Synonym:	3-(3,5-dichloro-4-methylbenzamido)-3-methyl-2-oxopentanoic acid (RH-163353)
Batch No.:	GD-003454-03
CAS No.:	401520-47-6
Purity:	100%
Storage Conditions:	Stored at room temperature in a dark, ventilated cabinet in the original container
Expiry Date:	9 March 2024
Date Received:	16 March 2023
Received From:	Globachem Discovery Ltd., Macclesfield, United Kingdom

Test Concentrations	
Nominal Concentrations:	3.1, 6.3, 13, 25, 50, and 100 mg/L
Mean Measured Concentrations	3.1, 5.9, 13, 26, 44, and 100 mg/L
Co-Solvent:	None
Co-Solvent Load:	Not Applicable
Interval(s) of Analytical Verification:	0 and 72 hours

Test Conditions	
Duration:	72-hours
Temperature:	24 to 25°C
Photoperiod:	None (continuous)
Photosynthetically-Active Radiation (PAR):	60 to 76 µE/m^2/S
Light Intensity:	4500 to 5200 lux
Agitation:	Continuous, 100 ± 10 rpm on an orbital shaker, rate monitored & recorded daily

Results and discussions/Conclusion

Results					
Biological Parameter	**Based on Mean Measured Concentrations (mg/L)**				
	NOEC[a]	**LOEC[a]**	**EC_{10} (95% CI[b])**	**EC_{20} (95% CI)**	**EC_{50} (95% CI)**
72-Hour Average Specific Growth Rate	100	>100	>100 (NA[c])	>100 (NA)	>100 (NA)
72-Hour Yield	100	>100	>100 (NA)	>100 (NA)	>100 (NA)

[a] Determined by Dunnett's Multiple Comparison Test.
[b] CI = Confidence Interval
[c] NA = Not Applicable; EC value was empirically estimated; therefore, a corresponding 95% confidence interval could not be determined.

Rationale for no further testing: The highest concentration tested meets the maximum testing requirement in the OECD 201 guideline.

Comments of zRMS:	The study was conducted according to OECD guideline 201 and principles of GLP. No deviations were noted in the study. All validity criteria were met. The analytical measurements demonstrated that the test item concentrations throughout the test was outside the range 80-120% of nominal and for this reason endpoints are expressed as geometric mean measured concentrations of RH-139432.

Reference:	KCA 8.2.6.1
Report	72-Hour Algal Inhibition Test with *Pseudokirchneriella subcapitata* (OECD 201) - Test Substance: RH-139432, Mikulas, J., Stillmeadow Inc, Report No.: 25770-22
Guideline(s):	OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/~~No/Supplementary~~

Materials and methods

This study was conducted to determine the toxic effects of the test item, RH-139432, on the growth of the freshwater algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 72-hour static test.

Test concentrations were determined by a preliminary range-finding test. The test item concentrations chosen for the definitive test (1.25 mg/L, 2.5 mg/L, 5.0 mg/L, 10.0 mg/L and 20.0 mg/L) were administered to the test system, *Pseudokirchneriella subcapitata*, in OECD media.

Analytical results

The concentration of the active ingredient, 3,5-Dichloro-4-methylbenzamide, in the test item was determined in each new test solution at 0 hours and in each old test solution at 72 hours

Table 8 - Dose Verification Detailed Results

Nominal Conc. (mg/L)	Injection Response				STD DEV	RSD	Conc. (ppm)	Calculation Based On
	1	2	3	Average				
0 Hour								
Control	ND	ND	ND	**NA**	NA	NA	0.000	Low curve
1.25	0.219	0.214	0.208	**0.214**	0.006	2.578	0.683	Low curve
2.5	0.385	0.369	0.388	**0.381**	0.010	2.683	1.226	Low curve
5.0	1.081	1.137	1.086	**1.101**	0.031	2.814	3.568	Low curve
10.0	1.760	1.707	1.757	**1.741**	0.030	1.710	5.245	High curve
20.0	3.429	3.367	3.401	**3.399**	0.031	0.913	10.572	High curve
72 Hour								
Control	ND	ND	ND	**NA**	NA	NA	0.000	Low curve
1.25	0.213	0.210	0.168	**0.197**	0.025	12.771	0.629	Low curve
2.5	0.399	0.393	0.389	**0.394**	0.005	1.279	1.268	Low curve
5.0	1.528	1.513	1.522	**1.521**	0.008	0.496	4.536	High curve
10.0	2.231	2.266	2.221	**2.239**	0.024	1.055	6.845	High curve
20.0	3.059	3.047	3.029	**3.045**	0.015	0.496	9.434	High curve

Conc. - Concentration; STD DEV - Standard Deviation; RSD - Relative Standard Deviation; NA - Not Applicable
ND - Not Detected

For each test concentration, four test flasks containing freshwater algae (10,000 cells/mL) were treated with the appropriate concentration of the test item. A control group consisted of six test flasks containing sterile medium and the test culture only. A positive control group consisted of three flasks treated with 20 mg/L zinc chloride. The cell density in each test and control container was measured at 24, 48 and 72 hours using a hemocytometer. Chamber temperature was measured daily with a calibrated thermometer. Daily maximum and minimum temperatures were also recorded. Light intensity was measured daily in at least 5 locations in the test areas at the height of the test solution in the test chambers. The pH of each test solution was determined at test initiation. The pH of each test and control container was determined at test termination. The test was terminated after 72 ± 1 hours of exposure.

Results and discussions/Conclusion

At 72 hours, the test concentration of RH-139432 that resulted in cell density Median Effective Concentration (EC_{50}) was determined to be 11.267 mg/L (**6.4980** mg/L) with 95% confidence limits of 9.782 - 12.567 mg/L (5.8937 - 7.0100 mg/L).
At 72 hours, the test concentration of that resulted in cell density (EC_{10}) was determined to be 5.117 mg/L (4.0690 mg/L) with 95% confidence limits of 0.000 - 6.031 mg/L (0.0000 - 4.3524 mg/L).
At 72 hours, the cell density No Observed Effect Concentration (NOEC) was determined to be 5 mg/L (4.023 mg/L).

At 72 hours, the test concentration of RH-139432 that resulted in the growth rate Median Effective Concentration (EC_{50}) was determined to be > 20 mg/L (**> 9.987** mg/L).
At 72 hours, the test concentration of that resulted in a growth rate (EC_{10}) was determined to be 10.172 mg/L (6.0606 mg/L) with 95% confidence limits of 9.222 - 10.744 mg/L (5.7225 - 6.3114 mg/L).
At 72 hours, the cell density No Observed Effect Concentration (NOEC) was determined to be 5 mg/L (4.023 mg/L). Parenthetical values are dose verified concentrations.

Comments of zRMS:	The study was conducted according to OECD guideline 201 and principles of GLP. No deviations were noted. Validity criteria were met: - the biomass in the control increased by a factor at least a 16-fold growth - the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture did not exceed 7%. - the mean coefficient of variation for the section-by-section growth rate in the control culture was 15.453 % (criterion: it must not exceed 35%). The analytical measurements demonstrated that the test item concentrations throughout the test was outside the range 80-120% of nominal and for this reason endpoints are expressed as geometric mean measured concentrations of RH-127450.

Reference:	KCA 8.2.6.1
Report	72-Hour Algal Inhibition Test with *Pseudokirchneriella subcapitata* (OECD 201) - Test Substance: RH-127450, Mikulas, J., Stillmeadow Inc, Report No.: 25834-22
Guideline(s):	OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes / ~~No / Supplementary~~

Materials and methods

This study was conducted to determine the toxic effects of the test item, RH-127450, on the growth of the freshwater algae *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) in a 72-hour static test.

Analytical measurements

Dose verification was performed during the study on new test solutions sampled at 0 hour and on old test solutions at 72 hours.

Table 2 - Dose Verification Results

Nominal Concentration (mg/L)	Calculated 3,5-dichloro-4-methyl-N-(3-methyl-2-oxopentan-3-yl)-benzamide		
	0 Hour	72 Hour	Geometric Mean
Control	0	0	**0**
1.5	0.648	2.689	**1.320**

The concentration needed for the limit test was determined by a preliminary range-finding test. The chosen concentration (1.5 mg/L) was administered to the test system, *Pseudokirchneriella subcapitata*, in OECD media. For the test concentration, six test flasks containing freshwater algae (10,000 cells/mL) were treated with 1.5 mg/L nominal concentration of the test item. A control group consisted of six test flasks containing sterile medium and the test culture only. A positive control group consisted of three flasks treated with 10 mg/L zinc chloride. The cell density in each test and control container was measured at 24, 48 and 72 hours using a hemocytometer. Chamber temperature was measured daily with a calibrated thermometer. Daily maximum and minimum temperatures were also recorded. Light intensity was measured daily in at least 5 locations in the test areas at the height of the test solution in the test chambers. The pH of each test solution was determined at test initiation. The pH of each test and control container was determined at test termination. The test was terminated after 72 ± 1 hours of exposure.

Results and discussions/Conclusion

Initial cell density was 10,000 cells/mL. There were no observable abnormalities in any of the test groups unless otherwise noted. Results presented are based on nominal concentrations. Daily cell counts, terminal pH and average growth rate are presented in the table below.

Table 3 - Limit Test Average Growth Rate Results

Test Conc. (mg/L)	Rep.	Cell Density x 10^4 cells/mL 24 Hours	48 Hours	72 Hours	Avg. Growth Rate	Growth Rate Stats	Avg. Growth Curve Area	Growth Curve Area Stats	Init. pH	Term. pH
Cont.	A	30	103	407	0.083	Mean: 0.083 SD: 0.001 CV: 1.065	8016	Mean: 7710.00 SD: 249.85 CV: 3.2	8.0	8.9
	B	36	91	387	0.083		7632			9.0
	C	20	84	409	0.084		7344			9.0
	D	15	102	421	0.084		7800			8.9
	E	25	121	373	0.082		7920			8.9
	F	29	110	356	0.082		7548			8.9
			Mean	392.2						
1.5*	A	25	97	386	0.083	Mean: 0.081 SD: 0.003 CV: 3.211	7500	Mean: 6771.00 SD: 993.678 CV: 14.675	7.8	8.9
	B	24	73	260	0.077		5388			8.0
	C	23	86	346	0.081		6708			8.9
	E	32	89	387	0.083		7488			8.5
			Mean	344.8						
		Percent Inhibition Yield: 12.09%				Percent Inhibition Growth Rate: 2.34%				
C2 10 mg/L $ZnCl_2$	A	3	3	2	0.010	Mean: 0.012 SD: 0.003 CV: 28.262	NA	NA	7.4	7.6
	B	5	3	3	0.015					7.7
	C	2	2	2	0.010					7.7
			Mean	2.33						

Note: All organisms had no observable abnormalities unless otherwise indicated.
Conc - Concentration; Rep - Replicate; Avg - Average; Init - Initial; Term - Terminal; SD - Standard Deviation; CV - Coefficient of Variation; Cont. - Control; C2 - Positive Control; NA - Not Applicable
*Replicates D and F considered outliers, removed from statistical analysis.

At 72 hours, the cell density and growth rate Median Effective Concentration (EC_{50}) and Lowest Observed Effect Concentration (LOEC) were determined to be > 1.5 mg/L (>1.32 mg/L) of the test item, RH-127450. At 72 hours, the growth rate EC_{10} was > 1.5 mg/L (> 1.32 mg/L) and the cell density EC_{10} was 1.2406 mg/L (1.0917 mg/L). At 72 hours, the cell density and growth rate No Observed Effect Concentration (NOEC) was determined to be 1.5 mg/L (1.32 mg/L). Parenthetical values are dose verified concentrations.

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No data submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No data submitted.

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 studies were conducted according to OECD guideline 213 and 214 and according to the principles of GLP. Following deviations to the guideline were noted: **Number of replications** Only 3 replicates were used for the control group instead of normally number of 5. However a minimum of three replicate test groups in the control group are still meeting the test guidelines criteria. **Application in the contact Test:** A 5 µL droplet of GLOB2007bF was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. Since all validity criteria were met the mentioned deviation had not effect on the results of both studies. Since the test item contains more than one active substance the dose level was based on product without taking into account the content of active ingredient. In the definitive test all the validity criteria were met as follows: − the mortality for the control was 3.3% in contact study and 0.0% in oral study at the end of the experiment (criterion: it must not exceed 10%). - LD_{50} values obtained with the reference item (dimethoate) were within the required ranges. The contact and oral LD_{50} (24 h) values for the reference item (dimethoate) were calculated to be 0.16 and 0.25 µg a.i./bee, respectively. The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: 48 h contact LD_{50} > 200.0 µg formulation/honeybee 48 h oral LD_{50} > 221.0 µg formulation/honeybee

Reference:	10.3.1.1
Report	GLOB2007bF: Effects (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory, Knautz, T., 2022, Ibacon GmbH, Report No.: 169561035
Guideline(s):	OECD 213, OECD 214
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes ~~No/Supplementary~~

Materials and methods

Test Item:	GLOB2007bF, Batch No.: LCM22012601, content: Zoxamide: 64.23 ± 0.07 g/L; Propamocarb-HCl: 445.8 ± 1.3 g/L (analytical), according to GLP certificate of analysis.
Test Species:	Honey bee (*Apis mellifera* L.); female worker bees; obtained from a healthy and queen-right colony, bred by ibacon, collected in the morning of use.

Test Design:	Acute contact and oral limit test; duration 48 hours (contact and oral test); 5 replicates for the test item, 3 replicates for the control and referenc item in the contact and oral test, each consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours; reference item: dimethoate 417 g/L (analysed).
Test Dose Levels:	Contact test: 200 µg prod./bee* Oral test (nominal): 200 µg prod./bee* Oral test (measured): 221.0 µg prod./bee* *since the test item contained more than one active ingredient the dose level was based on the product without taking into account the content of active ingredient.
Test Conditions:	Temperature: 24 - 26 °C; relative humidity: 54 - 63 %; photoperiod: 24 h darkness.

Results and discussions

Contact Test:

The tested dose of 200 µg product/bee led to no mortality at test termination (48 hours). There was 3.3 % mortality in the water control group (water + 0.1 % Triton X-100). One single affected bee was observed in the test item treatment group 24 hours and 48 hours after application.

Oral Test:

The target dose level of the test item (200 µg product/bee) was achieved. The actual oral consumed dose of 221.0 µg product/bee led to 2.0 % mortality at test termination (48 hours). No mortality occurred in the water control (50 % w/v sucrose solution = 500 g sucrose/L tap water). One single affected bee was observed in the test item treatment group 24 hours after application of the test item.

Table 3. Toxicity of GLOB2007bF to honey bees; laboratory test

Test Item	GLOB2007bF	
Test Species	*Apis mellifera* L.	
Exposure	contact (tap water and 0.1 % Triton X-100)	oral (50 % w/v sucrose solution)
Application rate [µg prod./bee]	200	Target: 200 Consumed: 221.0
LD_{50} [µg prod./bee]	24 hours: > 200 48 hours: > 200	24 hours: > 221.0 48 hours: > 221.0
NOED [µg prod./bee]	24 hours: 200 48 hours: 200	24 hours: 221.0 48 hours: 221.0

NOED: was determined using Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, α -= 0.05).

The contact and oral LD_{50} (24 h) values for the reference item (dimethoate) were calculated to be 0.16 and 0.25 µg a.i./bee, respectively.

Conclusions

The acute toxicity of GLOB2007bF on adult honey bees (*Apis mellifera* L.) was investigated in an acute contact and an acute oral, limit study under laboratory conditions.

The contact LD_{50} values (24 and 48 h) of GLOB2007bF were estimated to be both > 200 µg product/bee.

The oral LD_{50} values (24 and 48 h) of GLOB2007bF were estimated to be both > 221.0 µg product/bee.

The contact NOED values (24 and 48 h) were both 200 µg product/bee.

The oral NOED values (24 h and 48 h) were both 221.0 µg product/bee.

Comments of zRMS:	The study was conducted according OECD guideline 246 (acute contact toxicity) and OECD guideline 247 (oral) and) with the principles of GLP. In the definitive tests all the validity criteria were met. Following deviation was noted: In the contact toxicity study a 5 µL droplet was chosen in deviation to the guideline recommendation of 2 µL, since a higher volume ensures a more reliable dispersion of the test item. Since all validity criteria were met the mentioned deviations had not effect on the results of both studies The study is reliable and suitable for the risk assessment. Overall, the study is considered acceptable with following endpoints: 48 h contact LD_{50} > 200 µg formulation/bumblebee 48 h oral LD_{50} > 203.5 µg formulation/bumblebee

Reference:	10.3.1.1
Report	GLOB2007bF: Acute Contact and Oral Toxicity to Bumblebees (*Bombus terrestris* L.) in the Laboratory, Chwiesko, D., 2023, Ibacon GmbH, Report No.: 169561105
Guideline(s):	OECD 246, OECD 247 ~~OECD 213, OECD 214~~
Deviations:	Yes ~~No~~
GLP:	Yes
Acceptability:	Yes ~~No/Supplementary~~

Materials and methods

Test Item:	GLOB2007bF, Batch No.: LCM22012601, content: Zoxamide: 64.23 g/L (analysed) and Propamocarb-HCI: 445.8 g/L, according to certificate of analysis. The dose levels of the test item were based on the product without taking into consideration active substance content
Test Species:	Bumblebee (*Bombus terrestris* L.); female worker bumblebees; obtained from a commercial bumblebee breeding company (Koppert Deutschland GmbH, Zeppelinstr. 32, D-47638 Straelen, Germany).
Test Design:	<u>Acute Contact Limit Test:</u> Duration: 48 h; replicates: 50 for the test item and 50 for the water control treatment group, 30 for the reference item treatment group, each consisting of 1 bumblebee per cage per treatment; assessment of mortality and behavioural abnormalities: after 4 (± 0.5); 24 (± 2) and 48 (± 2) hours; reference item: dimethoate 417 g/L (analytical). Analytical verification of the concentration of the active ingredients Zoxamide and Propamocarb-HCI in the contact application solution of the single concentration. <u>Acute Oral Limit Test:</u> Duration: 48 h; replicates: 55 for the test item and 55 for the water control treatment group, 35 for the reference item treatment group, each consisting of 1 bumblebee per cage per treatment (individual bumblebees which did not take up at least 80 % of the mean food uptake per treatment group were excluded from the evaluation; see section 6.9 Result Evaluation); assessment of mortality and behavioural abnormalities: after 4 (± 0.5); 24 (± 2) and 48 (± 2) hours; reference item: dimethoate 417 g/L (analytical). Analytical verification of the concentration of the active ingredients Zoxamide and Propamocarb-HCI in the oral feeding solution of the single concentration.
Test Item Dose Levels:	<u>Contact & Oral Limit Test (nominal):</u> 200 µg product/bumblebee <u>Oral Limit Test achieved (target):</u> 200 µg product/bumblebee <u>Oral Limit Test achieved (mean consumption):</u> 203.5 µg product/bumblebee

Analytical Results of the Contact Test:	Contact test (200 µg product/ bumblebee):	Propamocarb: Zoxamide:	113 % 112%
Analytical Results of the Oral Test:	Oral test (200 µg product/ bumblebee):	Propamocarb - A-sample: Propamocarb - B-sample: Zoxamide – A-sample: Zoxamide - B-sample:	92 % 85 % 88 % 86 %

Test Conditions:

Contact Test:	Acclimatisation:	Temperature: 23 - 25 °C Relative Humidity: 55 – 60 %
	Exposure:	Temperature: 23 – 25 °C Relative Humidity: 55 – 62 %
Oral Test:	Acclimatisation:	Temperature: 23 - 25 °C Relative Humidity: 55 – 60 %
	Exposure:	Temperature: 23 – 25 °C Relative Humidity: 55 – 62 %
Photoperiod:	Photoperiod:	24 h darkness (except handling procedures, including treatment and observations).
Study Validity:	This study met the OECD 246 (2017) and OECD 247 (2017) validity criteria as the control mortality in both the oral and contact tests was ≤ 10 % and the mortality due to the reference item (dimethoate) was ≥ 50 % at test end.	

