REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: GWN-10616

Chemical active substances:

Zoxamide, 60 g/L

Potassium phosphonates, 750 g/L
Phosphonic acid equivalents, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: XXXX

Submission date: 31/10/2023

Evaluation date: 07/2024 (update 09/2024)

MS Finalisation date: 11/2024

Version history

When	What
July 2024	zRMS finalised dRR evaluation
September 2024	Update based on zRMS request of July 2024: Chapter 9.3 (higher-tier risk assessment grapevine). Chapter 9.5 (new PECsw calculations for potatoes) Chapter 9.6 (risk assessment according to EFSA (2013)).
November 2024	Revised version addressing the comments resived

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9 Ecotoxicology (KCP 10)

Review Comments:

This document describes the acceptable use conditions required for registration of GWN-10616, a SC formulation containing 60 g/L zoxamide and 755 g/L potassium phosphonates (=500 g/L phosphonic acid) for the use as fungicide in grapevine, pome fruit and potato.

This Part B document only reviews data 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.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	g 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 [illegible]	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	AT, BE, CZ, HU, NL, PL, RO, SI, SK	Grapevine (table and wine)	F	Downy mildew *Plasmopara viticola*	Broadcast foliar spray	BBCH 14-79	a) 3 b) 3	8-10	a) 3 b) 9	a) 180 (Z); 1500 (K) b) 540 (Z); 4500 (K)	200-1000 111 – 557 L/10000 m^2 tLWA	28	Collateral effects on *Botrytis cinerea* Assuming max. 18000 m^2 tLWA per ha ground area	A	A	R	A^	A	A	A
2	DE	Grapevine (table and wine)	F	Downy mildew *Plasmopara viticola*	Broadcast foliar spray	BBCH 14-79	a) 2 b) 2	8-10	a) 2.5 b) 5	a) 150 (Z); 1250 (K) b) 300 (Z); 2500 (K)	200-1000 111 – 557 L/10000 m^2 tLWA	28	Collateral effects on *Botrytis cinerea* Assuming max. 14970 m^2 tLWA per ha ground area	A	A	R	A^	A	A	A
3	AT, BE, CZ, HU, NL, PL, RO, SI, SK	Pome fruit	F	*Venturia* sp.	Broadcast foliar spray	BBCH 51-69	a) 2 b) 2	6-8	a) 3 b) 6	a) 180 (Z); 1500 (K) b) 360 (Z); 3000 (K)	200-1000 111 – 557 L/10000 m^2 tLWA	NR	Treatments within the end of flowering Assuming	A	A	R	A^	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
													max. 18000 m^2 tLWA per ha ground area							
4	DE	Pome fruit	F	*Venturia* sp.	Broadcast foliar spray	BBCH 51-69	a) 2 b) 2	6-8	a) 2.5 b) 5	a) 150 (Z); 1250 (K) b) 300 (Z); 2500 (K)	200-1000 111 – 557 L/10000 m^2 tLWA	NR	Treatments within the end of flowering Assuming max. 14970 m^2 tLWA per ha ground area	A	A	R	A^	A	A	A
5	AT, BE, CZ, IE, NL	Potato	F	Potato late blight *Phytophthora infestans*	Broadcast foliar spray	BBCH 21-89	a) 3 b) 3	7-8	a) 2.5 b) 7.5	a) 150 (Z); 1250 (K) b) 450 (Z); 3750 (K)	200-500	7		A	A	R#	A^	A	A	A
6	DE	Potato	F	Potato late blight *Phytophthora infestans*	Broadcast foliar spray	BBCH 21-89	a) 3 b) 3	7-8	a) 2 b) 6	a) 120 (Z); 1000 (K) b) 360 (Z); 3000 (K)	200-500	7		A	A	R#	A^	A	A	A

* 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

Considering LoEP input parameters: GWN-10616 applications close to surface water pose acceptable risk to aquatic organisms with appropriate risk mitigation measures, except R1 scenario, multiple applications in potatoes (BBCH 21). Since calculations have only been made for the BBCH 21 and 89, it is not possible to demonstrate from which growth stage of potato the multiple applications are acceptable. Therefore, it is currently only possible to accept a single application in potato for the R1 scenario.

Considering half-life on crop canopy of 5.8 days: GWN-10616 applications close to surface water pose acceptable risk to aquatic organisms with appropriate risk mitigation measures, for single and multiple applications in potatoes.

^ The evaluation of the acute and chronic risk for bees was performed by zRMS in accordance with the recommendations of the "EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)" (EFSA Journal 2013;11(7):3295; hereafter referred to as EFSA/2013/3295). Refinement of risk, where required, has been left to the national level.

Explanation for column 15 – 21 "Conclusion"

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required

C	To be confirmed by cMS
N	No safe use

Remarks table:

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

9.1.1 Overall conclusions

9.1.1.1 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
Based on screening and first-tier assessment steps, the calculated TER values for the acute and long-term risk resulting from an exposure of birds to Zoxamide and/or Phosphonic acid (oral exposure) according to the GAP of the formulation GWN-10616 do achieve the acceptability criteria TER ≥ 10 and TER ≥ 5, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for acute and chronic effects, respectively.
Based on the assessment of the risk arising from the uptake of Zoxamide and/or Phosphonic acid via drinking water, a TER calculation is not necessary. A low risk can be concluded.
Risk to vermivorous and piscivorous birds was assessed for Zoxamide and relevant metabolites RH-127450 and RH-24549 according to EFSA/2009/1438. Earthworm-eating and fish-eating birds are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

Terrestrial vertebrates other than birds
Based on screening, first-tier and higher-tier assessment steps, the calculated TER values for the acute and long-term risk resulting from an exposure of mammals to Zoxamide and/or Phosphonic acid (oral exposure) according to the GAP of the formulation GWN-10616 do achieve the acceptability criteria TER ≥ 10 and TER ≥ 5, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for acute and chronic effects, respectively.
Based on the assessment of the risk arising from the uptake of Zoxamide and/or Phosphonic acid via drinking water, a TER calculation is not necessary. A low risk can be concluded.
Risk to vermivorous and piscivorous mammals was assessed for Zoxamide and relevant metabolites RH-127450 and RH-24549 according to EFSA/2009/1438. Earthworm-eating and fish-eating mammals are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)
All relevant data with regard to birds and mammals are presented in the respective risk assessments (B.9.2 and B.9.3, respectively). No additional relevant information was identified in open literature which can be taken into account in the risk assessment.
Thus, the risk to reptiles and amphibians is considered to be covered by the risk assessment of birds (Chapter 9.2) and mammals (Chapter 9.3) as well as by the assessment of fish (Chapter 9.5).

Review Comments:

Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) were not evaluated by zRMS.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Studies on GWN-10616 are all available for product authorisation. A comparison of the results for GWN-10616 with the results for the single active substances revealed no additive or synergistic effects. Instead, the assessment confirms that Zoxamide drives the toxicity for aquatic organisms.

For the intended uses of GWN-10616 the calculated PEC/RAC ratios for Zoxamide indicate an acceptable risk for aquatic organisms considering drift reducing measures (drift reducing nozzles and buffer zones) and run-off reducing vegetated buffer zones, leading to a reduction of the exposure of surface water bodies. As a result, the implementation of the following measures is necessary:

Table 9.1-2: Risk mitigation measures to achieve acceptable risk for aquatic organisms

Use	Scenario	Mitigation
Grapevine	R1 Pond	No mitigation required
	D6 Ditch	50% drift reducing nozzles + 10 m buffer zone, or 20 m buffer zone
	~~D6 Ditch~~, R2 Stream	75% drift reducing nozzles + 10 m buffer zone, or 50% drift reducing nozzles + 15 m buffer zone, or 20 m buffer zone
	R1 Stream, R4 Stream	50% drift reducing nozzles + 10 m buffer zone or ~~20~~ 15 m buffer zone ~~(incl. vfs)~~
	R3 Stream	75% drift reducing nozzles + 10 m buffer zone (incl. vfs), or 50% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), or 20 m buffer zone (incl. vfs)
Pome fruit	~~R3 Stream~~	~~75% drift reducing nozzles + 10 m buffer zone (incl. vfs),~~ ~~or 50% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs),~~ ~~or 20 m buffer zone (incl. vfs)~~
	D3 Ditch, D4 Stream,	90% drift reducing nozzles + 15 m buffer zone, or 50 m buffer zone
	D4 Pond, D5 Pond, R1 Pond	~~75~~ 50% drift reducing nozzles + 10 m buffer zone, or ~~50% drift reducing nozzles +~~ 15 m buffer zone, ~~or 20 m buffer zone~~
	D5 Stream, R2 Stream, R3 Stream	90% drift reducing nozzles + 20 m buffer zone or 50 m buffer zone
	R1 Stream	90% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), or 75% drift reducing nozzles + 20 m buffer zone (incl. vfs), or 50 m buffer zone (incl. 20 m vfs)
	R4 Stream	90% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), 75% drift reducing nozzles + 20 m buffer zone (incl. vfs), or 50 m buffer zone (incl. 20 m vfs)
Potato	D4 Pond, R1 pond	No mitigation required
	D3 Ditch, D4 Stream, ~~R1 Pond~~ R2 Stream	10 m buffer zone
	~~R1 Stream, R2 Stream~~, R3 Stream	10 m buffer zone (incl. vfs)
	R1 Stream 3 appl. BBCH 21	No safe use (considering LoEP input parameters) 10 m buffer zone (incl. vfs) (considering half-life on crop canopy of 5.8 days)
	R1 Stream 1 appl. BBCH 21 covers single and multiple applications in BBCH 89	10 m buffer zone (incl. vfs)

Vfs: vegetated filter strip

Review Comments:

Considering LoEP input parameters:

GWN-10616 applications close to surface water pose acceptable risk to aquatic organisms with appropriate risk mitigation measures, except R1 scenario, multiple applications in potatoes (BBCH 21). Since calculations have only been made for the BBCH 21 and 89, it is not possible to demonstrate from which growth stage of potato the multiple applications are acceptable. Therefore, it is currently only

possible to accept a single application in potato for the R1 scenario.

During the comment stage the Applicant has the opportunity to provide an additional risk assessment for aquatic organisms to demonstrate acceptable risk for multiple applications in potato (R1 scenario).

With reference to zRMS request of July 2024, additional risk assessment for aquatic organisms for applications in potato were provided by the Applicant. The PECsw values performed with refined parameters were accepted by zRMS (please refer to Section B8).

Considering half-life on crop canopy of 5.8 days

GWN-10616 applications close to surface water pose acceptable risk to aquatic organisms with appropriate risk mitigation measures (R1: 10 m buffer zone (incl. vfs)), for single and multiple applications in potatoes.

The metabolites of zoxamide are of lower toxicity than the parent active substance. For all Zoxamide metabolites, PEC/RAC ratios are below 1 for all aquatic organism when $FOCUS_{SW}$ Step 1 and 2 exposure estimates are considered, indicating an acceptable risk.

9.1.1.3 Effects on bees (KCP 10.3.1)Effects on bees (KCP 10.3.1)

The risk assessments are performed following the approaches outlined in SANCO/10329/2002 rev. 2 final and the revised EPPO scheme.

The acute oral and contact toxicity hazard quotients are considerably less than 50, indicating that the active substances as well as the formulated product pose a low risk to bees. Therefore, a low risk to bees is expected from the application of GWN-10616 according to GAP. Based on the chronic oral adult toxicity study as well as the endpoint from the larval oral adult toxicity study, the respective TER values are considerably above 1. Thus, the chronic risk to adult and larval honey bees potentially exposed to GWN-10616 containing Zoxamide and Phosphonic acid via consumption of nectar and pollen after application of GWN-10616 is considered acceptable.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to GWN-10616.

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

The evaluation of the acute and chronic risk for bees was performed by zRMS in accordance with the recommendations of the "EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)" (EFSA Journal 2013;11(7):3295; hereafter referred to as EFSA/2013/3295). Refinement of risk, where required, has been left to the national level.

The risk assessment performed following the approaches outlined in SANCO/10329/2002 rev. 2 final and the revised EPPO scheme was not evaluated by zRMS.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

All hazard quotients for the in- and off-field assessment are considerably less than 2, indicating that the product poses a low risk to non-target arthropods. Therefore, a low in- and off-field risk to non-target arthropods is expected from the application of GWN-10616 in grapevine, pome fruit and potato according to the GAP.

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

Effects on earthworms
Acceptable acute and long-term risk (first-tier assessment step) for earthworms following exposure to Zoxamide metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses). Available earthworm field studies cover the application pattern of Zoxamide in grapevine (3 x 150 g a.s./ha, 8-days interval) resulting in an acceptable higher-tier risk.

Effects on other soil non-target macro-organisms
Acceptable long-term risk (first-tier assessment step) for non-target soil organisms other than earthworms following exposure to Zoxamide, its relevant metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses).