Results and discussions

Biological Results:

Contact Test:

In the contact test a droplet of 5 µL* containing the targeted dose level of 200 µg product/bumblebee was applied on the dorsal thorax of each exposed bumblebee. At the end of the contact toxicity test (48 hours after application) no mortality occurred in the test item treatment group. 2.0 % mortality occurred in the water control group (tap water containing 0.1 % v/v Triton X-100).

No test item related behavioural effects were observed at any time in the contact test.

The contact target dose level of the reference item of 10 µg dimethoate/bumblebee was applied on the dorsal thorax of each exposed bumblebee. The mortality in the reference item treatment group was 96.7 % (48 hours after application).

The contact test is considered valid as the water control mortality (tap water containing 0.1 % v/v Triton X-100) was ≤ 10 % and the reference item mortality (dimethoate) was ≥ 50 %.

*A 5 µL droplet was chosen in deviation to the guideline recommendation of 2 µL, since a higher volume ensures a more reliable dispersion of the test item; ibacon experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [according to internal ibacon experiments 2016 and 2022].

Oral Test:

In the oral test the targeted dose level of 200 µg product/bumblebee would have been achieved if an exact

amount of 40 mg treated feeding solution had been consumed by each exposed bumblebee. This was not the case and the actual food uptake per bumblebee in the different treatment groups varied between 3 and 50 mg. Therefore, bumblebees which did not consume at least 80 % of the mean food uptake were excluded from the derivation of the end points, as well as from the calculation of the actual mean oral doses in the test and reference item treatment groups. This was done to avoid potentially overestimating the final endpoints.

For the test item treatment group, 50 bumblebees were considered for the evaluation (≥ 80 % of the target food uptake). The actual mean consumed oral dose of the test item was 203.5 µg product/bumblebee. There was no mortality in the test item treatment group at test end (48 hours after application). No test item related behavioural effects were observed at any time in the oral test. For the water control group, 52 bumblebees were considered for the evaluation. 1.9 % mortality occurred in the water control group (50 % w/v aqueous sucrose solution).

Similarly, the reference item targeted dose level of 4.0 µg dimethoate/bumblebee would have been achieved if exactly 40 mg treated feeding solution had been consumed by each bumblebee. Considering bumblebees consuming a food uptake of at least 80 % of the mean food uptake, the mean consumption corresponded to an actual mean oral dose of 3.8 µg dimethoate/bumblebee. For the reference item treatment group, 27 bumblebees were considered for the evaluation. Under this condition, the mortality in the reference item treatment group was 100.0 % 48 hours after application.

The oral test is considered valid as the water control (50 % w/v sucrose solution) mortality was ≤ 10 % and the reference item (dimethoate) mortality was ≥ 50 %.

Table 4. Toxicity to Bumblebees; Laboratory Tests

Test Item	GLOB2007bF			
Test Species	*Bombus terrestris* L.			
Exposure	Contact (tap water containing 0.1 % v/v Triton X-100)		Oral [1] (50 % w/v sucrose solution)	
Target dose rate [µg product/bumblebee]	200		200	
Actual achieved dose rate [µg product/bumblebee]	n.a.		203.5	
Test Duration:	24 h	48 h	24 h	48 h
LD_{50} [µg product/bumblebee] [2,3]	> 200	> 200	> 203.5	> 203.5
NOED [µg product/bumblebee] [2, 4]	≥ 200	≥ 200	≥ 203.5	≥ 203.5

[1] For the 203.5 µg product/bumblebee test item treatment group, 50 bumblebees were considered for the evaluation.

[2] Results obtained from test item treated groups were compared to those obtained from the water control group.

[3] As the test item treatment groups in the contact and oral tests did not show mortality above 50.0 %, no statistical evaluation of the LD_{50} values was carried out. The contact and oral LD_{50} values were considered to be higher than the tested dose rates.

[4] The contact and oral NOED values were determined using Fisher's Exact Binomial Test (one-sided greater, α = 0.05).

n.a.= not applicable

Analytical Results:

The analytical recovery rates of the active substances Zoxamide and Propamocarb-HCI in the testing solutions were as follows:

Concentration/bumblebee	Nominal concentration in the solution	Recovery of the nominal value in the solution
Contact Test: Application solution (200 µg product/bumblebee*)	40 g product/L application solution	Propamocarb: 113% Zoxamide: 112%
Oral Test: Feeding solution (200 µg product/bumblebee*)	5 g product/kg feeding solution	Propamocarb: A-Sample 92% B- Sample 85% Zoxamide: A-Sample 88% B-Sample 86%

* The dose levels of the test item were based on the product without taking into consideration active substance content.

Conclusion

The toxicity of GLOB2007bF to bumblebee was tested in an acute contact and oral toxicity test.

As there was no mortality in the test item treatment group in the contact test, the contact LD_{50} (Lethal Dose causing 50 % mortality) (48 h) value was estimated to be > 200 µg product/bumblebee.

The contact NOED (No Observed Effect Dose) (48 h) value was calculated to be ≥ 200 µg product/bumblebee.

As there was no mortality in the test item treatment group in the oral test, the oral LD_{50} (48 h) value was estimated to be > 203.5 µg product/bumblebee. The oral NOED (48 h) value was calculated to be ≥ 203.5 µg product/bumblebee.

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Please refer to A 2.3.1.1.

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to A 2.3.1.1.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted to OECD guideline 245 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met as follows: After 10 days of continuous exposure, mortality in the solvent control was 0.0 % and in the untreated control 3.3 % and thus below the threshold of 15 %. Mortality in the reference treatment group was 100 % and thus above the threshold of 50 %. Overall, the study is considered acceptable with following endpoints: LC_{50} > 8000 mg product/kg feeding solution LDD_{50} ≥ 111.6 µg product/honeybee/day NOEDD ≥ 111.6 µg product/honeybee/day NOEC ≥ 8000 mg product/kg feeding solution.

Reference:	KCP 10.3.1.2
Report	GLOB2007bF: Chronic Oral Toxicity Test on the Honey Bee (Apis mellifera L.) in the Laboratory, Schabio, S., 2023, Report No.: 169561136
Guideline(s):	OECD 245
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~No/Supplementary~~

Materials and methods

Test Item:	GLOB2007bF Batch No.: LCM22012601 Zoxamide: 64.23 ± 0.07 g/L, Propamocarb-HCl: 445.8 ± 1.3 g/L, according to the certificate of analysis
Test Species:	Honey bee (*Apis mellifera* L.); freshly emerged young female worker bees; obtained from a healthy and queen-right colony, bred by ibacon. After hatch, the bees were collected and thereafter acclimatized under test conditions for one day.
Age of the Honey Bees:	Two days old worker bees.
Test Design:	10 days chronic oral feeding test in the laboratory (dose response test). Young honey bees were provided with 5 concentrations of the test item treated sugar solutions *ad libitum* over a period of 10 days. An untreated control, a xanthan control and a reference item (Danadim; 400 g/L dimethoate) were included in this study. 3 replicates per treatment, each consisting of 10 bees per test cage.
Endpoints:	Daily assessment of mortality and behavioural abnormalities up to day 10. Endpoints: LC_{50}, LDD_{50}, NOEC, NOEDD.
Test Concentrations:	Test item: 8000, 4000, 2000, 1000 and 500 mg prod./kg feeding solution Reference item: 1 ppm dimethoate (1 mg dimethoate/kg feeding solution)
Target Dose Level:	Test item: 160, 80.0, 40.0, 20.0 and 10.0 µg prod./bee/day* Reference item: 0.02 µg a.i./bee per day* * taking into account a mean uptake of feeding solution of 20 mg/bee/day; the

	exact dose per bee per day was calculated after determination of the definitive food uptake of the bees at test end (see "Actual Mean Dose Level").
Actual Mean Dose Level:	Test item: 111.6, 80.9, 43.0, 25.0 and 12.9 µg prod./bee/day** Reference item: 0.016 µg a.i./bee/day** ** based on daily actual intake taking into consideration loss by evaporation
Evaporation:	In order to adjust for possible evaporation of test solutions from the feeders, evaporation figure was subtracted from the calculated uptake to give the real uptake accounting the loss by evaporation.
Test Conditions:	Temperature: 23 - 33 °C; relative humidity: 50 - 59 % mean relative humidity: 58 %; photoperiod: 24 h darkness (except during observation).
Study Validity:	This study met the OECD 245 (2017) validity criteria as the control mortality was < 15 % and the mortality of the reference item (dimethoate) was ≥ 50 % at test end.

Results and discussions

The test item was administered daily to the bees in sugar solution at the following concentrations: 8000, 4000, 2000, 1000 and 500 mg product/kg feeding solution. These concentrations resulted in a daily mean dose of 111.6, 80.9, 43.0, 25.0 and 12.9 µg product/bee/day after 10 days (considering actual daily intake and evaporation).

Mortality occurred in the highest, intermediate and second lowest test item treated dose level ranging from 3.3 to 6.7 % at test end (10 days following the start of chronic exposure).

There was 3.3 % mortality in the untreated control (50 % w/v sucrose solution) and no mortality in the xanthan control (50 % w/v sucrose solution containing 0.1 % w/v xanthan).

The reference item (dimethoate) at a concentration of 1 ppm (1 mg dimethoate/kg feeding solution) corresponding to an actual consumed dose of 0.016 µg a.i./bee/day caused 100 % mortality on day 7.

Test item related behavioural abnormalities were observed in a single affected bee in the second lowest test item treatment on day 7.

Table 5. 10 days Chronic Oral Toxicity of GLOB2007bF to young honey bees; laboratory test

Test Organism		*Apis mellifera* L.	
Exposure		Oral 10 days chronic exposure	
Treatment Group	Concentration [mg prod./kg]	Dose Level [1] [µg prod./bee/day]	Mortality at day 10 [2] [% Mean]
GLOB2007bF	8000	111.6	3.3 (n.s.)
	4000	80.9	0.0 (n.s.)
	2000	43.0	3.3 (n.s.)
	1000	25.0	6.7 (n.s.)
	500	12.9	0.0 (n.s.)
Untreated control	0.0	0.0	3.3
Xanthan Control	0.0	0.0	0.0
Reference Item	1.0	0.016	100.0
Endpoint at test termination (day 10)			
LC_{50}	**LDD_{50}**	**NOEC**	**NOEDD**
> 8000 mg prod./kg	**> 111.6 µg prod./bee/day**	**≥ 8000 mg prod./kg**	**≥ 111.6 µg prod./bee/day**

1) mean dose per bee per day; dose measured based on consumed feeding solution adjusted for evaporation
2) Mortality at study termination 10 days after start of first feeding
Statistics:
NOEC/NOEDD: Chi² 2x2 Table with Bonferroni Correction (one-sided greater, $\alpha = 0.05$).
n.s. = no statistically significant difference compared to the control

The analytical recovery rates of the active ingredients of GLOB2007bF in the feeding solutions were as follows:

Concentration [2]	Recovery rate [%] [1]	
	Propamocarb	Zoxamide
Feeding Solution 500 ppm DAA8	80 %	80 %
Feeding Solution 8000 ppm DAA8	81 %	85 %

[1] recovery rate of the a.i. in feeding solution [ppm]
[2] nominal concentration of the a.i. in the feeding solution [ppm]
DAA = Days after 1st Application (1st Application = DAA0)

Conclusion

The chronic oral toxicity of GLOB2007bF on young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

All validity criteria for the study were met. After 10 days of continuous exposure, mortality in the solvent control was 0.0 % and in the untreated control 3.3 % and thus below the threshold of 15 %. Mortality in the reference treatment group was 100 % and thus above the threshold of 50 %.

The recovery rates of the active ingredients of GLOB2007bF in the highest and lowest test item concentration of Day 1 were within ± 20 % of the nominal concentrations.

The LDD_{50} was estimated to be > 111.6 µg product/bee/day.
The LC_{50} was estimated to be > 8000 product/kg feeding solution.

The NOEDD was determined to be ≥ 111.6 µg product/bee/day and the NOEC was ≥ 8000 mg product/kg feeding solution.

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

Comments of zRMS:	The study was conducted to guidance OECD 239 and according to the principles of GLP. The following deviations to the guideline were noted: The mortality assessment on D7 was not carried out for the following reason: on D7 no operation is required (there are no treatments and there is no feeding of the larvae) and performing a mortality assessment would have involved opening the dryer with a consequent decrease of temperature and humidity. Therefore, to avoid stress to the larvae (in a very delicate stage such as the transition to pre-pupa) the assessment was performed directly on D8. All the validity criteria were met as follows: a) in the control plate(s) the cumulative larval mortality from D3 to D8 is ≤ 15% across all replicates (actual value 0.00%); b) in the control plate(s) the adult emergence rate on D22 is ≥ 70% across all replicates (actual value 91.67%); c) in the reference item group (dimethoate) the larval mortality is ≥ 50% on D8 across all replicates (actual value 100%). Since all the validity criteria were met the study is considered to be reliable.

Reference:	KCP 10.3.1.3
Report	Effects of GLOB2007bF on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Colli, M., 2022, Ibacon GmbH, Report No.: BT127/22
Guideline(s):	OECD 239
Deviations:	The mortality assessment on D7 was not carried out for the following reason: on D7 no operation is required (there are no treatments and there is no feeding of the larvae) and performing a mortality assessment would have involved opening the dryer with a consequent decrease of temperature and humidity. Therefore, to avoid stress to the larvae (in a very delicate stage such as the transition to pre-pupa) the assessment was performed directly on D8.
GLP:	Yes
Acceptability:	Yes/~~No/ Supplementary~~

Materials and methods

A. MATERIALS	
1. Test item	
Name:	**GLOB2007bF**
Indication:	Agrochemical
Batch:	LCM22012601
Active substances:	Zoxamide [156052-68-5] Propamocarb HCl [25606-41-1]
Active substances content:	Zoxamide: 64.23 g/L Propamocarb HCl: 445.8 g/L
2. Test system	
Species:	*Apis mellifera ligustica*
Age:	3 days old bee larvae (D3)
Source:	Healthy colony maintained at BioTecnologie BT S.r.l. (colonies no. 6, 3, 1)
Diet:	Dependent on developmental stage: Diet A, Diet B and Diet C
3. Experimental conditions	
Temperature:	30.4 – 34.9°C (average measured during the test: 34.2°C)
Humidity:	from D1 to D8 = 82.5 – 98.3% (average measured during the test: 97.3%) from D8 to D15 = 82.6 - 86.2% (average measured during the test: 83.6%) from D15 to D22 = 40.8 – 82.0% (average measured during the test: 62.7%)
Photoperiod:	24 h darkness (except during observations)
B. STUDY DESIGN AND METHODS	
1. Experimental period: from 29th June to 2nd August 2022 (including the analytical phase)	
2. Study design	
The 22-day larval toxicity test with repeated exposure in the laboratory was performed as dose-response test: the test item was dissolved in water and then in the larval food (aqueous sugar solution mixed with royal jelly) and administered daily to the larvae from day 3 (D3) to 6 (D6) of the test. The stock solutions and the treated diet were prepared freshly each day of administration.	

The reference item Dimethoate was tested at a cumulative dose of 7.39 µg/larva.

An untreated control was run in parallel with the royal jelly-based diet.

For each test item treatment group, for the control group and the reference item treatment group, 3 replicates of 12 larvae each were set up.
The cumulative doses and the concentrations of test item and reference item used for the test are shown in tables below. ~~1 and Table 2 below~~.

Table 6 Trial layout - treatments expressed as doses

Groups	Cumulative doses		
	[µg test item/larva]	[µg Zoxamide /larva]	[µg Propamocarb HCl/larva]
Water control	0	0	0
Test item (T1)	6.25	0.37	2.54
Test item (T2)	12.50	0.73	5.07
Test item (T3)	25.00	1.46	10.14
Test item (T4)	50.00	2.92	20.29
Test item (T5)	100.00	5.85	40.57
Reference item	-	7.39 Dimethoate	

Table 7 Trial layout - treatments expressed as concentrations

Groups	Concentrations		
	[mg test item/kg diet]	[mg Zoxamide/kg diet]	[mg Propamocarb HCl/kg diet]
Water control	0	0	0
Test item (T1)	40.58	2.37	16.47
Test item (T2)	81.17	4.75	32.93
Test item (T3)	162.34	9.49	65.86
Test item (T4)	324.68	18.98	131.72
Test item (T5)	649.35	37.96	263.44
Reference item	-	Dimethoate: 48.0	

3. Observations

Assessments of mortality and any developmental/behavioral abnormality were performed daily from D4 to D8 (except on D7) and on D15 and on D22.
Pupal mortality and the emergence rate of adults were also assessed on D22.

4. Statistics

The Software Tox Rat Pro 3.3.0 was used to perform the statistics.

5. Deviation from the Guidance Document

The mortality assessment on D7 was not carried out for the following reason:
on D7 no operation is required (there are no treatments and there is no feeding of the larvae) and performing a mortality assessment would have involved opening the dryer with a consequent decrease of temperature and humidity.

Therefore, to avoid stress to the larvae (in a very delicate stage such as the transition to pre-pupa) the assessment was performed directly on D8.

Results and discussions

Table 8 Mortality (M) and Corrected Mortality (CM) of larvae (on D8)

Treatment	Cumulative dose [µg test item/larva]	Concentration [mg test item/kg diet]	Larvae mortality on D8		
			M - Mean [%]	CM - Mean [%]	Sign.
Water control	0.00	0.00	0.00	n.a.	n.a.
Test item (T1)	6.25	40.58	0.00	0.00	-
Test item (T2)	12.50	81.17	8.33	8.33	-
Test item (T3)	25.00	162.34	2.78	2.78	-
Test item (T4)	50.00	324.68	0.00	0.00	-
Test item (T5)	100.00	649.35	2.78	2.78	-

n.a. = not applicable
+ : significant; - : non-significant (Chi ^{2} 2x2 Table test with Bonferroni Correction- $\alpha = 0.05$, one-sided greater)

Table 9 Pupal Mortality

Treatment	Cumulative dose [µg test item/larva]	Concentration [mg test item/kg diet]	Pupal mortality from D8 to D15*	Pupal mortality from D8 to D22**
			Mean [%]	Mean [%]
Water control	0.00	0.00	0.00	8.33
Test item (T1)	6.25	40.58	8.33	16.67
Test item (T2)	12.50	81.17	5.56	5.56
Test item (T3)	25.00	162.34	8.59	19.70
Test item (T4)	50.00	324.68	5.56	22.22
Test item (T5)	100.00	649.35	8.33	16.92

*calculated as a percentage comparing the number of dead pupae from D8 to D15 with the number of alive pupae on D8
**calculated as a percentage comparing the number of dead pupae from D8 to D22 with the number of alive pupae on D8

Table 10 Total mortality (M) and corrected mortality (CM) from D3 to D22 and emergence (E) on D22

Treatment	Cumulative dose [µg test item/larva]	Concentration [mg test item/ kg diet]	Mortality (larvae + pupae) on D22			Adult emergence on D22	
			M - Mean [%]	CM - Mean [%]	Sign.	E - Mean [%]	Sign.
Water control	0.00	0.00	8.33	n.a.	n.a.	91.67	n.a.
Test item (T1)	6.25	40.58	16.67	9.09	-	83.33	-
Test item (T2)	12.50	81.17	13.89	6.06	-	86.11	-
Test item (T3)	25.00	162.34	22.22	15.15	-	77.78	-
Test item (T4)	50.00	324.68	22.22	15.15	-	77.78	-
Test item (T5)	100.00	649.35	19.44	12.12	-	80.56	-

n.a. = not applicable
+ : significant; - : non-significant (Chi ^{2} 2x2 Table test with Bonferroni Correction- $\alpha = 0.05$, one-sided greater).

Table 11 Reference item - mean mortality

Treatment	Dose [µg a.s/larva]	Concentration [mg a.s./kg diet]	Mortality on D8 Mean [%]
Reference item	7.39	48.00	100.00

Conclusion

The effects of the test item **GLOB2007bF** on the larval development and subsequent adult emergence of honeybees (*Apis mellifera* L.), were tested in a GLP compliant laboratory study.

The validity criteria of the GD OECD No. 239 (2021) with regards to control larval mortality on D8, control adults' emergence on D22 and toxicity on the reference item were met. Thus, the study is valid.

The content of active substances was analyzed in the lowest and highest test item concentrations of the stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values for all tested samples, therefore the endpoints were calculated based on nominal concentrations and doses.
The control was also analyzed, and no contamination was detected (<LOD).

Regarding the effects on larvae on D8 the test item **GLOB2007bF** did not cause statistically significant mortality up to the highest tested dose. Therefore, the NOED for larvae on D8 was determined to be 100.00 µg test item/larva (5.85 µg Zoxamide/larva and 40.57 µg Propamocarb HCl/larva) equivalent to a NOEC of 649.35 mg test item/kg diet (37.96 mg Zoxamide/kg and 263.44 mg Propamocarb HCl/kg).

Regarding the effects on adult emergence on D22, the test item **GLOB2007bF** did not cause statistically significant mortality up to the highest tested dose. The NOED and the NOEC for adult emergence were determined to be 100.00 µg test item/larva (5.885 µg Zoxamide/larva and 40.57 µg Propamocarb HCl/larva) and 649.35 mg test item/kg diet (37.96 mg Zoxamide/kg and 263.44 mg Propamocarb HCl/kg), respectively.

The relevant endpoints are summarized in the following tables.

Table 12 Summary of results for larval mortality on D8 after repeated exposure to the test item

Critical dose	Larval mortality on D8 [µg test item/larva]	Larval mortality on D8 [µg a.s./larva]
NOED	100.00	Zoxamide: 5.85 Propamocarb HCl: 40.57
Critical concentration	**Larval mortality on D8 [mg test item/kg diet]**	**Larval mortality on D8 [mg a.s./kg diet]**
NOEC	649.35	Zoxamide: 37.96 Propamocarb HCl: 263.44

Table 13 Summary of results for adult emergence on D22 after repeated exposure to the test item

Critical dose	Larval mortality on D8 [µg test item/larva]	Larval mortality on D8 [µg a.s./larva]
NOED	100.00	Zoxamide: 5.85 Propamocarb HCl: 40.57
Critical concentration	**Larval mortality on D8 [mg test item/kg diet]**	**Larval mortality on D8 [mg a.s./kg diet]**
NOEC	649.35	Zoxamide: 37.96 Propamocarb HCl: 263.44

Comments of zRMS:	The study was conducted to guidance OECD 239 and according to the principles of GLP. No significant deviations to the guideline were noted. All the validity criteria were met as follows: Controls The cumulative larval mortality from day 3 (D3) until day 8 (D8) was ≤ 15 % across all replicates (actual mean value 4.2 % for the negative control and 14.6 % for the solvent control). On day 22 (D22) the adult emergence rate was ≥ 70 % across all replicates (actual mean value 79.2 % for the negative control and 75.0 % for the solvent control). Reference The cumulative larval mortality was ≥ 50 % across all replicates on day 8 (D8) (actual mean value 89.6 %). Thus, the study is considered to be reliable

Reference:	KCA 8.3.1.3
Report	Zoxamide technical: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions, Aguilar-Alberola, J., 2023, Eurofins Trialcamp S.L.U., Report No.: S23-106642
Guideline(s):	OECD 239
Deviations:	The reduction of the relative humidity conditions from 95 ± 5 % to 80 ± 5 % was done on day 7 (D7) of the test instead of on day 8 (D8). The reported deviation to the guidance has no impact on the outcome of the study since validity criteria for the control were met.
GLP:	Yes
Acceptability:	Yes / ~~No / Supplementary~~

Materials and methods

Test item: Zoxamide technical; Batch code: L20210418Z; active substance (a.s.): zoxamide; content of a.s.: 98.86 ± 0.29 % w/w (content of active substance according to Certificate of Analysis); analytical certificate date: 24 May 2022; estimated retest date: 13 May 2026.

Reference item: BAS 152 I; Batch code: COD-002332; active substance (a.s.): dimethoate; content of a.s. analysed: 100.55 % w/w (100 % w/w was considered); expiry date: 25 Jan 2024.

Test organisms: Honey bee (*Apis mellifera* L.), synchronized first instar (L1) larvae not older than 30 hours at grafting time.

Source: Commercial beehives from the in-house test facility stock, adequately fed, healthy and as far as possible disease-free and queen-right. The hives from which the larvae were obtained were not previously exposed to any chemical treatments within four weeks of test initiation.

Preparation of test organisms On D-3, the queens from at least three colonies were isolated for one day within a queen excluder placed on a single frame with empty cells in their own hive, to provide

and larvae collection: known-aged eggs and subsequent larvae.

On D-2, maximum 30 hours after isolation, the queens were released. Frames containing eggs were left in the excluder cages until hatching (D1). Three frames from different hives, containing the highest number of synchronized larvae, were selected for grafting in the laboratory.

Test design: Dose response test with duration of 21 days from grafting on day 1 to the final assessment on day 22. From day 3 until day 6 of the test, 5 different concentrations of Zoxamide technical were applied to the larvae of the test item groups and one single concentration of the reference item was applied to the larvae of the reference item group. Both, test and reference item, were supplied in diet B (day 3) and C (days 4, 5 and 6). The daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period. The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva was considered for the calculation of the cumulative doses per larva.

Two control groups (negative and solvent controls) were included in the test and exposed for the same period of time under identical exposure conditions to the treatments. Each treatment group consisted of 48 larvae; 16 from each of three different colonies (each colony representing one replicate). Larval mortality assessments were on days 4, 5, 6, 7, and 8. The presence of uneaten food was qualitatively recorded on day 8. Assessment of mortality during pupation phase was on day 15 and assessment of emergence on day 22 was recorded.

Test concentrations and doses:

Controls: C1: Negative control (untreated diet).
C2: Solvent control (diet containing 1.5 % acetone).

Test item: 36.13, 72.25, 144.51, 289.01 and 578.02 mg test item/L diet (equivalent to 32.84, 65.68, 131.37, 262.74 and 525.47 mg test item/kg diet and 5.06, 10.12, 20.23, 40.46 and 80.92 µg test item/larva, respectively).

The equivalences in active substance are 35.71, 71.43, 142.86, 285.72 and 571.43 mg zoxamide/L diet (equivalent to 32.47, 64.94, 129.87, 259.74 and 519.48 mg zoxamide/kg diet and 5.00, 10.00, 20.00, 40.00 and 80.00 µg zoxamide/larva, respectively).

Reference item: R: 48.00 mg dimethoate/kg diet (equivalent to 7.39 µg dimethoate/larva).

Test conditions: Air Temperature: 33.7* – 35.0 °C

Relative humidity: 34.5* – 97.6 %

*Short term deviation (<2 hours).

Exposure to light: Constant darkness except during feeding and assessments.

Analytical verification: Samples of the highest and lowest concentrations of the test item treated larval diet were taken from D3 to D6, directly after preparation, and placed in the freezer at < -

18 ºC until shipment.

Analytical Phase was performed to verify the concentration of the samples taken. A method was validated and samples of diet were analysed for concentration determination of zoxamide.

Quantification was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). The limit of quantification (LOQ) of the analytical method was 3.00 mg/test item/kg (2.97 mg zoxamide/kg) with a limit of detection (LOD) set at 0.619 mg zoxamide/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).

Statistics: Mortality on D22 showed no statistically significant differences between the control groups (Fisher's exact binomial test, $\alpha = 0.05$, two sided), therefore, data of the solvent control (C2) was used for the analysis of this parameter.

In order to determine the NOEC, the Chi^2 2x2 table test with Bonferroni correction (one-sided greater, $\alpha = 0.05$) was used.

EC_x/ED_x values could not be calculated since no statistically significant concentration/response was obtained. Because on D22 the corrected mortality obtained was below 50 % in all the treatment groups, the EC_{50} was empirically estimated from the results.

Statistical calculations were made with MS Excel v.365 and the statistical program ToxRatPro® Version 3.3.0.

Results and discussions

The measured concentration in the samples from treatment T1 was within 20 % of nominal test concentration. The analysed concentrations of treatment T5 from D3 and D4 were lower than nominal (mean recovery values from A and R samples were 71.9 and 79.2 % of nominal, respectively), while the recoveries from D5 and D6 were within 20 % of nominal test concentration. Although the weighted average recovery of the samples was calculated as 89.4 % of the nominal value, as worst-case scenario, the treatment T5 values have been adjusted for the analysed concentration (corrected values: 510.78 mg zoxamide/L diet, 464.34 mg zoxamide/kg diet and 71.51 µg zoxamide/larva, these are the values shown in this section from now on).

On day 8, the cumulative larval mortality was 4.2 % for the negative control (C1) and 14.6 % for the solvent control (C2). On day 22, the adult emergence rate of the initial grafted larvae was 79.2 % for the negative control and 75.0 % for the solvent control. Therefore, the validity criteria for the control groups were met for both test periods: The D8 mortality was lower than 15 % and the D22 days emergence rate was greater than 70 %, across all replicates. Cumulative mortality in the Reference Item group also met the validity criteria (> 50 % at day 8, actual value 89.6 %).

Mean corrected cumulative larval mortality on day 8 (D8) of the test item treated groups relative to the solvent control (C2) was -4.9, 7.3, -2.4, 4.9 and -7.3 % in 32.47, 64.94, 129.87, 259.74 and 464.34 mg zoxamide/kg diet, respectively.

Mean corrected pupal mortality on day 15 (D15) of the test item treated groups relative to the solvent control (C2) was 17.9, 25.6, 20.5, 17.9 and 12.8 % in 32.47, 64.94, 129.87, 259.74 and 464.34 mg zoxamide/kg diet, respectively.

Mean corrected mortality at the end of the test (D22) of the test item treated groups relative to the solvent control (C2) was 16.7, 25.0, 16.7, 22.2 and 13.9 % in 32.47, 64.94, 129.87, 259.74 and 464.34 mg zoxamide/kg diet, respectively.

On day 8, one organism in treatment T4 (259.74 mg zoxamide/kg diet) and two organisms in treatment T5 (464.34 mg zoxamide/kg diet) were observed with uneaten food. All these organisms were recorded as dead from the D15 assessment. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were recorded as being affected (i.e. malformation).

Main results are shown in the table below.

Treatment Group		**Cumulative Mortality [%]**						
[mg a.s./kg diet]		**D4**	**D5**	**D6**	**D7**	**D8**	**D15**	**D22**
C1	[-]	0.0	2.1	2.1	2.1	4.2	20.8	20.8
C2	[-]	4.2	4.2	8.3	14.6	14.6	18.8	25.0
T1	[32.47]	2.1	6.3	6.3	10.4	10.4	33.3	37.5
T2	[64.94]	0.0	8.3	18.8	18.8	20.8	39.6	43.8
T3	[129.87]	2.1	2.1	6.3	12.5	12.5	35.4	37.5
T4	[259.74]	2.1	6.3	12.5	18.8	18.8	33.3	41.7
T5	[464.34]	4.2	4.2	6.3	8.3	8.3	29.2	35.4
R	[48.00][a]	39.6	54.2	72.9	85.4	89.6	100.0	100.0

a.s.: active substance (zoxamide).
[a] mg dimethoate/kg diet.

Treatment Group		**Corrected mortality [%][a]**						
[mg a.s./kg diet]		**D4**	**D5**	**D6**	**D7**	**D8**	**D15**	**D22**
C1	[-]	-4.3	-2.2	-6.8	-14.6	-12.2	2.6	-5.6
T1	[32.47]	-2.2	2.2	-2.3	-4.9	-4.9	17.9	16.7
T2	[64.94]	-4.3	4.3	11.4	4.9	7.3	25.6	25.0
T3	[129.87]	-2.2	-2.2	-2.3	-2.4	-2.4	20.5	16.7
T4	[259.74]	-2.2	2.2	4.5	4.9	4.9	17.9	22.2
T5	[464.34]	0.0	0.0	-2.3	-7.3	-7.3	12.8	13.9

a.s.: active substance (zoxamide).
[a] Corrected for solvent control (C2) according to Abbott's formula (1925), modified by Schneider-Orelli (1947).

Conclusion

The repeated exposure of the test item 'Zoxamide technical' to honey bee (*Apis mellifera* L.) was tested under laboratory conditions over a 21-day period.

All validity criteria were met and sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.

The test item concentrations of treatment T1 were analytically confirmed. Although the weighted average recovery of the samples was calculated as 89.4 % of the nominal value, as worst-case scenario, the treatment T5 values have been adjusted for the analysed concentration. Therefore, endpoints are shown adjusted for the mean analysed concentration.

On day 8, one organism in treatment T4 (259.74 mg zoxamide/kg diet) and two organisms in treatment T5 (464.34 mg zoxamide/kg diet) were observed with uneaten food. All these organisms were recorded as dead from the D15 assessment. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were recorded as being affected (i.e. malformation).

Main endpoints of the study are shown in the following table.

Endpoints	**Concentration**		**Dose**
	[mg a.s./L diet]	[mg a.s./kg diet] [a]	[µg a.s./larva] [b]
NOEC/NOED	≥510.78	≥464.34	≥71.51
LOEC/LOED	>510.78	>464.34	>71.51
EC_{10} / ED_{10}	Not determined	Not determined	Not determined
[95 % c.l.]	Not determined	Not determined	Not determined
EC_{20} / ED_{20}	Not determined	Not determined	Not determined
[95 % c.l.]	Not determined	Not determined	Not determined
EC_{50} / ED_{50}	>510.78	>464.34	>71.51
[95 % c.l.]	Not determined	Not determined	Not determined

a.s.: active substance (zoxamide; 98.86 ± 0.29 % w/w); c.l.: confidence limits.
[a] Based on the diet density (1.1 g/mL).
[b] Based on the cumulative application volume of 140 µL/larva.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No data submitted.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No data submitted.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No data 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

Comments of zRMS:	The study follows the guideline specified by Mead Briggs M.A. et al. (2000) and according to the principles of GLP. No deviations to the guideline were noted. In the definitive test all the validity criteria were met as follows: - The average mortality in the controls at 48h was 0.0 % (required: <13%) - Reference item mortality was 100% (required: >50%) - The average number of parasitised aphids per female in the controls was 44.5 (required: >5) with 1 zero value (required: <2) The study is reliable for risk Assessment purposes.

Reference:	KCP 10.3.2.1
Report	GLOB2007bF: Effects on the Parasitoid Aphidius rhopalosiphi (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold, J., 2022, Ibacon GmbH, Report No.: 169561001
Guideline(s):	Mead-Briggs et al. 2000 and Mead-Briggs et al. 2010
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF; batch no.: LCM22012601; analysed content of a.s.: 64.23 ± 0.07 g/L or 5.846 ± 0.007 % w/w zoxamide; 445.8 ± 1.3 g/L or 40.57 ± 0.12 % w/w propamocarb-HCL.
Test Species:	Parasitoid *(Aphidius rhopalosiphi)*, adults not older than 48 hours; source: Katz Biotech AG, Baruth, Germany.
Test Design:	This study encompassed 7 treatment groups (5 dose rates of the test item, control, reference item) with 4 replicates each containing 10 adult parasitoids. The parasitoids were exposed to dried residues on treated glass plates. Survival of the parasitoids was assessed after 2, 24 and 48 hours. After 48 hours, for treatment groups where the corrected mortality was ≤ 50 % the reproductive capacity was assessed by confining females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants left for a further 11 - 12 days before the numbers of aphid mummies that had developed were assessed.
Endpoints:	Mortality of exposed parasitoids; additionally, reproductive capacity for female survivors.
Validity Criteria:	– Control mortality should be ≤ 13 %. – Reference item mortality should be ≥ 50 % corrected mortality. – Mean reproduction rate of control treatment should be ≥ 5 mummies per female. – No more than 2 female parasitoids should produce zero values.
Reference Item:	Danadim Progress (nominal: 400 g dimethoate/L).
Test Rates:	Control, 1333, 2000, 3000, 4500 and 6750 mL product/ha and reference item. The reference item was applied at an application rate of 0.3 mL Danadim Progress/ha. All treatments were applied in 200 L spray volume/ha. The spraying solutions were sprayed onto glass plates *via* laboratory spraying

	equipment, which were then air dried.
Test Conditions:	Temperature: 20 - 21 °C; relative humidity: 73 - 81 % (acclimatisation and exposure period), 76 - 79 % (post-exposure period, within the test units); photoperiod: 16 h light : 8 h dark; light intensity: 800 - 1590 lux (acclimatisation, exposure and parasitisation period), 13190 - 16460 lux (post-parasitisation period).
Statistics:	Mortality: Step-down Cochran-Armitage Test, Fisher's Exact Binomial Test (both one-sided greater, $\alpha = 0.05$). Reproduction: Dunnett's t-Test (one-sided smaller, $\alpha = 0.05$).

Results and discussions

The mean mortality of *Aphidius rhopalosiphi* was 0.0 % in the control treatment. In the test item treatments, it ranged from 7.5 % to 25.0%, corresponding to a corrected mortality of 7.5 to 25.0 %. Mortality was not statistically significantly increased compared to the control up to and including the application rate of 3000 mL product/ha (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$, see Table 14).
The reference item applied at a rate of 0.3 mL Danadim Progress/ha produced a statistically significant corrected mortality of 100.0 % after 48 hours (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

Table 14. Mortality and parasitisation efficiency of the parasitoid wasp *Aphidius rhopalosiphi*

	Rate [1] **[mL product/ha]**	**Mortality** [2] **[%]**	**Mortality corr.** [3] **[%]**	**Reproduction** [4] **[mummies/female]**	**Effect on reproduction** [5] **[%]**
Control	0	0.0	--	44.5	--
GLOB2007bF	1333	7.5 n.s.	7.5	51.4 n.s.	-15.5
GLOB2007bF	2000	10.0 n.s.	10.0	47.5 n.s.	-6.7
GLOB2007bF	3000	7.5 n.s.	7.5	45.9 n.s.	-3.2
GLOB2007bF	4500	20.0 *	20.0	48.4 n.s.	-8.8
GLOB2007bF	6750	25.0 *	25.0	53.1 n.s.	-19.4

Endpoints [6]			
	[mL product/ha]	zoxamide [g a.s./ha]	propamocarb-HCL [g a.s./ha]
Mortality: LR_{50} Value	> 6750	> 434	> 3009
NOER for Mortality	3000	193	1337
LOER for Mortality	4500	289	2006
Reproduction: ER_{50} Value	> 6750	> 434	> 3009
NOER for Reproduction	≥ 6750	≥ 434	≥ 3009
LOER for Reproduction	> 6750	> 434	> 3009

1) Application rate in 200 L spray volume/ha
2) Mortality: after 48 hours of exposure to spray residues on glass plates,
(Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$; n.s. = not significant, * = significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of parasitised aphids/female,
(Dunnett's t-Test, one-sided smaller, $\alpha = 0.05$; n.s. = not significant)
5) Calculated on the exact raw data; negative values indicate better performance compared to the control
6) The LR_{50} and ER_{50} value were not calculated as no mortality or effect on reproduction above 50% was noted.