Effects on soil microbial activity
Acceptable risk for soil micro-organisms following exposure to Zoxamide, its relevant metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses).

Overall, an acceptable risk for soil organisms is demonstrated following the application of GWN-10616 in grapevine, pome fruit and potato according to GAP.

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

Limit tests at rates up to 4275 g/ha were conducted with GWN-10616 and effects were below the critical threshold. The limit test rates equal and/or cover the highest field application rate in grapevine, pome fruit and potato and are thus considered an indicator for an acceptable risk.
Therefore, a low risk to terrestrial non-target plants is expected from the application of GWN-10616 in grapevine, pome fruit and potato according to the GAP.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

The GAP consists of intended uses in vineyards, orchards (pome fruit) and potatoes, grouping of intended uses to support application of the risk envelope approach (according to SANCO/11244/2011) is presented below.

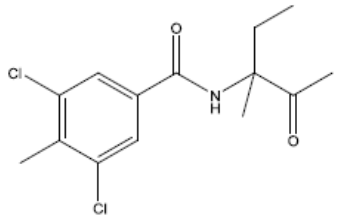
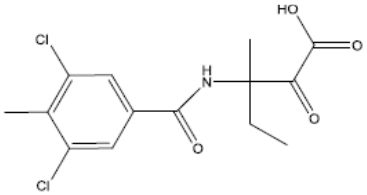
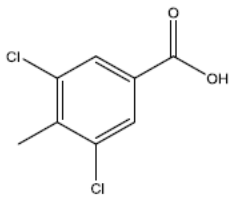
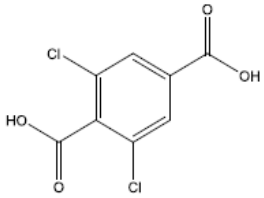
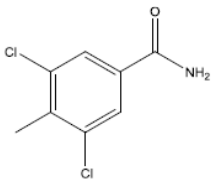
Table 9.1-3: Critical use pattern of GWN-10616

Group	Intended uses	Relevant use parameters
Grape	1, 2	Use 1 (3 x 3 L/ha with 8-day interval at BBCH 14-79) covering use 2 (2 x 2.5 L/ha with 8-day interval at BBCH 14-79)
Pome fruit	3, 4	Use 3 (2 x 3 L/ha with 6-day interval at BBCH 51-69) covering use 4 (2 x 2.5 L/ha with 6-day interval at BBCH 51-69)
Potato	5, 6	Use 5 (3 x 2.5 L/ha with 7-day interval at BBCH 21-89) covering use 6 (3 x 2 L/ha with 7-day interval at BBCH 21-89)

9.1.3 Consideration of metabolites

A list of metabolites of active substance Zoxamide found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of GWN-10616 is indicated in the table.
Regarding active substance Potassium phosphonates (formerly: phosphite; technical active substance) and Phosphonic acid (actual active substance) Phosphate is the only relevant metabolite in soil, surface water and sediment. Due to its ubiquitous nature no risk assessment for phosphate ions is presented.
$K^+ + HPO^3{}_{2-} / H_2PO_3^- + H_2O \rightarrow K^+ + PO_4^{3-} + H_3O^+$

Table 9.1-4 Metabolites of active substance Zoxamide

Metabolite	Molar mass	Chemical structure	Maximum observed occurrence in compartments	Exposure assessment required due to
RH-127450	302.15		Soil: Max. 15.1% AR after 7 days Water/sediment system: Max. 17.1% AR in surface water (day 28), max. 23.1% AR in sediment max. 39.3% AR in total system (after incubation at 10°C)	PEC_{soil}: occurrence in soil PEC_{gw}: leaching potential to groundwater $PEC_{sw/sed}$: occurrence in surface water
RH-163353	332.15		Soil: Max. 15% AR after 3 days Water/sediment: Max. 15.8% AR at day 28 in the water phase, max. 7.4% AR at day 106 in the sediment, max. 20.6% AR (day 56) in the total system	PEC_{soil}: occurrence in soil PEC_{gw}: leaching potential to groundwater $PEC_{sw/sed}$: occurrence in surface water
RH-24549	205.0		Soil: Max. 33.8% AR after 7 days Water/sediment: Max. 5% AR (whole system)	PEC_{soil}: occurrence in soil PEC_{gw}: leaching potential to groundwater $PEC_{sw/sed}$: occurrence in surface water
RH-141455	235.02		Soil: Max. 8.4% AR after 14 days Water/sediment: Max. 2.1% AR (whole system)	PEC_{soil}: occurrence in soil PEC_{gw}: leaching potential to groundwater $PEC_{sw/sed}$: occurrence in surface water
RH-139432	204.06		Soil: Max. 4.9% AR after 14 days Surface water: Max. 21.4% AR (day 28) in surface water of OECD 309 study (max. of 42.4% AR on day 30 in an aquatic photolysis study at pH 4 is regarded as environmentally not relevant).	$PEC_{sw/sed}$: occurrence in surface water

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with active substances Zoxamide and Phosphonic acid (active component of Potassium phosphonates). Full details of these studies are provided in the respective EU assessment reports and related documents.

Effects on birds of GWN-10616 were not evaluated as part of the EU assessment of the contained active substances. However, the provision of further data on the GWN-10616 is not considered essential, because both substances are not considerably toxic to vertebrates in general and specially to birds.
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

Speciesf	Substance	Exposure System	Results	Reference
Zoxamide				
Bobwhite quail *Colinus virginianus*	Zoxamide	Oral 1 d Acute	**LD_{50} > 2000 mg a.s./kg bw/d**	EFSA Journal 2017;15(9):4980
Bobwhite quail *Colinus virginianus*	Zoxamide	Dietary 8 d Short-term	LDD_{50} = 1889.3 mg a.s./kg bw/d	EFSA Journal 2017;15(9):4980
Mallard duck *Anas plathyrhynchos*	Zoxamide	Dietary 8 d Short-term	LDD_{50} = 1597.7 mg a.s./kg bw/d	EFSA Journal 2017;15(9):4980
Mallard duck *Anas plathyrhynchos*	Zoxamide	Dietary 22 weeks Reproduction	**NOEL = 122.8 mg a.s./kg bw/d**	EFSA Journal 2017;15(9):4980
Bobwhite quail *Colinus virginianus*	Zoxamide	Dietary 22 weeks Reproduction	NOEL = 170.9 mg a.s./kg bw/d	EFSA Journal 2017;15(9):4980
Phosphonic acid*				
Bobwhite quail *Colinus virginianus*	Potassium phosphonates	Oral 1 d Acute	**LD_{50} > 2250 mg a.s./kg bw/d (phosphonic acid equivalents)**	EFSA Journal 2012;10(12):2963
Bobwhite quail *Colinus virginianus*	Potassium phosphonates	Dietary 8 d Short-term	LDD_{50} > 1335 mg a.s./kg bw/d (phosphonic acid equivalents)	EFSA Journal 2012;10(12):2963
Mallard duck *Anas plathyrhynchos*	Potassium phosphonates	Dietary 8 d Short-term	LDD_{50} > 2363 mg a.s./kg bw/d (phosphonic acid equivalents)	EFSA Journal 2012;10(12):2963