One moribund parasitoid each was recorded at 6750 mL product/ha after 24 hours and at 2000 mL product/ha and 6750 mL product/ha after 48 hours. No further behavioural abnormalities (affected and/or moribund parasitoids) were observed at the remaining test item treatments at any observation.
Reproduction of *A. rhopalosiphi* was assessed in the control and at all test item application rates. The mean reproduction rate was 44.5 mummies per female in the control treatment. In the test item treatments, the mean reproduction ranged from 45.9 to 53.1 mummies per females, corresponding to -3.2 to -19.4 % reduction compared to the control. Reproduction was not statistically significantly reduced compared to the control up to and including the highest application rate of 6750 mL product/ha (Dunnett's t-test, one-sided smaller, $\alpha = 0.05$).

Validity criteria:
The reference item applied at a rate of 0.3 mL Danadim Progress/ha produced a statistically significant corrected mean mortality of 100.0 % after 48 hours (should be ≥ 50 % corrected mortality). The mean control mortality was 0.0 % after 48 hours of exposure (should not exceed 13 %). The mean control reproduction rate was 44.5 mummies per female (should be ≥ 5.0 mummies per female). One female parasitoid produced zero values in the control treatment (no more than 2 female parasitoids producing zero values). All validity criteria were met.

Conclusion

Under worst case laboratory conditions the LR_{50} value of GLOB2007bF is estimated to be greater than 6570 mL product/ha (equivalent to > 434 g zoxamide/ha and > 3009 g propamocarb-HCL/ha) in 200 L water/ha. The NOER (no observed effect rate) for mortality is 3000 mL product/ha (equivalent to 193 g zoxamide/ha and 1337 g propamocarb-HCL/ha) and the LOER (lowest observed effect rate) is 4500 mL product/ha (equivalent to 289 g zoxamide/ha and 2006 g propamocarb-HCL/ha) in 200 L water/ha.
Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at all test item application rates. Reproduction was not affected up to and including the highest test item rate of 6750 mL product/ha. The ER_{50} of GLOB2007bF is estimated to be greater than 6750 mL product/ha (equivalent to > 434 g zoxamide/ha and > 3009 g propamocarb-HCL/ha) in 200 L water/ha. The NOER is equal to or greater than 6750 mL product/ha (equivalent to ≥ 434 g zoxamide/ha and ≥ 3009 g propamocarb-HCL/ha) and the LOER is greater than 6750 mL product/ha (equivalent to > 434 g zoxamide/ha and > 3009 g propamocarb-HCL/ha) in 200 L water/ha.
All validity criteria were met. The study is considered valid.

Comments of zRMS:	The study follows the guideline specified by Blümel S. et al., 2000 and ac-cording to the principles of GLP. No deviations to the guideline were noted. All the validity criteria were met. - The reference item applied at a rate of 9.0 mL Danadim Progress/ha produced a statistically significant corrected mortality of 94.1 % after 7 days (should be ≥ 50 % corrected mortality). - The control mortality was 15.0 % after 7 days (should not exceed 20 %). - The mean control reproduction rate was 5.4 eggs per female after 14 days (should be ≥ 4 eggs per female in the second week). The study is reliable for Risk assessment purposes.

Reference:	KCP 10.3.2.1
Report	GLOB2007bF: Effects on the Predatory Mite Typhlodromus pyri (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold, J., 2022, Ibacon GmbH, Report No.: 169561063
Guideline(s):	Blümel *et al.* 2000
Deviations:	No

GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF; batch no.: LCM22012601; analysed content of a.s.: 64.23 ± 0.07 g/L or 5.846 ± 0.007 % w/w zoxamide; 445.8 ± 1.3 g/L or 40.57 ± 0.12 % w/w propamocarb-HCL.
Test Species:	Predatory Mite *(Typhlodromus pyri)*, protonymphs not older than 24 hours; source: Katz Biotech AG, Baruth, Germany.
Test Design:	This study comprised 7 treatment groups (5 application rates of the test item, control, reference item) with 3 replicates each containing 20 mites. The mites were exposed to dried residues on treated glass plates. Survival of the mites was assessed after 2 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where the corrected mortality was ≤ 50 % were sexed and the number of eggs per female was recorded at 3 assessment days within one week.
Endpoints:	Mortality after 7 days of exposure; additionally, reproduction capacity for survived mites.
Validity Criteria:	– Control mortality should not exceed 20 % on day 7 after exposure. – Reference item mortality should result in at least 50 % corrected mortality on day 7 after exposure. – Control reproductions (number of eggs per female) should be ≥ 4 eggs for the second week.
Reference Item:	Danadim Progress (nominal: 400 g dimethoate/L).
Test Rates:	Control, 600, 1200, 2400, 4800 and 9600 mL product/ha and reference item. The reference item was applied at an application rate of 9.0 mL Danadim Progress/ha. All treatments were applied in 200 L spray volume/ha. The spraying solutions were sprayed onto glass plates via laboratory spraying equipment, which were then air dried.
Test Conditions:	Temperature: 23 - 25 °C; relative humidity: 66 - 71 %; photoperiod: 16 h light : 8 h dark; light intensity: 350 - 420 lux.
Statistics:	Mortality: Step-down Cochran-Armitage Test, Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$), LR_{50} calculation by Spearman-Karber Procedure. Reproduction: Williams t-Test (one-sided smaller, $\alpha = 0.05$); ER_{50} calculation by Weibull Analysis

Results and discussions

The mean mortality of *Typhlodromus pyri* was 15.0 % in the control treatment. In the test item treatments, it ranged from 16.7 % to 73.3 %, corresponding to a corrected mortality of 2.0 to 68.6 %. Mortality was not statistically significantly increased compared to the control up to and including the application rate of 2400 mL product/ha (Step-down Cochran-Armitage Test, one-sided greater, $\alpha = 0.05$, see Table 15).
The reference item applied at a rate of 9.0 mL Danadim Progress/ha produced a statistically significant mortality of 95.0 % (corrected mortality 94.1 %) after 7 days (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$).

Table 15. Mortality and reproduction of the mites

	Rate [1] [mL product/ha]	Mortality [2] [%]	Mortality corr. [3] [%]	Reproduction [4] [eggs/female]	Effect on reproduction [5] [%]
Control	0	15.0	--	5.4	--
GLOB2007bF	600	33.3 n.s.	21.6	4.1 n.s.	23.2
GLOB2007bF	1200	16.7 n.s.	2.0	3.9 n.s.	26.8
GLOB2007bF	2400	26.7 n.s.	13.7	2.7 *	50.6
GLOB2007bF	4800	35.0 *	23.5	1.9 *	63.8
GLOB2007bF	9600	73.3 *	68.6	--	--

Endpoints [6]	[mL product/ha]	zoxamide [g a.s./ha]	propamocarb-HCL [g a.s./ha]
Mortality: LR_{50} Value (95% CL)	5536 (4756 – 6445)	355.6 (305.5 – 414.0)	2467.9 (2120.2 – 2873.2)
NOER for Mortality	2400	154.2	1069.9
LOER for Mortality	4800	308.3	2139.8
Reproduction: ER_{50} Value (95% CL)	2768 (2098 – 3825)	177.8 (134.8 – 245.7)	1234.0 (935.3 – 1705.2)
NOER for Reproduction	1200	77.1	535.0
LOER for Reproduction	2400	154.2	1069.9

1) Application rate in 200 L spray volume/ha
2) Mortality: after 7 days of exposure to spray residues on glass plates
(Step-down Cochran-Armitage Test; one-sided greater; $\alpha = 0.05$; n.s. = not significant, * = significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of eggs/female,
(Williams t-Test; one-sided smaller; $\alpha = 0.05$; n.s. = not significant, * = significant)
5) Calculated on the exact raw data
6) LR_{50} was calculated with Spearman-Karber Procedure, ER_{50} was calculated using the Weibull Analysis; CL = confidence limits

Reproduction of *T. pyri* was assessed in the control and at all test item application rates with exception of the highest test item rate of 9600 mL product/ha. The mean reproduction was 5.4 eggs per female in the control treatment. In the test item treatments, reproduction ranged from 1.9 to 4.1 eggs per female, corresponding to 23.2 to 63.8 % reduction compared to the control. Reproduction was not statistically significantly reduced compared to the control up to and including the test item application rate of 1200 mL product/ha (Williams t-Test, one-sided smaller, $\alpha = 0.05$).

Validity criteria:
The reference item applied at a rate of 9.0 mL Danadim Progress/ha produced a statistically significant corrected mortality of 94.1 % after 7 days (should be ≥ 50 % corrected mortality). The control mortality was 15.0 % after 7 days (should not exceed 20 %). The mean control reproduction rate was 5.4 eggs per female after 14 days (should be ≥ 4 eggs per female in the second week). All validity criteria were met.

Conclusion

Under worst case laboratory conditions the LR_{50} value of GLOB2007bF for mortality is 5536 mL product/ha, equivalent to 355.6 g zoxamide/ha and 2467.9 g propamocarb-HCL/ha, in 200 L water/ha (95 % confidence limits: 4756 – 6445 mL product/ha, equivalent to 305.5 – 414.0 g zoxamide/ha and 2120.2 – 2873.2 g propamocarb-HCL/ha). The NOER (no observed effect rate) for mortality is 2400 mL

product/ha (equivalent to 154.2 g zoxamide/ha and 1069.9 g propamocarb-HCL/ha) and the LOER (lowest observed effect rate) is 4800 mL product/ha (equivalent to 308.3 g zoxamide/ha and 2139.8 g propamocarb-HCL/ha) in 200 L water/ha.
Reproduction of *Typhlodromus pyri* was assessed in the control and at all test item application rates with exception of the highest test item rate of 9600 mL product/ha. Reproduction was not affected up to and including the application rate of 1200 mL product/ha. The ER_{50} value of GLOB2007bF for reproduction is 2768 mL product/ha (equivalent to 177.8 g zoxamide/ha and 1234.0 g propamocarb-HCL/ha) in 200 L water/ha (95 % confidence limits: 2098 – 3825 mL product/ha, equivalent to 134.8 – 245.7 g zoxamide/ha and 935.3 – 1705.2 g propamocarb-HCL/ha). The NOER is 1200 mL product/ha (equivalent to 77.1 g zoxamide/ha and 535.0 g propamocarb-HCL/ha) and the LOER is 2400 mL product/ha (equivalent to 154.2 g zoxamide/ha and 1069.9 g propamocarb-HCL/ha) in 200 L water/ha.
All validity criteria were met. The study is considered valid.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory tests

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

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met according to OECD 222 as follows: In the definitive test all the validity criteria were met according to OECD Guideline No. 222: - each replicate produced 50-96 juveniles (mean) at the end of the study (criterion: ≥ 30 juveniles by the end of the study), - the coefficient of variation of reproduction was 24.4 % (criterion: ≤ 30%), - adult mortality over the initial 4 weeks of the experiment was 0 % (criterion: ≤ 10%). The study is reliable.

Reference:	KCP 10.4.1.1
Report	GLOB2007bF (Zoxamide 67.5 g/L + propamocarb HCL 450 g/L SC): Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Straube, D., 2022, Ibacon GmbH, Report No.: 169561022
Guideline(s):	OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes / ~~No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF; batch no.: LCM22012601; content of a.i.: Zoxamide: 64.23 ± 0.07 g/L or 5.846 ± 0.007 % w/w (analysed); Propamocarb-HCL: 445.8 ± 1.3

	g/L or 40.57 ± 0.12 % w/w (analysed)
Test Species:	Earthworm (*Eisenia andrei*, Bouché, 1972), adult earthworms (with clitellum and weight range 300 mg to 584 mg), approximately 7 to 8 months old, source: from an in-house culture.
Test Design:	56-day test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Reference Item:	Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study.
Test Concentrations:	Control, 0.37, 0.60, 0.95, 1.53, 2.44, 3.91, 6.25 and 10.0 mg GLOB2007bF/kg soil dry weight.
Test Conditions:	Artificial soil according to OECD 222; initial pH 5.9 to 6.4, pH at experimental end 6.2 to 6.3; water content 25.2% to 26.3% (50.3% to 52.6% of maximum water holding capacity, WHC) at experimental start and 25.7% to 27.6% (51.4% to 55.1% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Statistics:	Standard procedures, Fisher’s Exact Test (mortality), Dunnett’s t-test (body weight changes and reproduction).

Results and discussions

The impact of the test item on mortality of the earthworms is presented in table below.

Table 3. Number of live adult earthworms and mortality after 28 days

Treatment Group	Container #	Number of Live Adults Start	Number of Live Adults 28 Days	Mortality of the Adults (28 days) % per Container	% Mean ± SD[1]	Signifi-cance
Control	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
	V	10	10	0		
	VI	10	10	0		
	VII	10	10	0		
	VIII	10	10	0		
0.37	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
0.60	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
0.95	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
1.53	I	10	10	0	2.5 ± 5.0	n.s.
	II	10	10	0		
	III	10	9	10		
	IV	10	10	0		
2.44	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
3.91	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
6.25	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
10.0	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		

Test item dosages are given as mg test item/kg artificial soil dry weight
- = Not relevant
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
n.s. = Not significantly different compared to the control, Fisher's Exact Test, α = 0.05, one-sided greater

Body weights of the living adult earthworms are presented in table below

Table 4. Body weight changes of the adult earthworms

Treatment Group	Container #	Mean Earthworm Body Weight At Start mg	Mean Earthworm Body Weight After 28 Days mg	Body Weight Change Mean per Earthworm mg	Mean per Earthworm %	Mean ± SD [1] mg	Mean ± SD [1] %	Significance
Control	I	347.9	491.1	143.2	41.2			
	II	372.0	530.1	158.1	42.5	169	44.1	-
	III	372.6	528.5	155.9	41.8	± 17	± 3.9	
	IV	381.1	563.9	182.8	48.0			
	V	381.2	572.0	190.8	50.1			
	VI	391.8	549.9	158.1	40.4			
	VII	392.0	579.9	187.9	47.9			
	VIII	425.0	599.5	174.5	41.1			
0.37	I	352.3	499.9	147.6	41.9			
	II	381.0	592.9	211.9	55.6	169	43.9	n.s.
	III	385.3	536.5	151.2	39.2	± 30	± 7.9	
	IV	423.0	588.1	165.1	39.0			
0.60	I	354.3	548.4	194.1	54.8			
	II	378.4	513.9	135.5	35.8	171	44.9	n.s.
	III	386.2	561.9	175.7	45.5	± 25	± 7.8	
	IV	407.7	585.5	177.8	43.6			
0.95	I	358.3	524.0	165.7	46.2			
	II	378.4	471.8	93.4	24.7	166	43.3	n.s.
	III	387.1	620.1	233.0	60.2	± 57	± 14.6	
	IV	407.0	577.5	170.5	41.9			
1.53	I	361.7	536.1	174.4	48.2			
	II	377.9	542.2	164.3	43.5	163	42.7	n.s.
	III	387.2	543.2	156.0	40.3	± 9	± 4.2	
	IV	402.5	558.7	156.2	38.8			
2.44	I	362.8	556.8	194.0	53.5			
	II	377.8	557.9	180.1	47.7	166	43.7	n.s.
	III	387.6	560.9	173.3	44.7	± 34	± 10.4	
	IV	399.6	515.9	116.3	29.1			
3.91	I	362.8	514.2	151.4	41.7			
	II	375.1	530.6	155.5	41.5	159	41.7	n.s.
	III	388.4	552.4	164.0	42.2	± 6	± 0.4	
	IV	399.3	563.8	164.5	41.2			
6.25	I	367.2	506.9	139.7	38.0			
	II	373.6	557.5	183.9	49.2	165	43.3	n.s.
	III	389.1	561.7	172.6	44.4	± 19	± 4.7	
	IV	396.7	562.4	165.7	41.8			
10.0	I	369.6	537.2	167.6	45.3			
	II	373.4	544.6	171.2	45.8	172	45.1	n.s.
	III	391.1	582.6	191.5	49.0	± 14	± 3.6	
	IV	393.4	551.8	158.4	40.3			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Dunnett' t-test, α = 0.05, two-sided

The numbers of juvenile earthworms found 56 days after application are shown in Table below.

Table 5. Reproduction of the earthworms after 56 days

Treatment Group	Container #	Number of Juvenile Earthworms Per Container	Mean ± SD [1]	% of Control	Significance
Control	I	50	65 ± 16	-	-
	II	50			
	III	61			
	IV	71			
	V	67			
	VI	50			
	VII	96			
	VIII	72			
0.37	I	59	57 ± 21	88.6	n.s.
	II	27			
	III	75			
	IV	68			
0.60	I	53	61 ± 10	94.0	n.s.
	II	70			
	III	51			
	IV	69			
0.95	I	64	75 ± 11	116.1	n.s.
	II	81			
	III	88			
	IV	67			
1.53	I	91	63 ± 19	97.5	n.s.
	II	59			
	III	53			
	IV	49			
2.44	I	85	81 ± 22	124.6	n.s.
	II	110			
	III	67			
	IV	60			
3.91	I	61	71 ± 18	109.1	n.s.
	II	63			
	III	60			
	IV	98			
6.25	I	63	69 ± 12	107.2	n.s.
	II	86			
	III	69			
	IV	59			
10.0	I	46	50 ± 18	77.0	n.s.
	II	42			
	III	35			
	IV	76			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Dunnett's t-test, α = 0.05, one-sided smaller

All study validity criteria were met.

OECD 222	Current study
Mortality rate: ≤10 %	0 %
Reproduction: number of juveniles per replicate ≥30	50-96
Coefficient of variation of reproduction: ≤30%	24.4 %

A slight mortality of 2.5% was found at the concentration of 1.53 mg test item/kg soil dry weight, which was not statistically significantly different compared to the control, where 0% of the earthworms died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).
The body weight changes of the earthworms after 28 days exposure to GLOB2007bF were not statistically significantly different compared to the control up to and including the highest test concentration of 10.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).
The reproduction rates were not statistically significantly different compared to the control up to and including the highest test concentration of 10.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller).
No behavioural abnormalities were observed in any of the treatment groups.
The feeding activity in all the treated groups was comparable to the control (see Table 1).

Table 16. Effect of GLOB2007bF on earthworms (*Eisenia andrei*) in a 56-day reproduction study

GLOB2007bF [mg test item/kg soil dry weight]	**Control**	**0.37**	**0.60**	**0.95**	**1.53**	**2.44**	**3.91**	**6.25**	**10.0**
Mortality (day 28) [%]	0	0	0	0	2.5	0	0	0	0
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Body weight change (day 28) [%]	44.1	43.9	44.9	43.3	42.7	43.7	41.7	43.3	45.1
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	65	57	61	75	63	81	71	69	50
Statistical Significance [3)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 56)	-	89	94	116	97	125	109	107	77
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg test item/kg soil dry weight]									
NOEC (day 28 mortality)	≥10.0								
LOEC (day 28 mortality)	>10.0								
LC_{50} [4)]	>10.0								
NOEC (day 28 weight)	≥10.0								
LOEC (day 28 weight)	>10.0								
NOEC (day 56 reproduction)	≥10.0								
LOEC (day 56 reproduction)	>10.0								
EC_{10}	n.d.								
EC_{20}	n.d.								
EC_{50} [4)]	>10.0								

The results represent rounded values calculated from the exact raw data.
- = not applicable
n.s. = not significantly different compared to the control
n.d. = not determined: the EC_{10} and EC_{20} values could not be determined statistically due to the lacking concentration response.
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Dunnett´s t-test, $\alpha = 0.05$, two-sided
[3)] Dunnett´s t-test, $\alpha = 0.05$, one-sided smaller
[4)] estimated value

Conclusion

In an earthworm reproduction and growth study with GLOB2007bF the No Observed Effect Concentration (NOEC) for mortality, weight changes and reproduction of the earthworm *Eisenia andrei* was determined to be ≥10.0 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be >10.0 mg test item/kg soil dry weight. The LC_{50} was estimated to be >10.0 mg test item/kg soil dry weight. The EC_{10} and the EC_{20} values could not be determined statistically due to the lacking concentration response. The EC_{50} was estimated to be >10.0 mg test item/kg soil dry weight.

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met according to OECD 222: - each replicate produced 67 to 142 juveniles (mean) at the end of the study (criterion: ≥ 30 juveniles by the end of the study), - the coefficient of variation of reproduction was 20.2 % (criterion: ≤ 30%), - adult mortality over the initial 4 weeks of the experiment was 0 % (criterion: ≤ 10%). In the study 8 different doses of the product were used for the determination of reproduction endpoint. Mortality was found at the concentration of 5.72 , 10.3 (2.5%) and at 33.3 (5%) mg test item/kg soil dry weight. At the test concentration of 10.3 mg test item/kg soil dry weight and above, reproduction was statistically significantly reduced compared to the control. Therefore, the NOEC for reproduction was determined to be 5.72 mg test item/kg soil dry weight.

Reference:	10.4.1.1
Report	GLOB2007bF (Zoxamide 67.5 g/L + propamocarb HCL 450 g/L SC): Effects on Reproduction and Growth of Earthworms Eisenia andrei in Artificial Soil, Straube, D., 2023, Ibacon Gmbh, Report No.: 169562022
Guideline(s):	OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supportive~~

Materials and methods

Test Item:	GLOB2007bF; batch no.: LCM22012601; content of a.i.: Zoxamide: 64.23 ± 0.07 g/L or 5.846 ± 0.007 % w/w (analysed); Propamocarb-HCL: 445.8 ± 1.3 g/L or 40.57 ± 0.12 % w/w (analysed)
Test Species:	Earthworm (*Eisenia andrei*, Bouché, 1972), adult earthworms (with clitellum and weight range 301 mg to 597 mg), approximately 8 to 9 months old, source: from an in-house culture.
Test Design:	56-day test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Reference Item:	Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study.
Test Concentrations:	Control, 0.98, 1.76, 3.18, 5.72, 10.3, 18.5, 33.3 and 60.0 mg GLOB2007bF/kg soil dry weight.
Test Conditions:	Artificial soil according to OECD 222; initial pH 5.9 to 6.2, pH at

	experimental end 6.0 to 6.2; water content 24.6% to 25.6% (49.3% to 51.2% of maximum water holding capacity, WHC) at experimental start and 25.0% to 26.7% (50.0% to 53.5% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Dunnett's t-test (body weight changes), Williams t-test (reproduction).

Results and discussions

Mortality results are presented below.

Table 3. Number of live adult earthworms and mortality after 28 days

Treatment Group	Container #	Number of Live Adults Start	Number of Live Adults 28 Days	Mortality of the Adults (28 days) % per Container	% Mean ± SD[1]	Signifi-cance
Control	I	10	10	0		
	II	10	10	0	0.0	-
	III	10	10	0	± 0.0	
	IV	10	10	0		
	V	10	10	0		
	VI	10	10	0		
	VII	10	10	0		
	VIII	10	10	0		
0.98	I	10	10	0		
	II	10	10	0	0.0	n.s.
	III	10	10	0	± 0.0	
	IV	10	10	0		
1.76	I	10	10	0		
	II	10	10	0	0.0	n.s.
	III	10	10	0	± 0.0	
	IV	10	10	0		
3.18	I	10	10	0		
	II	10	10	0	0.0	n.s.
	III	10	10	0	± 0.0	
	IV	10	10	0		
5.72	I	10	10	0		
	II	10	10	0	2.5	n.s.
	III	10	9	10	± 5.0	
	IV	10	10	0		
10.3	I	10	10	0		
	II	10	10	0	2.5	n.s.
	III	10	9	10	± 5.0	
	IV	10	10	0		
18.5	I	10	10	0		
	II	10	10	0	0.0	n.s.
	III	10	10	0	± 0.0	
	IV	10	10	0		
33.3	I	10	9	10		
	II	10	9	10	5.0	n.s.
	III	10	10	0	± 5.8	
	IV	10	10	0		
60.0	I	10	10	0		
	II	10	10	0	0.0	n.s.
	III	10	10	0	± 0.0	
	IV	10	10	0		

Test item dosages are given as mg test item/kg artificial soil dry weight
- = Not relevant
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
n.s. = Not significantly different compared to the control, Fisher's Exact Test, α = 0.05, one-sided greater

Body weight changes

Table 4. Body weight changes of the adult earthworms

Treatment Group	Container #	Mean Earthworm Body Weight At Start mg	Mean Earthworm Body Weight After 28 Days mg	Body Weight Change Mean per Earthworm mg	Mean per Earthworm %	Mean ± SD [1] mg	Mean ± SD [1] %	Significance
Control	I	393.8	555.6	161.8	41.1	114 ± 36	27.1 ± 9.7	-
	II	412.9	569.4	156.5	37.9			
	III	414.0	552.6	138.6	33.5			
	IV	425.6	487.7	62.1	14.6			
	V	426.6	547.6	121.0	28.4			
	VI	436.0	535.5	99.5	22.8			
	VII	441.6	534.5	92.9	21.0			
	VIII	467.8	548.8	81.0	17.3			
0.98	I	396.7	497.5	100.8	25.4	123 ± 30	28.7 ± 6.5	n.s.
	II	424.9	583.6	158.7	37.3			
	III	427.0	522.4	95.4	22.3			
	IV	466.1	604.2	138.1	29.6			
1.76	I	397.1	566.7	169.6	42.7	131 ± 30	30.9 ± 8.6	n.s.
	II	424.6	552.5	127.9	30.1			
	III	428.7	524.3	95.6	22.3			
	IV	460.8	592.4	131.6	28.6			
3.18	I	399.2	504.8	105.6	26.5	117 ± 33	27.5 ± 8.1	n.s.
	II	424.5	585.8	161.3	38.0			
	III	428.7	545.1	116.4	27.2			
	IV	460.3	544.2	83.9	18.2			
5.72	I	401.9	545.8	143.9	35.8	134 ± 26	31.4 ± 7.2	n.s.
	II	423.0	583.7	160.7	38.0			
	III	433.4	564.1	130.7	30.2			
	IV	454.4	553.2	98.8	21.7			
10.3	I	405.2	527.6	122.4	30.2	114 ± 11	26.7 ± 2.7	n.s.
	II	422.2	524.6	102.4	24.3			
	III	433.7	542.0	108.3	25.0			
	IV	453.9	577.9	124.0	27.3			
18.5	I	406.3	486.7	80.4	19.8	120 ± 40	28.0 ± 9.3	n.s.
	II	418.8	584.7	165.9	39.6			
	III	435.1	527.4	92.3	21.2			
	IV	453.4	595.1	141.7	31.3			
33.3	I	407.9	567.7	159.8	39.2	119 ± 32	28.2 ± 8.5	n.s.
	II	417.4	540.4	123.0	29.5			
	III	435.3	518.5	83.2	19.1			
	IV	446.8	558.4	111.6	25.0			
60.0	I	408.2	567.1	158.9	38.9	136 ± 20	32.0 ± 5.1	n.s.
	II	414.2	526.1	111.9	27.0			
	III	435.4	565.1	129.7	29.8			
	IV	444.7	588.8	144.1	32.4			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Dunnett' t-test, α = 0.05, two-sided

Reproduction

Table 5. Reproduction of the earthworms after 56 days

Treatment Group	Container #	Number of Juvenile Earthworms Per Container	Mean ± SD [1]	% of Control	Significance
Control	I	114			
	II	105	109	-	-
	III	142	± 22		
	IV	95			
	V	109			
	VI	67			
	VII	117			
	VIII	125			
0.98	I	111			
	II	120	95	87.0	n.s.
	III	55	± 29		
	IV	94			
1.76	I	81			
	II	96	99	90.8	n.s.
	III	99	± 17		
	IV	121			
3.18	I	99			
	II	81	89	81.2	n.s.
	III	100	± 13		
	IV	75			
5.72	I	95			
	II	101	100	91.3	n.s.
	III	79	± 19		
	IV	124			
10.3	I	81			
	II	53	64	58.8	*
	III	72	± 15		
	IV	51			
18.5	I	64			
	II	96	71	65.2	*
	III	57	± 17		
	IV	68			
33.3	I	65			
	II	84	89	81.7	*
	III	92	± 21		
	IV	116			
60.0	I	30			
	II	39	47	43.0	*
	III	55	± 15		
	IV	64			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller
* = Significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller

All study validity criteria were met.
A slight mortality of 2.5% was found at the test concentrations of 5.72 mg test item/kg soil dry weight and 10.3 mg test item/kg soil dry weight and which was not statistically significantly different compared to the control, where 0% of the earthworms died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater). At the test concentration of 33.3 mg test item/kg soil dry weight a mortality of 5% was observed, which was not statistically significantly different compared to the control.
The body weight changes of the earthworms after 28 days exposure to GLOB2007bF were not statistically significantly different compared to the control up to and including the highest test concentration of 60.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).
The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 5.72 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 10.3 mg test item/kg soil dry weight and above, reproduction was statistically significantly reduced compared to the control.

No behavioural abnormalities were observed in any of the treatment groups.
The feeding activity in all the treated groups was comparable to the control (see Table 1).

Table 17. Effect of GLOB2007bF on earthworms (*Eisenia andrei*) in a 56-day reproduction study

GLOB2007bF [mg test item/kg soil dry weight]	**Control**	**0.98**	**1.76**	**3.18**	**5.72**	**10.3**	**18.5**	**33.3**	**60.0**
Mortality (day 28) [%]	0	0	0	0	2.5	2.5	0	5.0	0
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Body weight change (day 28) [%]	27.1	28.7	30.9	27.5	31.4	26.7	28.0	28.2	32.0
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	109	95	99	89	100	64	71	89	47
Statistical Significance [3)]	-	n.s.	n.s.	n.s.	n.s.	*	*	*	*
Reproduction in [%] of control (day 56)	-	87	91	81	91	59	65	82	43
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg test item/kg soil dry weight]									
NOEC (day 28 mortality)	≥60.0								
LOEC (day 28 mortality)	>60.0								
LC_{50} [4)]	>60.0								
NOEC (day 28 weight)	≥60.0								
LOEC (day 28 weight)	>60.0								
NOEC (day 56 reproduction)	5.72								
LOEC (day 56 reproduction)	10.3								
EC_{10}	n.d.								
EC_{20}	n.d.								
EC_{50}	n.d.								

The results represent rounded values calculated from the exact raw data.
- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
n.d. = the EC_{10}, EC_{20}, and EC_{50} could not be statistically determined or estimated as no statistically significant concentration/response was found
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Dunnett´s t-test, $\alpha = 0.05$, two-sided
[3)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[4)] estimated value

Conclusion

In an earthworm reproduction and growth study with GLOB2007bF the No Observed Effect Concentration (NOEC) for mortality and weight changes of the earthworm *Eisenia andrei* was determined to be ≥60.0 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be >60.0 mg test item/kg soil dry weight. The LC_{50} was estimated to be >60.0 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 5.72 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 10.3 mg test item/kg soil dry weight. The EC_{10}, EC_{20}, and EC_{50} could not be statistically determined or estimated as no statistically significant concentration/response was found.

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met according to OECD 222: Each replicate produced 105 to 175 juveniles (mean) at the end of the study (criterion: ≥ 30 juveniles by the end of the study), - the coefficient of variation of reproduction was 16.8 % (criterion: ≤ 30%), - adult mortality over the initial 4 weeks of the experiment was 0 % (criterion: ≤ 10%). The study is accepted and reliable for risk assessment purposes.

Reference:	10.4.1.1
Report	GLOB2007bF (Zoxamide 67.5 g/L + propamocarb HCL 450 g/L SC): Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Natural Soil, Straube, D., 2023, Ibacon Gmbh, Report No.: 169563022
Guideline(s):	OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF; batch no.: LCM22012601; content of a.i.: Zoxamide: 64.23 ± 0.07 g/L or 5.846 ± 0.007 % w/w (analysed), Propamocarb-HCL: 445.8 ± 1.3 g/L or 40.57 ± 0.12 % w/w (analysed)
Test Species:	Earthworm (*Eisenia andrei*, Bouché, 1972), adult earthworms (with clitellum and weight range 301 to 579 mg), approximately 6 months old, source: from an in-house culture.
Test Design:	56-day test in treated natural soil LUFA 2.2; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated natural soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Reference Item:	Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study.
Test Concentrations:	Control, 4.57, 8.23, 14.8, 26.7, 48.0, 86.4, 155.5 and 279.9 mg GLOB2007bF/kg soil dry weight.
Test Conditions:	Natural soil LUFA 2.2; initial pH 6.2 to 6.4, pH at experimental end 6.3 to 6.7; water content 26.6% to 27.1% (51.2% to 52.1% of maximum water holding capacity, WHC) at experimental start and 26.6% to 29.5% (51.2% to 56.8% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Dunnett's t-test (body weight changes), Williams t-test (reproduction), 3-param. normal CDF (EC

values).

Results and discussions

Mortality results are presented below.

Table 3. Number of live adult earthworms and mortality after 28 days

Treatment Group	Container #	Number of Live Adults Start	Number of Live Adults 28 Days	Mortality of the Adults (28 days) % per Container	% Mean ± SD[1]	Signifi-cance
Control	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
	V	10	10	0		
	VI	10	10	0		
	VII	10	10	0		
	VIII	10	10	0		
4.57	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
8.23	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
14.8	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
26.7	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
48.0	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
86.4	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
155.5	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
279.9	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		

Test item dosages are given as mg test item/kg natural soil dry weight
- = Not relevant
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
n.s. = Not significantly different compared to the control, Fisher's Exact Test, α = 0.05, one-sided greater

Changes in body weights are presented below.

Table 4. Body weight changes of the adult earthworms

Treatment Group	Container #	Mean Earthworm Body Weight At Start mg	Mean Earthworm Body Weight After 28 Days mg	Body Weight Change Mean per Earthworm mg	Mean per Earthworm %	Mean ± SD [1] mg	Mean ± SD [1] %	Significance
Control	I	362.8	483.7	120.9	33.3	104 ± 22	26.5 ± 6.4	-
	II	376.5	485.0	108.5	28.8			
	III	377.7	471.1	93.4	24.7			
	IV	390.4	474.3	83.9	21.5			
	V	393.6	532.6	139.0	35.3			
	VI	408.3	532.1	123.8	30.3			
	VII	408.6	485.8	77.2	18.9			
	VIII	458.9	546.2	87.3	19.0			
4.57	I	365.5	512.5	147.0	40.2	104 ± 30	26.9 ± 9.4	n.s.
	II	388.7	481.9	93.2	24.0			
	III	393.9	493.8	99.9	25.4			
	IV	424.5	501.7	77.2	18.2			
8.23	I	366.8	504.9	138.1	37.6	123 ± 36	31.7 ± 10.2	n.s.
	II	387.7	532.7	145.0	37.4			
	III	395.0	534.8	139.8	35.4			
	IV	424.2	494.1	69.9	16.5			
14.8	I	367.7	468.0	100.3	27.3	107 ± 6	27.1 ± 0.5	n.s.
	II	386.9	489.9	103.0	26.6			
	III	399.6	510.6	111.0	27.8			
	IV	421.3	534.5	113.2	26.9			
26.7	I	368.1	510.8	142.7	38.8	112 ± 22	28.7 ± 6.9	n.s.
	II	386.3	479.1	92.8	24.0			
	III	400.3	510.4	110.1	27.5			
	IV	419.9	522.8	102.9	24.5			
48.0	I	370.2	518.0	147.8	39.9	121 ± 24	30.9 ± 6.8	n.s.
	II	382.6	483.0	100.4	26.2			
	III	404.8	507.0	102.2	25.2			
	IV	415.4	549.9	134.5	32.4			
86.4	I	371.5	518.2	146.7	39.5	119 ± 31	30.5 ± 9.3	n.s.
	II	381.4	523.1	141.7	37.2			
	III	405.5	486.8	81.3	20.0			
	IV	414.7	519.4	104.7	25.2			
155.5	I	372.2	522.1	149.9	40.3	115 ± 26	29.6 ± 8.1	n.s.
	II	381.4	501.1	119.7	31.4			
	III	406.1	508.1	102.0	25.1			
	IV	410.8	500.2	89.4	21.8			
279.9	I	374.4	524.2	149.8	40.0	143 ± 7	36.4 ± 2.9	n.s.
	II	380.3	517.6	137.3	36.1			
	III	407.1	555.3	148.2	36.4			
	IV	409.7	545.0	135.3	33.0			

Test item dosages are given as mg test item/kg natural soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Dunnett's t-test, α = 0.05, two-sided

The numbers of juvenile earthworms found 56 days after application are shown below.

Table 5. Reproduction of the earthworms after 56 days

Treatment Group	Container #	Number of Juvenile Earthworms Per Container	Mean ± SD [1]	% of Control	% Reduction Compared to Control	Significance
Control	I	105				
	II	164	136	-	-	-
	III	131	± 23			
	IV	130				
	V	123				
	VI	140				
	VII	123				
	VIII	175				
4.57	I	150				
	II	172	151	110.4	-10.4	n.s.
	III	129	± 18			
	IV	151				
8.23	I	152				
	II	110	131	95.9	4.1	n.s.
	III	118	± 20			
	IV	143				
14.8	I	147				
	II	153	145	106.0	-6.0	n.s.
	III	126	± 13			
	IV	152				
26.7	I	109				
	II	140	116	84.9	15.1	*
	III	90	± 21			
	IV	124				
48.0	I	110				
	II	113	113	82.5	17.5	*
	III	111	± 3			
	IV	116				
86.4	I	89				
	II	101	76	55.9	44.1	*
	III	54	± 22			
	IV	61				
155.5	I	83				
	II	58	76	55.9	44.1	*
	III	92	± 15			
	IV	72				
279.9	I	37				
	II	47	41	30.2	69.8	*
	III	61	± 17			
	IV	20				

Test item dosages are given as mg test item/kg natural soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller
* = Significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller

All study validity criteria were met.
No mortality was observed in any treatment group.
The body weight changes of the earthworms after 28 days exposure to GLOB2007bF were not statistically significantly different compared to the control up to and including the highest test concentration of 279.9 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).
The reproduction rates were not statistically significantly different compared to the control up to and the test concentration of 14.8 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 26.7 and above, the reproduction was statistically significantly reduced compared to the control. No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control (see Table 1).

Table 18. Effect of GLOB2007bF on earthworms (*Eisenia andrei*) in a 56-day reproduction study

GLOB2007bF [mg test item/kg soil dry weight]	**Control**	**4.57**	**8.23**	**14.8**	**26.7**	**48.0**	**86.4**	**155.5**	**279.9**
Mortality (day 28) [%]	0	0	0	0	0	0	0	0	0
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Body weight change (day 28) [%]	26.5	26.9	31.7	27.1	28.7	30.9	30.5	29.6	36.4
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	136	151	131	145	116	113	76	76	41
Statistical Significance [3)]	-	n.s.	n.s.	n.s.	*	*	*	*	*
Reproduction in [%] of control (day 56)	-	110	96	106	85	82	56	56	30
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg test item/kg soil dry weight]									
NOEC (day 28 mortality)	≥279.9								
LOEC (day 28 mortality)	>279.9								
LC_{50} [4)]	>279.9								
NOEC (day 28 weight)	≥279.9								
LOEC (day 28 weight)	>279.9								
NOEC (day 56 reproduction)	14.8								
LOEC (day 56 reproduction)	26.7								
EC Values (reproduction) [5)]	EC_{10}			EC_{20}			EC_{50}		
	22.7			42.4			140.0		
95% confidence limits	18.1 – 27.5			36.0 – 49.4			124.3 – 161.2		

The results represent rounded values calculated from the exact raw data.
- = not applicable
n.s. = not significantly different compared to the control
* = significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Dunnett´s t-test, $\alpha = 0.05$, two-sided
[3)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[4)] estimated value
[5)] 3-param. normal CDF

Conclusion

In an earthworm reproduction and growth study with GLOB2007bF the No Observed Effect Concentration (NOEC) for mortality and weight changes of the earthworm *Eisenia andrei* was determined to be ≥279.9 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be >279.9 mg test item/kg soil dry weight. The LC_{50} was estimated to be >279.9 mg test item/kg soil dry weight.
The NOEC for reproduction was determined to be 14.8 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 26.7 mg test item/kg soil dry weight.
The EC_{10} was determined to be 22.7 mg test item /kg soil dry weight, the EC_{20} was determined to be 42.4 mg test item/kg soil dry weight, and the EC_{50} was determined to be 140.0 mg test item/kg soil dry weight.