Speciesf	Substance	Exposure System	Results	Reference
Bobwhite quail *Colinus virginianus*	Fosetyl-aluminium	Dietary Reproduction	**NOEL = 149.04 mg a.s./kg bw/d (phosphonic acid equivalents)**	EFSA Journal 2012;10(12):2963

*Phosphonic acid (also Phosphorous acid) is the actual active substance of the technical item Potassium phosphonates (formerly: phosphite; technical active substance).

Relevance of metabolites

Metabolites further considered in the birds and mammals risk assessment were identified based on the results of the plant metabolism studies and for the metabolites regarded potentially relevant for the ecotoxicological risk assessment (see table in chapter 9.1.3).
In the metabolism studies with Zoxamide (see RAR 2017 Volume 3-CA B.7.2) conducted on grapes, tomatoes, cucumbers and peas all metabolites found generally occurred at levels < 10 % TRR. The major residue observed in all studies was always the parent compound. Only one exception was found for tomatoes: In tomato green and red fruits **RH-141452** occurred at 15 % and 11.2 % AR, respectively. Metabolites RH-141452 and **RH-141455** were identified as major metabolites (> 10 % TRR) in the potato metabolism study (Reibach PH and Spencer WO, 1998; RAR 2017). However, these metabolites occur only at levels < LOQ in potato tubers in relevant supervised field trials.
Both RH-141452 and RH-141455 are rat metabolites found at low levels in urine (Swenson, R.E., Frederick, C.B., Graves, D.D. 1998a, Report No: 94R-235, ER Ref No: 24.1).
RH-141452 (designated M-17 in the rat metabolism study) was isolated from rat urine by acid/base extraction and identified by HPLC and TLC by comparison with an authentic reference standard. Analysis by LC-MS gave a molecular weight consistent with RH-141452. The identity was confirmed by derivatisation (methylation with diazomethane) followed by GC/MSD analysis. RH-141452 (M-17) was estimated to account for 0.37 % of the administered dose in the low dose female group. RH-141455 was not detected by this method.
Both RH-141452 and RH-141455 were, however, found in rat urine using a non-radiolabelled residue method. Rat urine was diluted with water, acidified and extracted with ethyl acetate to separate the acidic components. After concentration and derivatization (methylation) with diazomethane, the metabolites were identified by GC/MSD and quantified using GC-ECD. Using this method, RH-141455 was found at 0.006 % of dose in males and 0.004 % of dose in females. RH-141452 was found at higher levels of 0.037 % of dose in males and 0.034 % of dose in females.
Metabolism of Zoxamide to RH-141452 and RH-141455 has the effect of increasing the polarity of the residue and thereby increasing water solubility, which facilitates excretion. From the structures of RH-141452 and RH-141455, which are small molecules containing aromatic carboxylic acids, it would be predicted that these compounds would be readily excreted largely unchanged. Rat metabolism studies have been performed with both RH-141452 and RH-141455, which confirm this expectation (Wu, D., Gu, Z., 1998a, Report No: 97RC-154, ER Ref No: 27.1 and Wu, D., Gu, Z. 1998b, Report No: 98RC-017, ER Ref No: 27.2).

Following oral administration of RH-141452 to rats, the majority of the RH-141452 was eliminated unchanged through urine, accounting for > 94 % of the administered dose. Three minor conjugates, M-2, M-3 (glucuronide conjugates), and M-4 (glycine conjugate) were also found in the urine samples, accounting for ~3 % of the administered dose. An additional 1.6 % of the administered radioactivity was excreted in the faeces as the parent chemical.
Following oral administration of RH-141455 to rats, greater than 96 % of radioactivity excreted in faeces (73 %) and urine (11 %) was identified to be unchanged parent. Some very minor metabolites were also observed in urine samples but were not identified due to their extremely low percentage of dose.

The hydrolysis of Zoxamide and the subsequent oxidation steps to form RH-141452 and RH-141455 are regarded as detoxification reactions and therefore, both metabolites would be expected to be less toxic than parent Zoxamide. In acute oral toxicity studies in male and female mice, the acute oral LD_{50} of RH-141452 and RH-141455 in male and female mice were both > 5000 mg/kg bw.
In addition, a comparison of the toxicological profile of Zoxamide and the two metabolites, RH-141452 and RH-141455 has been made using OECD QSAR Toolbox version 3.4.0.17 (Pellizzaro, M. and Da Silva, M., 2017; see RAR (2017)). This analysis also indicates that both metabolites are expected to have a lower toxicity than the parent Zoxamide. Also, genotoxicity testing of both metabolites revealed no harmful effects.

A 90-days dietary toxicity study plus with RH-141455 in rats revealed a NOAEL of > 1000 mg/kg bw/d (i.e. NOAEL = 16000 ppm, which is equivalent to 924 and 1119 mg/kg body weight/day for the males and females, respectively; see Satish Kumar 2020, report no. U-19102 presented in dRR Part B.6).

Taking into account that RH-141452
- is not genotoxic,
- showed an acute oral toxicity of LC_{50} > 5000 mg/kg bw/d (see EFSA, 2017), which is above 2000 mg/kg bw/d, the dose for classification and
- is predicted to have a similar toxicity as RH-141455 (which is regarded as toxicologically non relevant)

this metabolite can also be regarded as (eco)toxicologically not relevant without any further proof (i.e. without further repeated dose toxicity studies).

During AIR, the metabolites **RH-127450** and **RH-24549** were both identified as relevant for the soil and aquatic risk assessments. RH-127450 and RH-24549 together with the parent compound have log P_{ow} values > 3 and have the potential to bioaccumulate. Therefore, an assessment of secondary poisoning is required for these metabolites and Zoxamide.

9.2.1.1 Justification for new endpoints

All endpoints are in agreement with EU review data.

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).