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met according to OECD 222: Each replicate produced 79 to 138 juveniles (mean) at the end of the study (criterion: ≥ 30 juveniles by the end of the study), - the coefficient of variation of reproduction was 17.6 % (criterion: ≤ 30%), - adult mortality over the initial 4 weeks of the experiment was 0 % (criterion: ≤ 10%). Study is accepted.

Reference:	KCA 8.4.1
Report	RH-24549: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Straube, D., 2023, Ibacon GmbH, Report No.: 166191022
Guideline(s):	OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	RH-24549; batch no.: C47360/1; purity: 97.9% by titration
Test Species:	Earthworm (*Eisenia andrei*, Bouché, 1972), adult earthworms (with clitellum and weight range 302 to 598 mg), approximately 8 to 9 months old, source: from an in-house culture.
Test Design:	56-day test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Reference Item:	Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study.
Test Concentrations:	Control, 0.408, 0.735, 1.32, 2.38, 4.29, 7.72, 13.9 and 25.0 mg RH-24549/kg soil dry weight.
Test Conditions:	Artificial soil according to OECD 222; initial pH 5.9 to 6.1, pH at experimental end 6.1 to 6.3; water content 25.3% to 26.5% (50.6% to 53.0% of maximum water holding capacity, WHC) at experimental start and 24.4% to 26.3% (48.9% to 52.5% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Statistics:	Standard procedures, Dunnett's t-test (body weight changes), Williams t-test (reproduction).

Results and discussions

The numbers of surviving earthworms are shown below.

Table 3. Number of live adult earthworms and mortality after 28 days

Treatment Group	Container #	Number of Live Adults Start	Number of Live Adults 28 Days	Mortality of the Adults (28 days) % per Container	% Mean ± SD[1]	Signifi-cance
Control	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
	V	10	10	0		
	VI	10	10	0		
	VII	10	10	0		
	VIII	10	10	0		
0.408	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
0.735	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
1.32	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
2.38	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
4.29	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
7.72	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
13.9	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
25.0	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		

Test item dosages are given as mg test item/kg artificial soil dry weight
- = Not relevant
[1] = Mean ± standard deviation of 4 replicates (8 in the control)

No mortality was observed in any treatment group.
The body weight changes of the adult earthworms after 28 days exposure to RH-24549 are shown below.

Table 4. Body weight changes of the adult earthworms

Treatment Group	Container #	Mean Earthworm Body Weight		Body Weight Change				
		At Start	After 28 Days	Mean per Earthworm		Mean ± SD [1]		Signifi-cance
		mg	mg	mg	%	mg	%	
Control	I	355.2	569.6	214.4	60.4			
	II	381.7	620.5	238.8	62.6	212	54.0	-
	III	382.4	619.5	237.1	62.0	± 20	± 7.0	
	IV	392.5	589.8	197.3	50.3			
	V	393.8	611.5	217.7	55.3			
	VI	407.8	599.6	191.8	47.0			
	VII	408.0	591.2	183.2	44.9			
	VIII	427.0	639.2	212.2	49.7			
0.408	I	364.8	641.6	276.8	75.9			
	II	392.4	659.1	266.7	68.0	240	61.5	n.s.
	III	394.3	619.9	225.6	57.2	± 39	± 13.4	
	IV	424.8	615.9	191.1	45.0			
0.735	I	373.6	618.7	245.1	65.6			
	II	390.0	613.7	223.7	57.4	230	58.3	n.s.
	III	395.2	608.1	212.9	53.9	± 14	± 5.1	
	IV	423.3	661.5	238.2	56.3			
1.32	I	376.7	581.7	205.0	54.4			
	II	389.1	616.0	226.9	58.3	213	54.1	n.s.
	III	396.6	609.8	213.2	53.8	± 10	± 3.5	
	IV	417.4	625.5	208.1	49.9			
2.38	I	378.6	631.4	252.8	66.8			
	II	388.9	544.7	155.8	40.1	208	52.8	n.s.
	III	398.0	604.2	206.2	51.8	± 40	± 10.9	
	IV	413.8	631.0	217.2	52.5			
4.29	I	379.7	545.1	165.4	43.6			
	II	386.5	612.1	225.6	58.4	184	46.7	n.s.
	III	398.4	601.3	202.9	50.9	± 38	± 10.5	
	IV	413.2	553.3	140.1	33.9			
7.72	I	379.8	605.4	225.6	59.4			
	II	386.3	685.0	298.7	77.3	245	62.3	n.s.
	III	399.7	613.4	213.7	53.5	± 38	± 10.4	
	IV	412.1	655.6	243.5	59.1			
13.9	I	380.5	561.9	181.4	47.7			
	II	384.9	600.9	216.0	56.1	190	48.2	n.s.
	III	402.5	614.5	212.0	52.7	± 31	± 8.6	
	IV	411.6	561.9	150.3	36.5			
25.0	I	381.6	598.0	216.4	56.7			
	II	384.0	570.7	186.7	48.6	205	52.0	n.s.
	III	407.3	633.6	226.3	55.6	± 19	± 4.9	
	IV	409.0	601.1	192.1	47.0			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Dunnett's t-test, α = 0.05, two-sided

The body weight changes of the earthworms after 28 days exposure to RH-24549 were not statistically significantly different compared to the control up to and including the highest test concentration of 25.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).

The numbers of juvenile earthworms found 56 days after application are shown below.

Table 5. Reproduction of the earthworms after 56 days

Treatment Group	Container #	Number of Juvenile Earthworms Per Container	Mean ± SD [1]	% of Control	Significance
Control	I	98	102 ± 18	-	-
	II	107			
	III	138			
	IV	79			
	V	91			
	VI	93			
	VII	113			
	VIII	95			
0.408	I	93	110 ± 18	108.4	n.s.
	II	123			
	III	96			
	IV	129			
0.735	I	85	94 ± 16	92.6	n.s.
	II	78			
	III	101			
	IV	113			
1.32	I	78	82 ± 14	80.3	n.s.
	II	74			
	III	103			
	IV	72			
2.38	I	71	91 ± 14	89.4	n.s.
	II	99			
	III	100			
	IV	94			
4.29	I	86	93 ± 15	91.2	n.s.
	II	102			
	III	75			
	IV	108			
7.72	I	110	96 ± 11	94.6	n.s.
	II	98			
	III	83			
	IV	94			
13.9	I	71	92 ± 14	89.9	n.s.
	II	96			
	III	105			
	IV	94			
25.0	I	98	75 ± 15	74.0	*
	II	68			
	III	68			
	IV	67			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller
* = Significantly different compared to the control, Williams t-test, α = 0.05, one-sided smaller

The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 13.9 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one sided smaller). At the test concentration of 25.0 mg test item/kg soil dry weight reproduction was statistically significantly reduced compared to the control.

All study validity criteria were met.
No mortality was observed in any treatment group.
The body weight changes of the earthworms after 28 days exposure to RH-24549 were not statistically significantly different compared to the control up to and including the highest test concentration of 25.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).

The reproduction rates were not statistically significantly different compared to the control up to and including the test concentration of 13.9 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 25.0 mg test item/kg soil dry weight reproduction was statistically significantly reduced compared to the control.
No behavioural abnormalities were observed in any of the treatment groups.
The feeding activity in all the treated groups was comparable to the control ~~(see Table 1)~~

Table 19. Effect of RH-24549 on earthworms (*Eisenia andrei*) in a 56-day reproduction study

RH-24549 [mg test item/kg soil dry weight]	**Control**	**0.408**	**0.735**	**1.32**	**2.38**	**4.29**	**7.72**	**13.9**	**25.0**
Mortality (day 28) [%]	0	0	0	0	0	0	0	0	0
Statistical Significance	-	-	-	-	-	-	-	-	-
Body weight change (day 28) [%]	54.0	61.5	58.3	54.1	52.8	46.7	62.3	48.2	52.0
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	102	110	94	82	91	93	96	92	75
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Reproduction in [%] of control (day 56)	-	108	93	80	89	91	95	90	74
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg test item/kg soil dry weight]									
NOEC (day 28 mortality)	≥25.0								
LOEC (day 28 mortality)	>25.0								
LC_{50} [3)]	>25.0								
NOEC (day 28 weight)	≥25.0								
LOEC (day 28 weight)	>25.0								
NOEC (day 56 reproduction)	13.9								
LOEC (day 56 reproduction)	25.0								
EC_{10}	n.d.								
EC_{20}	n.d.								
EC_{50} [3)]	>25.0								

The results represent rounded values calculated from the exact raw data.
- = not applicable
n.s. = not significantly different compared to the control
n.d. = The EC_{10} and EC_{20} could not be statistically determined or estimated as no statistically significant concentration/response was found
* = significantly different compared to the control
[1)] Dunnett´s t-test, $\alpha = 0.05$, two-sided
[2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value

Conclusion

In an earthworm reproduction and growth study with RH-24549 the No Observed Effect Concentration (NOEC) for mortality and weight changes of the earthworm *Eisenia andrei* was determined to be ≥25.0 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) for mortality and weight changes was estimated to be >25.0 mg test item/kg soil dry weight. The LC_{50} was estimated to be >25.0 mg test item/kg soil dry weight.
The NOEC for reproduction was determined to be 13.9 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 25.0 mg test item/kg soil dry weight. The EC_{10} and EC_{20} could not be statistically determined or estimated as no statistically significant concentration/response was found. The EC_{50} was estimated to be >25.0 mg test item/kg soil dry weight.

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met according to OECD 222: Each replicate produced 138 to 184 juveniles (mean) at the end of the study (criterion: ≥ 30 juveniles by the end of the study), - the coefficient of variation of reproduction was 10.3 % (criterion: ≤ 30%), - adult mortality over the initial 4 weeks of the experiment was 0 % (criterion: ≤ 10%). Study is accepted.

Reference:	KCA 8.4.1
Report	RH-127450: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Straube, D., 2023, Ibacon GmbH, Report No.: 175161022
Guideline(s):	OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	RH-127450; batch no.: GD-003486-01; purity: 100%
Test Species:	Earthworm (*Eisenia andrei*, Bouché, 1972), adult earthworms (with clitellum and weight range 329 to 600 mg), approximately 10 months old, source: from an in-house culture.
Test Design:	56-day test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Reference Item:	Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study.
Test Concentrations:	Control, 0.408, 0.735, 1.32, 2.38, 4.29, 7.72, 13.9 and 25.0 mg RH-127450/kg soil dry weight.
Test Conditions:	Artificial soil according to OECD 222; initial pH 6.0 to 6.2, pH at experimental end 5.9 to 6.1; water content 24.8% to 26.2% (49.6% to 52.4% of maximum water holding capacity, WHC) at experimental start and 25.5% to 27.1% (51.0% to 54.3% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Williams t-test (body weight changes), Dunnett's t-test (reproduction).

Results and discussions

The numbers of surviving earthworms are shown in table below.

Table 3. Number of live adult earthworms and mortality after 28 days

Treatment Group	Container #	Number of Live Adults Start	Number of Live Adults 28 Days	Mortality of the Adults (28 days) % per Container	% Mean ± SD[1]	Signifi-cance
Control	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
	V	10	10	0		
	VI	10	10	0		
	VII	10	10	0		
	VIII	10	10	0		
0.408	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
0.735	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
1.32	I	10	9	10	2.5 ± 5.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
2.38	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
4.29	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
7.72	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
13.9	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
25.0	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		

Test item dosages are given as mg test item/kg artificial soil dry weight
- = Not relevant
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
n.s. = Not significantly different compared to the control, Fisher's Exact Test, $\alpha = 0.05$, one-sided greater

A slight mortality of 2.5% was found at the test concentration of 1.32 mg test item/kg soil dry weight, which was not statistically significantly different compared to the control, where 0% of the earthworms died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

The body weight changes of the adult earthworms after 28 days exposure to RH-127450 are shown below.

Table 4. Body weight changes of the adult earthworms

Treatment Group	Container #	Mean Earthworm Body Weight At Start mg	Mean Earthworm Body Weight After 28 Days mg	Body Weight Change Mean per Earthworm mg	Body Weight Change Mean per Earthworm %	Body Weight Change Mean ± SD [1] mg	Body Weight Change Mean ± SD [1] %	Significance
Control	I	460.1	560.2	100.1	21.8	99 ± 29	19.6 ± 5.8	-
	II	487.9	608.4	120.5	24.7			
	III	489.0	593.3	104.3	21.3			
	IV	509.3	541.2	31.9	6.3			
	V	510.0	607.1	97.1	19.0			
	VI	517.6	622.4	104.8	20.2			
	VII	518.4	645.3	126.9	24.5			
	VIII	556.8	662.2	105.4	18.9			
0.408	I	466.4	579.4	113.0	24.2	112 ± 23	22.2 ± 5.0	n.s.
	II	507.9	645.0	137.1	27.0			
	III	511.2	625.4	114.2	22.3			
	IV	538.1	620.1	82.0	15.2			
0.735	I	470.0	585.8	115.8	24.6	110 ± 48	22.0 ± 9.7	n.s.
	II	507.6	660.5	152.9	30.1			
	III	513.1	643.3	130.2	25.4			
	IV	532.4	575.1	42.7	8.0			
1.32	I	476.9	571.2	94.3	19.8	81 ± 10	16.1 ± 2.7	n.s.
	II	505.4	587.1	81.7	16.2			
	III	513.4	590.2	76.8	15.0			
	IV	531.8	603.1	71.3	13.4			
2.38	I	481.8	606.2	124.4	25.8	134 ± 24	26.5 ± 4.5	n.s.
	II	504.1	622.7	118.6	23.5			
	III	513.5	683.0	169.5	33.0			
	IV	528.5	653.4	124.9	23.6			
4.29	I	483.8	635.3	151.5	31.3	129 ± 23	25.5 ± 5.2	n.s.
	II	501.7	616.3	114.6	22.8			
	III	514.4	659.8	145.4	28.3			
	IV	525.1	628.9	103.8	19.8			
7.72	I	485.1	625.6	140.5	29.0	136 ± 25	27.0 ± 5.6	n.s.
	II	500.3	669.7	169.4	33.9			
	III	516.0	640.4	124.4	24.1			
	IV	524.8	635.2	110.4	21.0			
13.9	I	485.1	692.1	207.0	42.7	151 ± 61	30.2 ± 12.9	n.s.
	II	500.2	698.7	198.5	39.7			
	III	516.6	629.9	113.3	21.9			
	IV	522.4	608.3	85.9	16.4			
25.0	I	486.6	670.5	183.9	37.8	133 ± 46	26.4 ± 9.6	n.s.
	II	496.6	616.7	120.1	24.2			
	III	516.8	667.1	150.3	29.1			
	IV	520.9	597.6	76.7	14.7			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Williams t-test, $\alpha = 0.05$, one-sided smaller

The body weight changes of the earthworms after 28 days exposure to RH-127450 were not statistically significantly different compared to the control up to and including the highest test concentration of 25.0 mg test item/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller).

The numbers of juvenile earthworms found 56 days after application are shown below.

Table 5. Reproduction of the earthworms after 56 days

Treatment Group	Container #	Number of Juvenile Earthworms Per Container	Mean ± SD [1]	% ofControl	Significance
Control	I	176	156 ± 16	-	-
	II	143			
	III	184			
	IV	148			
	V	150			
	VI	157			
	VII	152			
	VIII	138			
0.408	I	123	117 ± 12	74.8	*[a]
	II	103			
	III	112			
	IV	129			
0.735	I	179	156 ± 21	100.0	n.s.
	II	128			
	III	156			
	IV	161			
1.32	I	179	176 ± 8	113.0	n.s.
	II	169			
	III	170			
	IV	187			
2.38	I	106	134 ± 22	85.9	n.s.
	II	161			
	III	135			
	IV	134			
4.29	I	119	133 ± 24	85.4	n.s.
	II	137			
	III	111			
	IV	166			
7.72	I	150	124 ± 30	79.3	n.s.
	II	103			
	III	93			
	IV	149			
13.9	I	120	124 ± 16	79.3	n.s.
	II	122			
	III	107			
	IV	146			
25.0	I	79	115 ± 29	73.4	*
	II	115			
	III	149			
	IV	115			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
a = statistically significantly reduced compared to the control, but determined to be incidental and not biological relevant as at the higher test concentrations no statistically significant effect on reproduction was observed
n.s. = Not significantly different compared to the control, Dunnett's t-test, α = 0.05, one-sided smaller
* = Significantly different compared to the control, Dunnett's t-test, α = 0.05, one-sided smaller

The reproduction rates were not statistically significantly different compared to the control up to and including the highest test concentration of 25.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller).
At the test concentration of 0.408 mg test item/kg soil dry weight an incidental reduction of reproduction of 25.2% was observed, which was statistically significantly reduced compared to the control, but determined to be not biological relevant as at the higher test concentrations (up to and including 13.9 mg test item/kg soil dry weight) no reduction of reproduction was observed.
All study validity criteria were met.
No behavioural abnormalities were observed in any of the treatment groups.
The feeding activity in all the treated groups was comparable to the control (see Table 1).

Table 20. Effect of RH-127450 on earthworms (*Eisenia andrei*) in a 56-day reproduction study

RH-127450 [mg test item/kg soil dry weight]	**Control**	**0.408**	**0.735**	**1.32**	**2.38**	**4.29**	**7.72**	**13.9**	**25.0**
Mortality (day 28) [%]	0	0	0	2.5	0	0	0	0	0
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Body weight change (day 28) [%]	19.6	22.2	22.0	16.1	26.5	25.5	27.0	30.2	26.4
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	156	117	156	176	134	133	124	124	115
Statistical Significance [3)]	-	*[a]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Reproduction in [%] of control (day 56)	-	74.8	100	113	85.9	85.4	79.3	79.3	73.4
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg test item/kg soil dry weight]									
NOEC (day 28 mortality)	≥25.0								
LOEC (day 28 mortality)	>25.0								
LC_{50} [4)]	>25.0								
NOEC (day 28 weight)	≥25.0								
LOEC (day 28 weight)	>25.0								
NOEC (day 56 reproduction)	13.9								
LOEC (day 56 reproduction)	25.0								
EC_{10}	n.d.								
EC_{20}	n.d.								
EC_{50} [4)]	>25.0								

The results represent rounded values calculated from the exact raw data.
- = not applicable
[a] = statistically significantly reduced compared to the control, but determined to be incidental and not biological relevant as at the higher test concentrations no statistically significant effect on reproduction was observed
n.s. = not significantly different compared to the control
n.d. = The EC_{10} and EC_{20} could not be statistically determined or estimated as no statistically significant concentration/response was found
* = significantly different compared to the control
1) Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
2) Williams t-test, $\alpha = 0.05$, one-sided smaller
3) Dunnett´s t-test, $\alpha = 0.05$, one-sided smaller
4) estimated value

Conclusion

In an earthworm reproduction and growth study with RH-127450 the No Observed Effect Concentration (NOEC) for mortality and weight changes of the earthworm *Eisenia andrei* was determined to be ≥25.0 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be >25.0 mg test item/kg soil dry weight. The LC_{50} was estimated to be >25.0 mg test item/kg soil dry weight.
The NOEC for reproduction was determined to be 13.9 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 25.0 mg test item/kg soil dry weight.
The EC_{10} and EC_{20} could not be statistically determined or estimated as no statistically significant concentration/response was found. The EC_{50} was estimated to be >25.0 mg test item/kg soil dry weight.

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. No deviation were noted during the study. In the definitive test all the validity criteria were met: Each replicate produced 92 to 148 juveniles (mean) at the end of the study (criterion: ≥ 30 juveniles by the end of the study), - the coefficient of variation of reproduction was 20.1 % (criterion: ≤ 30%), - adult mortality over the initial 4 weeks of the experiment was 0 % (criterion: ≤ 10%). Study is accepted.

Reference:	KCA 8.4.1
Report	RH-163353: Effects on Reproduction and Growth of Earthworms *Eisenia andrei* in Artificial Soil, Straube, D., 2023, Ibacon GmbH, Report No.: 175171022
Guideline(s):	OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

Test Item:	RH-163353; batch no.: GD-003454-02; Purity: 100%
Test Species:	Earthworm (*Eisenia andrei*, Bouché, 1972), adult earthworms (with clitellum and weight range 328 to 600 mg), approximately 9 months old, source: from an in-house culture.
Test Design:	56-day test in treated artificial soil prepared according to OECD; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, control); 4 replicates for the test item treatments and 8 replicates for the control with 10 earthworms each. Assessment of adult earthworm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, feeding activity and reproduction rate were determined.
Reference Item:	Carbendazim (600 g/L nominal). The effects of the reference item were investigated in a separate GLP study.
Test Concentrations:	Control, 0.408, 0.735, 1.32, 2.38, 4.29, 7.72, 13.9 and 25.0 mg RH-163353/kg soil dry weight.
Test Conditions:	Artificial soil according to OECD 222; initial pH 5.9 to 6.2, pH at experimental end 6.2 to 6.3; water content 26.2% to 27.2% (53.5% to 55.4% of maximum water holding capacity, WHC) at experimental start and 25.3% to 27.0% (51.7% to 55.2% of the maximum WHC) at experimental end; temperature: within the range of 18 °C to 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 400 lux to 800 lux.
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Dunnett's t-test (body weight changes and reproduction).

Results and discussions

The numbers of surviving earthworms are shown in table below.

Table 3. Number of live adult earthworms and mortality after 28 days

Treatment Group	Container #	Number of Live Adults Start	Number of Live Adults 28 Days	Mortality of the Adults (28 days) % per Container	% Mean ± SD[1]	Signifi-cance
Control	I	10	10	0	0.0 ± 0.0	-
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
	V	10	10	0		
	VI	10	10	0		
	VII	10	10	0		
	VIII	10	10	0		
0.408	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
0.735	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
1.32	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
2.38	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
4.29	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
7.72	I	10	10	0	2.5 ± 5.0	n.s.
	II	10	9	10		
	III	10	10	0		
	IV	10	10	0		
13.9	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		
25.0	I	10	10	0	0.0 ± 0.0	n.s.
	II	10	10	0		
	III	10	10	0		
	IV	10	10	0		

Test item dosages are given as mg test item/kg artificial soil dry weight
- = Not relevant
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
n.s. = Not significantly different compared to the control, Fisher's Exact Test, α = 0.05, one-sided greater

A slight mortality of 2.5 ~~3~~% was found at the concentration of 7.72 mg test item/kg soil dry weight, respectively, which was not statistically significantly different compared to the control, where 0% of the earthworms died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).

The body weight changes (table below) of the earthworms after 28 days exposure to RH-163353 were not statistically significantly different compared to the control up to and including the highest test concentration of 25.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, two-sided).

Table 4. Body weight changes of the adult earthworms

Treatment Group	Container #	Mean Earthworm Body Weight At Start mg	Mean Earthworm Body Weight After 28 Days mg	Body Weight Change Mean per Earthworm mg	Mean per Earthworm %	Mean ± SD [1] mg	Mean ± SD [1] %	Significance
Control	I	470.2	560.4	90.2	19.2	107 ± 23	21.2 ± 4.6	-
	II	491.2	622.6	131.4	26.8			
	III	494.6	586.4	91.8	18.6			
	IV	505.5	654.1	148.6	29.4			
	V	505.8	610.5	104.7	20.7			
	VI	514.7	603.5	88.8	17.3			
	VII	515.8	628.0	112.2	21.8			
	VIII	531.8	618.6	86.8	16.3			
0.408	I	475.8	582.2	106.4	22.4	105 ± 8	20.8 ± 2.2	n.s.
	II	504.9	616.8	111.9	22.2			
	III	506.3	612.9	106.6	21.1			
	IV	527.3	620.7	93.4	17.7			
0.735	I	485.3	641.3	156.0	32.1	132 ± 34	26.1 ± 7.2	n.s.
	II	503.0	601.2	98.2	19.5			
	III	506.7	671.7	165.0	32.6			
	IV	527.0	633.8	106.8	20.3			
1.32	I	485.8	595.8	110.0	22.6	107 ± 31	21.3 ± 6.4	n.s.
	II	502.3	637.6	135.3	26.9			
	III	506.8	626.3	119.5	23.6			
	IV	525.9	589.4	63.5	12.1			
2.38	I	486.3	630.9	144.6	29.7	144 ± 18	28.5 ± 3.2	n.s.
	II	501.5	620.8	119.3	23.8			
	III	507.2	659.3	152.1	30.0			
	IV	525.9	686.3	160.4	30.5			
4.29	I	487.9	672.4	184.5	37.8	148 ± 36	29.4 ± 7.7	n.s.
	II	499.3	664.4	165.1	33.1			
	III	508.8	611.0	102.2	20.1			
	IV	525.4	664.3	138.9	26.4			
7.72	I	489.6	586.2	96.6	19.7	116 ± 30	22.9 ± 5.7	n.s.
	II	497.6	594.7	97.1	19.5			
	III	509.5	669.7	160.2	31.4			
	IV	522.9	632.9	110.0	21.0			
13.9	I	490.1	596.5	106.4	21.7	131 ± 20	26.0 ± 3.8	n.s.
	II	497.3	642.3	145.0	29.2			
	III	509.6	658.8	149.2	29.3			
	IV	521.4	645.6	124.2	23.8			
25.0	I	490.7	611.2	120.5	24.6	134 ± 21	26.6 ± 4.5	n.s.
	II	495.0	660.2	165.2	33.4			
	III	511.4	639.4	128.0	25.0			
	IV	519.6	642.1	122.5	23.6			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Dunnett's t-test, $\alpha = 0.05$, two-sided

The reproduction rates (table below) were not statistically significantly different compared to the control up to and including the highest test concentration of 25.0 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller).

Table 5. Reproduction of the earthworms after 56 days

Treatment Group	Container #	Number of Juvenile Earthworms Per Container	Mean ± SD [1]	% of Control	Significance
Control	I	115	114 ± 23	-	-
	II	98			
	III	93			
	IV	134			
	V	148			
	VI	92			
	VII	94			
	VIII	137			
0.408	I	99	111 ± 32	97.7	n.s.
	II	156			
	III	81			
	IV	109			
0.735	I	140	138 ± 5	121.0	n.s.
	II	142			
	III	139			
	IV	130			
1.32	I	101	120 ± 31	105.6	n.s.
	II	108			
	III	105			
	IV	167			
2.38	I	134	129 ± 23	112.8	n.s.
	II	156			
	III	123			
	IV	101			
4.29	I	91	102 ± 18	89.4	n.s.
	II	83			
	III	122			
	IV	111			
7.72	I	115	92 ± 20	81.0	n.s.
	II	100			
	III	84			
	IV	70			
13.9	I	111	107 ± 26	93.5	n.s.
	II	136			
	III	106			
	IV	73			
25.0	I	110	116 ± 7	101.9	n.s.
	II	121			
	III	123			
	IV	110			

Test item dosages are given as mg test item/kg artificial soil dry weight
The results represent rounded values calculated on the exact raw data
[1] = Mean ± standard deviation of 4 replicates (8 in the control)
- = Not relevant
n.s. = Not significantly different compared to the control, Dunnett's t-test, α = 0.05, one-sided smaller

No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control ~~(see Table 1).~~

Table 21. Effect of RH-163353 on earthworms (*Eisenia andrei*) in a 56-day reproduction study

RH-163353 [mg test item/kg soil dry weight]	**Control**	**0.408**	**0.735**	**1.32**	**2.38**	**4.29**	**7.72**	**13.9**	**25.0**
Mortality (day 28) [%]	0	0	0	0	0	0	3	0	0
Statistical Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Body weight change (day 28) [%]	21.2	20.8	26.1	21.3	28.5	29.4	22.9	26.0	26.6
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	114	111	138	120	129	102	92	107	116
Statistical Significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction in [%] of control (day 56)	-	98	121	106	113	89	81	94	102
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0

Table 21. Effect of RH-163353 on earthworms (*Eisenia andrei*) in a 56-day reproduction study

RH-163353 [mg test item/kg soil dry weight]	**Control**	**0.408**	**0.735**	**1.32**	**2.38**	**4.29**	**7.72**	**13.9**	**25.0**
Endpoints [mg test item/kg soil dry weight]									
NOEC (day 28 mortality and weight)	≥25.0								
LOEC (day 28 mortality and weight)	>25.0								
LC_{50} [3)]	>25.0								
NOEC (day 56 reproduction)	≥25.0								
LOEC (day 56 reproduction)	>25.0								
EC_{50} [3)]	>25.0								

The results represent rounded values calculated from the exact raw data.
- = not applicable
n.s. = not significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Dunnett´s t-test, $\alpha = 0.05$, two-sided for weight changes and one-sided smaller for reproduction
[3)] estimated value

Conclusion

All study validity criteria were met.
In an earthworm reproduction and growth study with RH-163353 the No Observed Effect Concentration (NOEC) for mortality, weight changes and reproduction of the earthworm *Eisenia andrei* was determined to be ≥25.0 mg test item/kg soil dry weight, *i.e.* the highest concentration tested. The Lowest Observed Effect Concentration (LOEC) was estimated to be >25.0 mg test item/kg soil dry weight. The LC_{50} and EC_{50} were estimated to be >25.0 mg test item/kg soil dry weight.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

No data submitted.

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

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:	The study was conducted to OECD guideline 232 and according to the principles of GLP. No deviations from the guideline occurred. The results are considered valid because the following criteria were satisfied in the controls: - mean adult mortality: 4.0 % (criterion: ≤ 20%), - the mean number of juveniles per vessel at the end of the test: 1420 (criterion: ≥100 juveniles at the end of the test), - the coefficient of variation calculated for the number of juveniles: 18.8 % (criterion: ≤ 30%)

Reference: 10.4.2.1

Report: GLOB2007bF (Zoxamide 67.5 g/L + propamocarb HCL 450 g/L SC): Effects on Reproduction of Collembola (*Folsomia candida*) in Artificial Soil, Straube, D ~~Daniela, S~~., 2022, Ibacon Gmbh, Report No.: 169561016

Guideline(s):	OECD 232, 2016 and ISO 11267, 2014
Deviations:	No
GLP:	Yes
Acceptability:	Yes/ ~~No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF; batch no.: LCM22012601; content: Zoxamide: 64.23 ± 0.07 g/L or 5.846 ± 0.007 % w/w (analysed); Propamocarb-HCL: 445.8 ± 1.3 g/L or 40.57 ± 0.12 % w/w (analysed)
Test Species:	Collembola *Folsomia candida*, 10 - 12 days old, from cultures held at the laboratory.
Test Design:	28-day exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was placed into glass vessels before the Collembola were introduced on top of the soil; 8 concentrations and one control were tested; 4 replicates/concentration with 10 Collembola each (8 replicates for the control). Feeding of Collembola with approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioural effects and reproduction was performed after 28 days.
Endpoints:	Mortality of adult Collembola, behavioural effects, number of juveniles.
Reference Item:	Boric acid (the effects of the reference item were investigated in a separate GLP study).
Test Concentrations:	Control, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg GLOB2007bF/kg soil dry weight.
Test Conditions:	Artificial soil based on OECD 226; initial pH 5.8 to 5.9, pH at experimental end 6.1 to 6.2; water content at experimental start 17.4% to 18.1% (51.1% to 53.3% of the maximum water holding capacity); at experimental end 14.8% to 16.3% (43.4% to 48.1% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux).
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Dunnett's t-test (reproduction).

Results and discussions

All validity criteria for the study were met.
A mortality of up to 10% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 4% of the Collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the Collembolan exposed to GLOB2007bF was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg test item/kg soil dry weight (Dunnett's t-test, $\alpha = 0.05$, one-sided smaller).
No behavioural abnormalities were observed in any of the treatment groups.
The results are shown in Table 22.

Table 22. Summary of the Effects of GLOB2007bF on Collembola (*Folsomia candida*) in a 28-day Reproduction Study

GLOB2007bF [mg/kg soil dry weight]	**Control**	**16.3**	**29.4**	**52.9**	**95.3**	**171**	**309**	**556**	**1000**
Mean mortality (day 28) [%]	4	10	8	8	0	8	3	0	5
Significance [1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean no. of juveniles (day 28)	1420	1408	1388	1456	1547	1547	1330	1393	1323
Reproduction in [%] of control (day 28)	-	99	98	102	109	109	94	98	93
Statistical significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

	Endpoints [mg/kg soil dry weight]
NOEC (mortality)	≥1000
LOEC (mortality)	>1000
LC_{50} (mortality) [3)]	>1000
NOEC (reproduction)	≥1000
LOEC (reproduction)	>1000
EC_{10} (reproduction) [3)]	>1000
EC_{20} (reproduction) [3)]	>1000
EC_{50} (reproduction) [3)]	>1000

n.s. = not significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Dunnett's t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value
- not applicable

Conclusion

GLOB2007bF caused no statistically significant effects on mortality and reproduction of *Folsomia candida* up to and including the concentration of 1000 mg test item/kg soil dry weight.
Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC_{50} was estimated to be >1000 mg test item/kg soil dry weight. The EC_{10}, EC_{20}, and EC_{50} values were estimated to be >1000 mg test item/kg soil dry weight.

Comments of zRMS:	The study was conducted to OECD guideline 226 and according to the principles of GLP. The results are considered valid because the following criteria were satisfied in the control: - mean adult mortality: 9 % (criterion: ≤ 20%), - the mean number of juveniles per vessel at the end of the test: 199 (criterion: ≥ 50 juveniles at the end of the test), - the coefficient of variation for the number of juveniles: 12.1 % (criterion: ≤ 30%).

Reference: 10.4.2.1

Report: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil, Straube, D ~~Daniela, S.~~, 2022, Ibacon Gmbh, Report No.: 169561089

Guideline(s): OECD 226, 2016

Deviations: No

GLP:	Yes
Acceptability:	Yes/ ~~No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF; batch no.: LCM22012601; content: Zoxamide: 64.23 ± 0.07 g/L or 5.846 ± 0.007 % w/w (analysed); Propamocarb-HCL: 445.8 ± 1.3 g/L or 40.57 ± 0.12 % w/w (analysed)
Test Species:	Predatory mite *Hypoaspis aculeifer*, adult females, approximately 14 days after reaching the adult stage (35 days after placing adult females in clean rearing vessels and the start of the egg laying period in the synchronisation), cultured by ibacon.
Test Design:	14 days exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil, which was filled into glass vessels before the predatory mites were introduced on top of the soil; 8 concentrations and one control were tested; 4 replicates per test item concentration and 8 replicates for the control, with 10 female predatory mites in each replicate. Feeding of the mites with cheese mites (*Tyrophagus putrescentiae) ad libitum* at test start and on day 2, 4, 7, 9 and 11. Assessment of adult mortality and reproduction performed after 14 days.
Endpoints:	Adult mortality, number of juveniles.
Reference Item:	Dimethoate (the effects of the reference item were investigated in a separate GLP study).
Test Concentrations:	Control, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg GLOB2007bF/kg soil dry weight.
Test Conditions:	Artificial soil based on OECD 226; initial pH 5.8 to 5.9, pH at experimental end 6.0 to 6.1; water content at experimental start 17.4% to 18.1% (51.1% to 53.3% of the maximum water holding capacity); at experimental end 15.7% to 16.6% (46.3% to 48.8% of the maximum water holding capacity); temperature: within the range of 18°C to 22°C; illumination: 16 h light : 8 h dark (within the range of 400 to 800 lux).
Statistics:	Standard procedures, Fisher's Exact Test (mortality), Williams t-test (reproduction).

Results and discussions

All validity criteria for the study were met.
A mortality of up to 13% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 9% of the adult mites died (Fisher's Exact Test, $\alpha = 0.05$, one-sided greater).
Reproduction of the predatory mites exposed to GLOB2007bF was not statistically significantly different compared to the control up to and including the test concentration of 556 mg/kg soil dry weight (Williams t-test, $\alpha = 0.05$, one-sided smaller). At the test concentration of 1000 mg test item/kg soil dry weight reproduction was statistically significantly reduced compared to the control.
No behavioural abnormalities were observed in any of the treatment groups.
The results are shown in Table 23.

Table 23. Summary of the Effects of GLOB2007bF on the Predatory Mite *Hypoaspis aculeifer* in a 14-day Reproduction Study

GLOB2007bF [mg/kg soil dry weight]	**Control**	**16.3**	**29.4**	**52.9**	**95.3**	**171**	**309**	**556**	**1000**
Mortality (day 14) [%]	9	3	3	3	5	8	3	3	13
Statistical significance[1)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 14)	199	228	205	189	202	189	172	188	165
Reproduction in [%] of control (day 14)	-	115	103	95	101	95	86	94	83
Statistical significance [2)]	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
	Endpoints [mg/kg soil dry weight]								
NOEC (mortality)	≥1000								
LOEC (mortality)	>1000								
LC_{50} (mortality) [3)]	>1000								
NOEC (reproduction)	556								
LOEC (reproduction)	1000								
EC_{10} (reproduction)	n.d.								
EC_{20} (reproduction) [3)]	>1000								
EC_{50} (reproduction) [3)]	>1000								

n.s. = not significantly different compared to the control
[1)] Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
[2)] Williams t-test, $\alpha = 0.05$, one-sided smaller
[3)] estimated value
- not applicable

Conclusion

GLOB2007bF caused no statistically significant effects on mortality of *Hypoaspis aculeifer* up to and including the test concentration of 1000 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) of GLOB2007bF for mortality was determined to be ≥1000 mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be >1000 mg test item/kg soil dry weight. The LC_{50} was estimated to be >1000 mg test item/kg soil dry weight. The NOEC of GLOB2007bF for reproduction of *Hypoaspis aculeifer* was determined to be 556 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight. The EC_{10}, EC_{20} and EC_{50} values for reproduction were not determined by statistical analysis since there was no adequate concentration response. The EC_{20} and EC_{50} values were estimated to be >1000 mg test item/kg soil dry weight.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was carried out to the OECD Guideline 216 (2000) according to the principles of GLP. The test item is assumed to be uniformly distributed in the top 5 cm of soil (penetration depth 0.05 m) according to OECD 216 and EU Method C.21. According to OECD 216 the rate of nitrate formation in treated samples is compared with the rate in the controls, and the percent deviation of the treated from the control is calculated. At day 28 differences to the control of nitrate formation were -3.58% and -10.61% in the 8.79 mg and 44.0 mg test item/kg soil dry weight treatment, respectively. On the basis of the results, it was concluded that GLOB2007bF at the concentrations corresponding to the PEC: 8.79 mg of the test item/kg dry weight of soil and 5 x PEC: 44.0 mg of the test item/kg dry weight of soil did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils..