Assessment of combined toxicity

Toxicity studies for birds with formulated products are typically not conducted without further necessity. Data on the toxicity to avian species are therefore only available for the active substances. Accordingly, the risk assessment was based on these data, which were considered sufficient.

Zoxamide and Phosphonic acid have different chemical structures and mechanisms of action against the target organisms and display their toxicological effect on different target organs. Moreover, the ecotoxicological studies with the formulated product GWN-10616 discussed in this section give no indication of increased toxicity for the formulated product compared to data for the active substances alone, indicating that the two actives are unlikely to display synergistic effects.

According to the conservative approach indicated by EFSA Guidance Document, the acute risk for formulated products containing more than one active substance should be evaluated assuming additive

properties of the actives. For the assessment of acute effects, a surrogate LD_{50} is calculated using the formula:

LD_{50} (mix) = ($\sum$ X(a.s.$_i$) / LD_{50} (a.s. $_i$))$^{-1}$

with:

X (a.s.$_i$) = fraction of the active substance [i] in the mixture (the sum of fraction (a.s.$_i$) must be 1)
LD_{50} (a.s.$_i$) = acute toxicity for the active substance [i]

The formulated product contains two active substances, the fraction X (a.s.$_i$) for Zoxamide and Phosphonic acid is 0.11 and 0.89, respectively. Using the above presented ecotoxicological endpoints in the formula, the surrogate LD_{50} is calculated, and the results are presented in the following table.

Table 9.2-2: Calculation of surrogate $LD_{50\ mix}$ value for acute effect assessment of the product GWN-10616 containing Zoxamide and Phosphonic acid

Substance	LD_{50} (mg a.s./kg bw)	Amount in product	X (a.s.)	TU (%)	$LD_{50\ mix}$ (mg/kg bw)
Zoxamide	>2000	60 g/L	0.11	12	2220.3
Phosphonic acid	>2250	500 g/L	0.89	88	

TU = toxic units

For acute risk assessment a $LD_{50\ mix}$ of 2220.3 mg/kg bw was calculated for the combined toxicity of active substances Zoxamide and Phosphonic acid in the product GWN-10616. No substance is driving the toxicity of the mixture (i.e. TU > 90%) therefore a respective combined risk assessment is presented below.

With regard to the reproductive risk from exposure to more than one active substance, it is currently not recommended to consider the use of predicted toxicity values as surrogate in the risk assessment Appendix B of the Guidance Document (EFSA, 2009). Regardless, select Member States have requested evaluation of the need for combined risk assessment considering reproductive endpoints for birds as well. Due to the lack of an overall evaluation approach, the method provided by EFSA/2009/1438 (described above) is also used for chronic combination toxicity. Sublethal effects and effects on reproduction are assessed on a case-by-case basis.

Table 9.2-3: Calculation of surrogate $NOEL_{mix}$ value for long-term effect assessment of the product GWN-10616 containing Zoxamide and Phosphonic acid

Substance	NOEL (mg a.s./kg bw/d)	Amount in product	X (a.s.)	TU (%)	$NOEL_{mix}$ (mg/kg bw/d)
Zoxamide	122.8	60 g/L	0.11	13	145.7
Phosphonic acid	149.04	500 g/L	0.89	87	

TU = toxic units

For long-term risk assessment a $NOEL_{mix}$ of 145.7 mg/kg bw/d was calculated for the combined toxicity of active substances Zoxamide and Phosphonic acid in the product GWN-10616. No substance is driving the toxicity of the mixture (i.e. TU > 90%) therefore a respective combined risk assessment is presented below.

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

Birds may be exposed to residues of Zoxamide and Phosphonic acid mainly by the consumption of

contaminated feed following the dilution and spraying of the formulated product. The exposure towards Potassium phosphonates in the following is expressed in equivalents of Phosphonic acid.
The potential exposure of birds to GWN-10616 was estimated following 3 applications at 3 L product/ha with an 8-day interval in vine, 2 applications at 3 L product/ha with a 6-day interval in pome fruit and 3 applications at 2.5 L product/ha with a 7-day interval in potato.

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

Table 9.2-4: Screening assessment of the acute and long-term risk for birds due to the use of GWN-10616 – Zoxamide

Acute toxicity [mg a.s./kg bw]	> 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg a.s./kg bw/d]**	**TER_A**
Grapevine BBCH 14-79 3 × 0.18 kg a.s./ha	Small omniv. bird	95.3	1.57 (8-day interval)	26.93	> 74.3
Pome fruit BBCH 51-69 2 × 0.18 kg a.s./ha	Small insectiv. bird	46.8	1.46 (6-day interval)	12.30	> 162.6
Potato BBCH 21-89 3 × 0.15 kg a.s./ha	Small omniv. bird	158.8	1.64 (7-day interval)	39.06	> 51.2
Reprod. toxicity [mg a.s./kg bw/d]	122.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg a.s./kg bw/d]**	**TER_{LT}**
Grapevine BBCH 14-79 3 × 0.18 kg a.s./ha	Small omniv. bird	38.9	1.01 (8-day interval)	7.07	17.4
Pome fruit BBCH 51-69 2 × 0.18 kg a.s./ha	Small insectiv. bird	18.2	0.9 (6-day interval)	2.95	41.6
Potato BBCH 21-89 3 × 0.15 kg a.s./ha	Small omniv. bird	64.8	1.06 (7-day interval)	10.30	11.9

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.

Acceptable acute and long-term risk (screening step) for birds from exposure to Zoxamide after application of GWN-10616 is demonstrated for the intended uses in grapevine, pome fruit and potato according to GAP.

Table 9.2-5: Screening and first-tier risk assessment of the acute and long-term risk for birds due to the use of GWN-10616 – Phosphonic acid