Reference:	KCP 10.5
Report	GLOB2007bF: Effects on the Activity of the Soil Microflora in the Laboratory (Nitrogen Transformation), Hammesfahr, U., 2022, Ibacon GmbH, Report No.: 169561080
Guideline(s):	OECD 216
Deviations:	No
GLP:	Yes
Acceptability:	Yes/ ~~No / Supplementary~~

Materials and methods

Test Item:	GLOB2007bF, Lot No. LCM22012601
Test System:	Biologically active agricultural soil: Loamy sand
Test Design:	Determination of nitrogen-transformation in soil enriched with lucerne meal. Comparison of test item treated soil with a non-treated soil. Three replicates per treatment. NH_4-, NO_2- and NO_3-nitrogen formed in the nitrification process was determined by continuous flow analysis. Sampling scheme: 0, 7, 14 and 28 days after treatment
Test Rates:	Control 8.79 mg GLOB2007bF/kg soil dry weight 44.0 mg GLOB2007bF/kg soil dry weight
Endpoints:	Effects on NO_3-nitrogen production after 28 days exposure (soil nitrogen transformation).
Reference Item:	Effects of sodium chloride were determined at a rate of 16 g/kg dry soil in a separate study (ibacon study code: 116526080) once a year.
Test Conditions:	Moisture: 47% to 49% of maximum water holding capacity (WHC_{max}). Temperature: 20°C ± 2°C, in the dark.
Statistics:	Calculation of mean values per treatment, standard deviation and coefficient of variation. Normality and homogeneity of variances were tested using the R/S-Test ($\alpha = 0.01$) and Levene´s test ($\alpha = 0.01$), respectively and pair-wise comparisons of treated and control values according to Student t-test ($\alpha = 0.05$) were conducted.

Results and discussions

Nitrogen Transformation - Nitrate Content:	No adverse effects of the test item on nitrate content in soil were observed at day 28. At day 28 differences to the control were -6.85% and -11.77% in the 8.79 mg and 44.0 mg test item/kg soil dry weight treatment, respectively. The results are summarized in Table 24.
Nitrogen Transformation - Mineral Nitrogen Content:	The mineral nitrogen contents in soil were within the trigger range of ± 25% set by EPPO and SETAC guidelines at day 28. At day 28 differences to the control were -6.85% and -11.77% in the 8.79 mg and 44.0 mg test item/kg soil dry weight treatment, respectively. A summary of the results is shown in in Table 24.
Nitrogen Transformation - Nitrate Formation Rates:	The cumulative soil nitrate formation rates did not exceed the trigger range of ± 25% set by OECD guideline 216 at the 0 - 28 day determination. Differences to the control were -3.58% and -10.61% in the 8.79 mg and 44.0 mg test item/kg soil dry weight treatment, respectively. The incremental soil nitrate formation rates did not exceed the trigger

range of ± 25% set by OECD guideline 216 at the 14 - 28 day determination. Differences to the control were -6.07% and -10.14% in the 8.79 mg and 44.0 mg test item/kg soil dry weight treatment, respectively. A summary of the results is shown in Table 24.

Validity Criteria: The variation between the replicate control samples did not exceed the validity criterion of 15% throughout the study.

Table 24. Effects of the test item on Nitrogen Transformation in a Loamy Sand Soil

Nitrogen Transformation - NO_3 – Nitrogen (mg / kg soil dry weight) Mean Values						
	Control		8.79 mg GLOB2007bF/kg soil dw		44.0 mg GLOB2007bF/kg soil dw	
Sampling	Nitrate-N Content	Replicate Variation[1]	Nitrate-N Content	Deviation[2]	Nitrate-N Content	Deviation[2]
Day 0	14.070	0.44	12.414*	-11.77	12.173*	-13.48
Day 7	8.115	3.48	5.349*	-34.09	5.743*	-29.23
Day 14	16.961	4.96	15.661	-7.66	14.667*	-13.53
Day 28	35.187	4.75	32.778	-6.85	31.044*	-11.77
Nitrogen Transformation - Mineral Nitrogen[3] (mg / kg soil dry weight) Mean Values						
	Control		8.79 mg GLOB2007bF/kg soil dw		44.0 mg GLOB2007bF/kg soil dw	
Sampling	Mineral-N Content	Replicate Variation[1]	Mineral -N Content	Deviation[2]	Mineral -N Content	Deviation[2]
Day 0	20.729	0.97	20.912	0.88	20.737	0.04
Day 7	9.356	3.38	8.348*	-10.77	8.089*	-13.54
Day 14	16.961	4.97	15.662	-7.66	14.667*	-13.53
Day 28	35.187	4.75	32.778	-6.85	31.044*	-11.77
Nitrogen Transformation - NO_3 – Nitrogen Formation Rate (mg / kg soil dry weight per day)[4]						
	Control		8.79 mg GLOB2007bF/kg soil dw		44.0 mg GLOB2007bF/kg soil dw	
Interval[4]	Nitrate-N Formation		Nitrate-N Formation	Deviation[2]	Nitrate-N Formation	Deviation[2]
Day 0 - 7	-0.851		-1.009	-18.57	-0.919	-7.99
Day 0 - 14	0.206		0.232	12.62	0.178	-13.59
Day 0 - 28	0.754		0.727	-3.58	0.674	-10.61
Nitrogen Transformation - NO_3 – Nitrogen Formation Rate (mg / kg soil dry weight per day)[5]						
	Control		8.79 mg GLOB2007bF/kg soil dw		44.0 mg GLOB2007bF/kg soil dw	
Interval[5]	Nitrate-N Formation		Nitrate-N Formation	Deviation[2]	Nitrate-N Formation	Deviation[2]
Day 0 - 7	-0.851		-1.009	-18.57	-0.919	-7.99
Day 7 - 14	1.264		1.473	16.53	1.275	0.87
Day 14 - 28	1.302		1.223	-6.07	1.170	-10.14

[1] = % variation within control replicates (coefficient of variation, calculated as standard deviation / mean value x 100)
[2] = % deviation to control
[3] = mineral nitrogen = sum of nitrite- nitrate- and ammonium-nitrogen
[4] = related to test start
[5] = related to successive intervals between samplings
positive values = stimulatory effect; negative values = inhibitory effect
dw = dry weight
* statistically significantly different from control (Student t-test; $\alpha = 0.05$)

Conclusion

Nitrogen Transformation — The test item had no impact on nitrogen transformation (nitrate content, mineral nitrogen content and nitrate

formation rates) of soil microorganisms when applied at 8.79 mg and 44.0 mg test item/kg soil dry weight treatment.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The seedling emergence study was conducted to OECD guideline 208 and according to the principles of GLP. No deviations from OECD Guideline No. 208 were noted. In the definitive test all the validity criteria were met as follows: - Emergence in the untreated control pots exceeded 70%. - Untreated control seedlings did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation). Untreated control seedlings only exhibited normal variation in growth and morphology for that particular species. - The mean survival of emerged untreated control seedlings was at least 90 % for the duration of the study. - Environmental conditions for a particular species were identical and the growing media contained the same amount of soil matrix, support media or substrate from the same source. The study is acceptable and reliable for risk assessment purposes.

Reference:	KCP 10.6.2
Report	GLOB2007bF: OECD Terrestrial Plant Test - Seedling Emergence and Seedling Growth Test, Stead, A., 2023, Stockbridge Technology Centre Ltd., Report No.: STC/22/E1556
Guideline(s):	OECD 208
Deviations:	No
GLP:	Yes
Acceptability:	Yes/ ~~No / Supplementary~~

Materials and methods

A glasshouse study was conducted by Stockbridge Technology Centre Ltd to generate dose response data for GLOB2007bF when applied pre-emergence to ten species of non-target plants. The methodology for the study was based on OECD Guideline 208 (July 2006) Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test. ER_{10}, ER_{25}, ER_{50}, NOEC and LOEC values based on seedling emergence, percentage survival, shoot fresh weight reduction and percentage visual injury assessment at harvest were ascertained from the dose response data and will be used to assess the risk of GLOB2007bF to terrestrial non-target plant species.
The test species consisted of four monocotyledon species (oats, onion, corn and ryegrass) and six dicotyledon species (sugar beet, sunflower, soybean, tomato, oilseed rape and cucumber). Species tested

represented the plant families of Poaceae, Liliaceae, Chenopodiaceae, Asteraceae, Fabaceae, Solanaceae, Brassicaceae and Cucurbitaceae.
The Batch Number for the sample of GLOB2007bF used in this study was LCM22012601.
Applications were made pre-emergence to all ten species.
All species were treated with the test item on 23rd June 2022. GLOB2007bF was applied to oats, onion, corn, ryegrass, sugar beet, sunflower, soybean, tomato, oilseed rape and cucumber at five different rates (0.5, 1, 2, 4 and 8 L product/ha) and compared with an untreated water only control. The application water volume was 200 L/ha.

Results and discussions

ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq. values) NOEC and LOEC values, based on seedling emergence are summarized below:

Species	ER_{10}# GLOB2007bF (L/ha)	ER_{25} GLOB2007bF (L/ha)	ER_{50} GLOB2007bF (L/ha)	R-Sq.	NOEC GLOB2007bF (L/ha)	LOEC GLOB2007bF (L/ha)
Oats	>8	>8	>8	N/A	8	8
Onion	>8	>8	>8	N/A	8	8
Corn	>8	>8	>8	N/A	8	8
Ryegrass	>8	>8	>8	N/A	8	8
Sugar beet	>8	>8	>8	N/A	8	8
Sunflower	>8	>8	>8	N/A	8	8
Soybean	>8	>8	>8	N/A	8	8
Tomato	>8	>8	>8	N/A	8	8
Oilseed rape	>8	>8	>8	N/A	8	8
Cucumber	>8	>8	>8	N/A	8	8

ER_{10} values should be treated with caution due to natural plant to plant variability.
N/A = not appropriate owing to tolerance of these species to GLOB2007bF.

ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq. values) NOEC and LOEC values, based on percentage survival are summarized below:

Species	ER_{10}# GLOB2007bF (L/ha)	ER_{25} GLOB2007bF (L/ha)	ER_{50} GLOB2007bF (L/ha)	R-Sq.	NOEC GLOB2007bF (L/ha)	LOEC GLOB2007bF (L/ha)
Oats	>8	>8	>8	N/A	8	8
Onion	>8	>8	>8	N/A	8	8
Corn	>8	>8	>8	N/A	8	8
Ryegrass	>8	>8	>8	N/A	8	8
Sugar beet	>8	>8	>8	N/A	8	8
Sunflower	>8	>8	>8	N/A	8	8
Soybean	>8	>8	>8	N/A	8	8
Tomato	>8	>8	>8	N/A	8	8
Oilseed rape	>8	>8	>8	N/A	8	8
Cucumber	>8	>8	>8	N/A	8	8

ER_{10} values should be treated with caution due to natural plant to plant variability.
N/A = not appropriate owing to tolerance of these species to GLOB2007bF.

ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq. values) NOEC and LOEC values, based on shoot fresh weight reduction are summarized below:

Species	ER_{10}# GLOB2007bF (L/ha)	ER_{25} GLOB2007bF (L/ha)	ER_{50} GLOB2007bF (L/ha)	R-Sq.	NOEC GLOB2007bF (L/ha)	LOEC GLOB2007bF (L/ha)
Oats	>8	>8	>8	N/A	8	8
Onion	>8	>8	>8	N/A	8	8
Corn	>8	>8	>8	N/A	8	8
Ryegrass	>8	>8	>8	N/A	8	8
Sugar beet	>8	>8	>8	N/A	8	8
Sunflower	>8	>8	>8	N/A	8	8
Soybean	>8	>8	>8	N/A	8	8
Tomato	>8	>8	>8	N/A	8	8
Oilseed rape	>8	>8	>8	N/A	8	8
Cucumber	>8	>8	>8	N/A	8	8

ER_{10} values should be treated with caution due to natural plant to plant variability.
N/A = not appropriate owing to tolerance of these species to GLOB2007bF.

ER_{10}, ER_{25} and ER_{50} values (with corresponding R-Sq. values) NOEC and LOEC values, based on percentage visual injury assessment at harvest are summarized below:

Species	ER_{10}# GLOB2007bF (L/ha)	ER_{25} GLOB2007bF (L/ha)	ER_{50} GLOB2007bF (L/ha)	R-Sq.	NOEC GLOB2007bF (L/ha)	LOEC GLOB2007bF (L/ha)
Oats	>8	>8	>8	N/A	8	8
Onion	>8	>8	>8	N/A	8	8
Corn	>8	>8	>8	N/A	8	8
Ryegrass	>8	>8	>8	N/A	8	8
Sugar beet	>8	>8	>8	N/A	8	8
Sunflower	>8	>8	>8	N/A	8	8
Soybean	>8	>8	>8	N/A	8	8
Tomato	>8	>8	>8	N/A	8	8
Oilseed rape	>8	>8	>8	N/A	8	8
Cucumber	>8	>8	>8	N/A	8	8

ER_{10} values should be treated with caution due to natural plant to plant variability.
N/A = not appropriate owing to tolerance of these species to GLOB2007bF.

Conclusion

Based on seedling emergence:

- All ten species had an ER_{10}, ER_{25} and ER_{50} value of >8 L product/ha and NOEC and LOEC values of 8 L product/ha.

Based on percentage survival:

- All ten species had an ER_{10}, ER_{25} and ER_{50} value of >8 L product/ha and NOEC and LOEC values of 8 L product/ha.

Based on shoot fresh weight reduction:

- All ten species had an ER_{10}, ER_{25} and ER_{50} value of >8 L product/ha and NOEC and LOEC values of 8 L product/ha.

Based on percentage visual injury assessment at harvest:

- All ten species had an ER_{10}, ER_{25} and ER_{50} value of >8 L product/ha and NOEC and LOEC values of 8 L product/ha.

Comments of zRMS:	The Vegetative vigour study was conducted to OECD guideline 227 and according to the principles of GLP. No deviations were noted. In the definitive test all the validity criteria were met as follows: - The percentage of seedling emergence of each seed lot is at least 70 %. - Untreated control plants must not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation). Plants exhibit only normal variation in growth and morphology for that particular species. - The mean survival of untreated control plants 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. The study is accepted and reliable for risk assessment purposes.

Reference:	KCP 10.6.2
Report	GLOB2007bF: OECD Terrestrial Plant Test – Vegetative Vigour Test, Davies, C., 2023, Stockbridge Technology Centre Ltd., Report No.: STC/22/E1555
Guideline(s):	OECD 227
Deviations:	No
GLP:	Yes
Acceptability:	Yes ~~/ No / Supplementary~~

Materials and methods

A glasshouse study was conducted by Stockbridge Technology Centre Ltd to generate dose response data for GLOB2007bF when applied post-emergence to four monocotyledon species and six dicotyledon species. The methodology for the study was based on OECD Guideline For The Testing of Chemicals, 227 Terrestrial Plant Test: Vegetative Vigour Test (July 2006). ER_{10}, ER_{25}, ER_{50}, NOEC and LOEC values based on percentage survival, shoot fresh weight reduction and percentage visual injury at harvest were ascertained from the dose response data and used to assess the risk of GLOB2007bF to terrestrial non-target plant species.

The test species consisted of four monocotyledon species (oats, onion, corn and ryegrass) and six dicotyledon species (sugar beet, sunflower, soybean, tomato, oilseed rape and cucumber). Species tested represented the plant families of Poaceae, Liliaceae, Chenopodiaceae, Asteraceae, Fabaceae, Solanaceae, Brassicaceae and Cucurbitaceae.

All species were treated with the test item on 16th June 2022. GLOB2007bF was applied at five different rates to all species at (0.5, 1, 2, 4 and 8 L product/ha) and compared with an untreated water only control. The water application rate was 200 L/ha.

The Batch Number for the sample of GLOB2007bF used in this study was LCM22012601.

Applications were made post-emergence to all ten species at growth stage BBCH 12-14 (2 to 4 true leaves).

No species displayed visual injury except soybean.

Results and discussions

NOEC levels, LOEC levels and ER_{10}, ER_{25} and ER_{50} values for GLOB2007bF based on plant survival are given below, with their corresponding R-Sq values and statistical model functions used.

Species	L GLOB2007bF/ha					R-Sq	Statistical Model Function
	NOEC	LOEC	ER_{10}*	ER_{25}	ER_{50}		
Oats	8	8	>8	>8	>8	N/A	N/A
Onion	8	8	>8	>8	>8	N/A	N/A
Corn	8	8	>8	>8	>8	N/A	N/A
Ryegrass	8	8	>8	>8	>8	N/A	N/A
Sugar beet	8	8	>8	>8	>8	N/A	N/A
Sunflower	8	8	>8	>8	>8	N/A	N/A
Soybean	8	8	>8	>8	>8	N/A	N/A
Tomato	8	8	>8	>8	>8	N/A	N/A
Oilseed rape	8	8	>8	>8	>8	N/A	N/A
Cucumber	8	8	>8	>8	>8	N/A	N/A

NOEC levels, LOEC levels and ER_{10}, ER_{25} and ER_{50} values for GLOB2007bF based on shoot fresh weight reduction at harvest are given below, with their corresponding R-Sq values and statistical model functions used.

Species	L GLOB2007bF/ha					R-Sq	Statistical Model Function
	NOEC	LOEC	ER_{10}*	ER_{25}	ER_{50}		
Oats	8	8	>8	>8	>8	N/A	N/A
Onion	8	8	>8	>8	>8	N/A	N/A
Corn	8	8	>8	>8	>8	N/A	N/A
Ryegrass	8	8	>8	>8	>8	N/A	N/A
Sugar beet	8	8	>8	>8	>8	N/A	N/A
Sunflower	8	8	>8	>8	>8	N/A	N/A
Soybean	8	8	>8	>8	>8	N/A	N/A
Tomato	8	8	>8	>8	>8	N/A	N/A
Oilseed rape	8	8	>8	>8	>8	N/A	N/A
Cucumber	8	8	>8	>8	>8	N/A	N/A

NOEC levels, LOEC levels and ER_{10}, ER_{25} and ER_{50} values for GLOB2007bF based on percentage visual injury at harvest are given below, with their corresponding R-Sq values and statistical model functions used.

Species	L GLOB2007bF/ha					R-Sq	Statistical Model Function
	NOEC	LOEC	ER_{10}*	ER_{25}	ER_{50}		
Oats	8	>8	>8	>8	>8	N/A	N/A
Onion	8	>8	>8	>8	>8	N/A	N/A
Corn	8	>8	>8	>8	>8	N/A	N/A
Ryegrass	8	>8	>8	>8	>8	N/A	N/A
Sugar beet	8	>8	>8	>8	>8	N/A	N/A
Sunflower	8	>8	>8	>8	>8	N/A	N/A
Soybean	4	8	>8	>8	>8	N/A	N/A
Tomato	8	>8	>8	>8	>8	N/A	N/A
Oilseed rape	8	>8	>8	>8	>8	N/A	N/A
Cucumber	8	>8	>8	>8	>8	N/A	N/A

Conclusion

Based on plant survival:

- All species had an ER_{10}, ER_{25} and ER_{50} value > 8 L product/ha (the highest rate tested) following post-emergence application of GLOB2007bF.

- All species had a NOEC and LOEC value of 8 L product/ha (the highest rate tested) following post-emergence application of GLOB2007bF.

Based on shoot fresh weight reduction at harvest:

- All species had an ER_{10}, ER_{25} and ER_{50} value > 8 L product/ha (the highest rate tested) following post-emergence application of GLOB2007bF.

- All species had a NOEC and LOEC value of 8 L product/ha (the highest rate tested) following post-emergence application of GLOB2007bF.

Based on percentage visual injury at harvest:

- All species had an ER_{10}, ER_{25} and ER_{50} value > 8 L product/ha (the highest rate tested) following post-emergence application of GLOB2007bF.

- Based on NOEC values the most sensitive dicotyledon species to post-emergence application of GLOB2007bF was soybean with a NOEC value of 4 L product/ha and a LOEC value of 8 L product/ha.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No data submitted.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No data submitted.

A 2.8 KCP 10.8 Monitoring data

No data submitted.