Acute toxicity [mg a.s./kg bw]	> 2250				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg a.s./kg bw/d]**	**TER_A**
Grapevine BBCH ~~15~~ 14-79 3 × 1.5 kg a.s./ha	Small omniv. bird	95.3	1.57 (8-day interval)	224.43	> 10.0
Pome fruit BBCH 51-69 2 × 1.5 kg a.s./ha	Small insectiv. bird	46.8	1.46 (6-day interval)	102.49	> 21.95
Potato BBCH 21-89 3 × 1.25 kg a.s./ha	Small omniv. bird	158.8	1.64 (7-day interval)	325.54	**> 6.9**
BBCH 10-39 covering BBCH ≥ 40	Small omniv. bird “Lark”	24.0	1.64	49.20	> 45.7
BBCH ≥ 20	Small insectiv. bird “Wagtail”	25.2		51.66	> 43.6
Reprod. toxicity [mg a.s./kg bw/d]	149.04				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg a.s./kg bw/d]**	**TER_{LT}**
Grapevine BBCH ~~15~~ 14-79 3 × 1.5 kg a.s./ha	Small omniv. bird	38.9	1.01 (8-day interval)	58.76	**2.5**
BBCH 10-19 covering BBCH ≥ 20	Small insectivorous species “Redstart”	11.5	1.01 (8-day interval)	17.371	8.6
BBCH 10-19 covering BBCH 20-39 and BBCH ≥ 40	Small granivorous bird “Finch”	6.9		10.422	14.3
Ripening	Frugivorous bird “Trush/Starling”	14.4		21.751	6.9
BBCH 10-19 Covering BBCH 20-39 and BBCH ≥ 40	Small omnivorous bird “Lark”	6.5		9.818	15.2
Pome fruit BBCH 51-69 2 × 1.5 kg a.s./ha	Small insectiv. bird	18.2	0.9 (6-day interval)	24.57	6.1
Potato BBCH 21-89 3 × 1.25 kg a.s./ha	Small omniv. bird	64.8	1.06 (7-day interval)	85.86	**1.7**
BBCH 10-39 covering BBCH ≥ 40	Small omniv. bird “Lark”	10.9	1.06	14.44	10.3
BBCH ≥ 20	Small insectiv. bird “Wagtail”	9.7		12.85	11.6

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

Acceptable acute (screening and first-tier assessment step) and long-term risk (first-tier assessment step) for birds from exposure to Phosphonic acid after application of GWN-10616 is demonstrated for the intended uses in grapevine, pome fruit and potato according to GAP.

Following the assessment of single active substances, an assessment on combined toxicity is presented.

Table 9.2-6: Screening and first-tier assessment of the acute risk for birds due to the use of GWN-10616 containing active substances Zoxamide and Phosphonic acid – combined toxicity

Active substance/product		GWN-10616			
Acute toxicity [mg a.s./kg bw]		EP (mix) = 2220.3			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg a.s./kg bw/d]**	**TER_A**
Grapevine BBCH ~~15~~ 14-79 3 × 3 L/ha (3 × 1680 g sum of a.s./ha = 180 g Zoxamide + 1500 g Phosphonic acid)					
Screening	Small omniv. bird	95.3	1.57	251.63	**8.8**
BBCH 10-19 covering BBCH ≥ 20	Small insectivorous species “Redstart”	27.4	1.57 (8-day interval)	72.347	30.7
BBCH 10-19 covering BBCH 20-39 and BBCH ≥ 40	Small granivorous bird “Finch”	14.8		39.078	56.8
Ripening	Frugivorous bird “Trush/Starling”	28.9		76.307	29.1
BBCH 10-19 Covering BBCH 20-39 and BBCH ≥ 40	Small omnivorous bird “Lark”	14.4		38.022	58.4
Pome fruit BBCH 51-69 2 × 3 L/ha (2 × 1680 g sum of a.s./ha = 180 g Zoxamide + 1500 g Phosphonic acid)					
Screening	Small insectiv. bird	46.8	1.46 (6-day interval)	114.791	19.3
Potato BBCH 21-89 3 × 2.5 L/ha (3 × 1400 g sum of a.s./ha = 150 g Zoxamide + 1250 g Phosphonic acid)					
Screening	Small omniv. bird	158.8	1.64	364.60	**6.1**
BBCH 10-39 covering BBCH ≥ 40	Small omniv. bird “Lark”	24.0	1.64 (7-day interval)	55.10	40.3
BBCH ≥ 20	Small insectiv. bird “Wagtail”	25.2		57.86	38.4

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

Acceptable acute risk for birds from exposure to Zoxamide and Phosphonic acid (combined toxicity considering surrogate $LD_{50\ mix}$ of 2220.3 mg/kg bw) after application of GWN-10616 is demonstrated for the intended uses in grapevine, pome fruit and potato according to GAP.

Table 9.2-7: Screening and first-tier assessment of the long-term risk for birds due to the use of GWN-10616 containing active substances Zoxamide and Phosphonic acid – combined toxicity

Active substance/product		GWN-10616			
Reprod. toxicity [mg a.s./kg bw/d]		EP (mix) = 145.7			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg a.s./kg bw/d]**	**TER_{LT}**
Grapevine BBCH 15 14-79 3 × 3 L/ha (3 × 1680 g sum of a.s./ha = 180 g Zoxamide + 1500 g Phosphonic acid)					
Screening	Small omniv. bird	38.9	1.01	65.81	**2.2**
BBCH 10-19 covering BBCH ≥ 20	Small insectivorous species "Redstart"	11.5	1.01 (8-day interval)	19.455	7.5
BBCH 10-19 covering BBCH 20-39 and BBCH ≥ 40	Small granivorous bird "Finch"	6.9		11.673	12.5
Ripening	Frugivorous bird "Trush/Starling"	14.4		24.361	6.0
BBCH 10-19 Covering BBCH 20-39 and BBCH ≥ 40	Small omnivorous bird "Lark"	6.5		10.996	13.2
Pome fruit BBCH 51-69 2 × 3 L/ha (2 × 1680 g sum of a.s./ha = 180 g Zoxamide + 1500 g Phosphonic acid)					
Screening	Small insectiv. bird	18.2	0.9 (6-day interval)	27.518	5.3
Potato BBCH 21-89 3 × 2.5 L/ha (3 × 1400 g sum of a.s./ha = 150 g Zoxamide + 1250 g Phosphonic acid)					
Screening	Small omniv. bird	64.8	1.06	96.16	**1.5**
BBCH 10-39 covering BBCH ≥ 40	Small omniv. bird "Lark"	10.9	1.06 (7-day interval)	16.18	9.0
BBCH ≥ 20	Small insectiv. bird "Wagtail"	9.7		14.39	10.1

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.

Acceptable long-term risk for birds from exposure to Zoxamide and Phosphonic acid (combined toxicity considering surrogate $NOEL_{mix}$ of 145.7 mg/kg bw/d) after application of GWN-10616 is demonstrated for the intended uses in grapevine, pome fruit and potato according to GAP.

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since GWN-10616 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha; considering multiple application) 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 1179, Zoxamide belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	180			
Acute toxicity (mg/kg bw) =	> 2000	quotient	=	0.09
Reprod. toxicity (mg/kg bw/d) =	122.8	quotient	=	1.5

With a K(f)oc of 721, Potassium phosphonates/ Phosphonic acid belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	1500 (Phosphonic acid equivalents)			
Acute toxicity (mg/kg bw) =	> 2250	quotient	=	0.7
Reprod. toxicity (mg/kg bw/d) =	149.04	quotient	=	10.1

Review Comments:

The effective application rate should be calculated using following equations:

AR_{eff} = Application rate (g/ha) x MAF_{mean}

where

MAFm = $(1-e^{-nki}) / (1-e^{-ki})$ with k = $ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

As the applicant used the cumulative dose in risk assessment, the calculations were not adjusted.

Since for both active substances the trigger of 3000 is not exceeded, no specific calculations of exposure and TER are necessary.

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. In addition, Zoxamide metabolites RH-127450 and RH-24549 have log P_{ow} values > 3 and were both identified as being relevant for the soil and aquatic risk assessments. They have the potential to bioaccumulate and therefore a secondary poisoning assessment is presented.
According to the relevant guidance document, in the absence of data on metabolites an additional safety factor of 10 is applied to the endpoint for the parent compound. Therefore, for the metabolites RH-24549 and RH-127450, the toxicity endpoint to be used is:
Chronic endpoint birds: **12.28 mg/kg bw/d**

The log P_{ow} of Potassium phosphonates/ Phosphonic acid is very low (-0.7699; pH = 7), 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 predicted concentrations in soil.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, always the scenario with the highest PEC value was taken into account for the risk assessment.

Table 9.2-8: Assessment of the risk for earthworm-eating birds due to exposure to Zoxamide and relevant metabolites RH-127450 and RH-24549 via bioaccumulation in earthworms (secondary poisoning)

Parameter	Zoxamide	RH-127450	RH-24549	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.2220*	0.0204*	0.0299*	See Part B, Section 8.7.2
log P_{ow} / P_{ow}	3.76 / 5754.4	3.5 / 3162	3.83 / 6760	EFSA (2017) + RAR (2017)
Koc	1179	593	~~161~~ 90.5	Geomean (n = 4; n= 3; n= 3)
Foc	0.02	0.02	0.02	Default
BCF_{worm}	2.964	3.270	~~25.45~~ 45.28	$BCF_{worm/soil}$ = ($PEC_{worm,ww}/PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	0.658	0.067	~~0.761~~ 1.35	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.691	0.070	~~0.799~~ 1.42	DDD = PEC_{worm} × 1.05
NOEL (mg/kg bw/d)	122.8	12.28**	12.28**	
TER_{lt}	177.7	175.4	~~15.4~~ 8.6	Trigger = 5

*Maximum PEC_{soil} twa values from application in grapevine.
**In the absence of toxicity data, the metabolite is considered as 10 times more toxic than the parent compound.
TER values shown in **bold** fall below the relevant trigger.

As a result, earthworm-eating birds are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

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.
To achieve a concise risk assessment, the risk envelope approach is applied. Here, always the scenario with the highest PEC value was taken into account for the risk assessment.

Table 9.2-9: Assessment of the risk for fish-eating birds due to exposure to Zoxamide and relevant metabolites RH-127450 and RH-24549 via bioaccumulation in fish (secondary poisoning)

Parameter	Zoxamide	RH-127450	RH-24549	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0087*	0.0077*	0.0036*	See Part B, Section 8.9.2
BCF_{fish}	136	136	136	EFSA (2017)
BMF	-	-	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	1.182	1.047	0.490	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.188	0.166	0.078	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	122.8	12.28**	12.28**	
TER_{lt}	653.2	74.0	157.4	Trigger = 5

*Max. FOCUS Step 2 PEC_{sw} twa values from application in pome fruit (Zoxamide, RH-127450) and grapevine (RH-24549).
**In the absence of toxicity data, the metabolite is considered as 10 times more toxic than the parent compound.
TER values shown in **bold** fall below the relevant trigger.

As a result, fish-eating birds are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

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

Risk assessment for exposure via directly contaminated diet
Based on screening and first-tier assessment steps, the calculated TER values for the acute and long-term risk resulting from an exposure of birds to Zoxamide and/or Phosphonic acid (oral exposure) according to the GAP of the formulation GWN-10616 do achieve the acceptability criteria TER ≥ 10 and TER ≥ 5, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for acute and chronic effects, respectively.

Drinking water exposure
Based on the assessment of the risk arising from the uptake of Zoxamide and/or Phosphonic acid via drinking water, a TER calculation is not necessary. A low risk can be concluded.

Risk assessment for exposure via secondary poisoning
Risk to vermivorous and piscivorous birds was assessed for Zoxamide and relevant metabolites RH-127450 and RH-24549 according to EFSA/2009/1438. Earthworm-eating and fish-eating birds are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

Review Comments:

The acute and chronic risks of GWN-10616 to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with active ingredients, and maximum residues occurring on food items.

All TER values exceed the relevant triggers indicating that GWN-10616 does not pose an unacceptable risk to birds following applications according to recommended use pattern.

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

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 active substances Zoxamide and Phosphonic acid (active component of Potassium phosphonates). Full details of these studies are provided in the respective EU assessment reports and related documents. New toxicity data submitted for zoxamide metabolites are summarised in Part B Section 6.

Effects on mammals of GWN-10616 were not evaluated as part of the EU assessment of the contained active substances. The provision of further acute oral data on GWN-10616 is not considered essential, because both substances are not considerably toxic to vertebrates in general and specially to mammals. because there is no indication of synergistic effects justifying additional vertebrate testing. For more details, please refer to Part B Section 6.

Endpoints used for the risk assessment are summarised below. The selection of studies and endpoints for the risk assessment is in line with 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
Zoxamide				
Rat	Zoxamide	Oral Acute	**LD_{50} > 5000 mg/kg bw**	EFSA (2017)
Rat	Z oxamide	Long-term (parental)	NOAEL = 5000 mg/kg bw (= 360 mg/kg bw/d)	EFSA (2017)
Rat	Zoxamide	Reproductive	NOAEL > 20000 mg/kg bw (= 1 474 mg/kg bw/d)	EFSA (2017)
Rat	Zoxamide	Long-term (offspring)	NOAEL = 5000 mg/kg bw (= 360 mg/kg bw/d)	EFSA (2017)
Rabbit	Zoxamide	Long-term (development)	NOAEL = 1000 mg/kg bw/d	EFSA (2017)
Rat	Zoxamide	Long term (development)	NOAEL = 1000 mg/kg bw/d	EFSA (2017)

Species	Substance	Exposure System	Results	Reference
Rat	Zoxamide		**NOAEL = 71** (Value agreed in the Peer review meeting 160 by experts)	EFSA (2017)
Phosphonic acid*				
Rat	Potassium phosphonates	Oral Acute	**LD_{50} > 1736 mg/kg bw (phosphonic acid equivalents)**	EFSA (2012)
Rat	Fosetyl-aluminium	Long-term (offspring)	**NOEL = 302.9 mg a.s./kg bw/d (phosphonic acid equivalents)**	EFSA (2012)

*Phosphonic acid (also Phosphorous acid) is the actual active substance of the technical item Potassium phosphonates (formerly: phosphite; technical active substance).
Bold letters: endpoints used for the risk assessment

Relevance of metabolites
Please refer to information provided in chapter 9.2.1.

9.3.1.1 Justification for new endpoints

All endpoints are in agreement with EU review data.

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).

Assessment of combined toxicity

Data on the toxicity to mammalian species are only available for the active substances. Accordingly, the risk assessment was based on these data, which were considered sufficient.

Zoxamide and Phosphonic acid have different chemical structures and mechanisms of action against the target organisms and display their toxicological effect on different target organs. Moreover, the ecotoxicological studies with the formulated product GWN-10616 discussed in this section give no indication of increased toxicity for the formulated product compared to data for the active substances alone, indicating that the two actives are unlikely to display synergistic effects.

According to the conservative approach indicated by EFSA Guidance Document, the acute risk for formulated products containing more than one active substance should be evaluated assuming additive properties of the actives. For the assessment of acute effects, a surrogate LD_{50} is calculated using the formula:

$$LD_{50}\ (mix) = (\sum X(a.s._i) / LD_{50}\ (a.s._i))^{-1}$$

with:

X (a.s.$_i$) = fraction of the active substance [i] in the mixture (the sum of fraction (a.s.$_i$) must be 1)
LD_{50} (a.s.$_i$) = acute toxicity for the active substance [i]

The formulated product contains two active substances, the fraction X (a.s.$_i$) for Zoxamide and Phosphonic acid is 0.11 and 0.89, respectively. Using the above presented ecotoxicological endpoints in the formula, the surrogate LD_{50} is calculated and the results are presented in the following table.

Table 9.3-2: Calculation of surrogate $LD_{50\ mix}$ value for acute effect assessment of the product GWN-10616 containing Zoxamide and Phosphonic acid

Substance	LD_{50} (mg a.s./kg bw)	Amount in product	X (a.s.)	TU (%)	$LD_{50\ mix}$ (mg/kg bw)
Zoxamide	>5000	60 g/L	0.11	4	1868.9
Phosphonic acid	>1736	500 g/L	0.89	96	

TU = toxic units

For acute risk assessment a $LD_{50\ mix}$ of 1868.9 mg/kg bw was calculated for the combined toxicity of active substances Zoxamide and Phosphonic acid in the product GWN-10616. Phosphonic acid is driving the toxicity of the mixture (i.e. TU > 90%) therefore a respective combined risk assessment is not considered necessary and the acute risk assessment of Phosphonic acid is covering the potential acute effects of the mixture.

With regard to the reproductive risk from exposure to more than one active substance, it is currently not recommended to consider the use of predicted toxicity values as surrogate in the risk assessment Appendix B of the Guidance Document (EFSA, 2009). Regardless, select Member States have requested evaluation of the need for combined risk assessment considering reproductive endpoints for mammals as well. Due to the lack of an overall evaluation approach, the method provided by EFSA/2009/1438 (described above) is also used for chronic combination toxicity. Sublethal effects and effects on reproduction are assessed on a case-by-case basis.

Table 9.3-3: Calculation of surrogate $NOEL_{mix}$ value for long-term effect assessment of the product GWN-10616 containing Zoxamide and Phosphonic acid

Substance	NOEL (mg a.s./kg bw/d)	Amount in product	X (a.s.)	TU (%)	$NOEL_{mix}$ (mg/kg bw/d)
Zoxamide	71	60 g/L	0.11	34	223.4
Phosphonic acid	302.9	500 g/L	0.89	66	

TU = toxic units

For long-term risk assessment a $NOEL_{mix}$ of 223.4 mg/kg bw/d was calculated for the combined toxicity of active substances Zoxamide and Phosphonic acid in the product GWN-10616. No substance is driving the toxicity of the mixture (i.e. TU > 90%) therefore a respective combined risk assessment is presented below.

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

Terrestrial mammals may be exposed to residues of Zoxamide and Phosphonic acid mainly by the consumption of contaminated feed following the dilution and spraying of the formulated product. The exposure towards Potassium phosphonates in the following is expressed in equivalents of Phosphonic acid.
The potential exposure of mammals to GWN-10616 was estimated following 3 applications at 3 L

product/ha with an 8-day interval in grapevine and 2 applications at 3 L product/ha with a 6-day interval in pome fruit and 3 applications at 2.5 L product/ha with a 7-day interval in potato.

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

Table 9.3-4: Screening assessment step of the acute and long-term risk for mammals due to the use of GWN-10616 - Zoxamide

Acute toxicity [mg a.s./kg bw]		> 5000			
TER criterion		10			
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90}** **[mg a.s./kg bw/d]**	**TER_A**
Grapevine **BBCH 14-79** 3 × 0.18 kg a.s./ha	Small herbiv. mammals	136.4	1.57 (8-day interval)	38.55	> 129.7
Pome fruit **BBCH 51-69** 2 × 0.18 kg a.s./ha			1.46 (6-day interval)	35.85	> 139.5
Potato **BBCH 21-89** 3 × 0.15 kg a.s./ha	Small herbiv. mammals	118.4	1.64 (7-day interval)	29.13	> 171.6
Reprod. toxicity [mg a.s./kg bw/d]		71			
TER criterion		5			
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m** **[mg a.s./kg bw/d]**	**TER_{LT}**
Grapevine **BBCH 14-79** 3 × 0.18 kg a.s./ha	Small herbiv. mammals	72.3	1.01 (8-day interval)	13.11	5.4
Pome fruit **BBCH 51-69** 2 × 0.18 kg a.s./ha			0.9 (6-day interval)	11.71	6.1
Potato **BBCH 21-89** 3 × 0.15 kg a.s./ha	Small herbiv. mammals	48.3	1.06 (7-day interval)	7.68	9.2

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

Acceptable acute and long-term risk (screening step) for mammals from exposure to Zoxamide after application of GWN-10616 is demonstrated for the intended uses in grapevine, pome fruit and potato according to GAP.

Table 9.3-5: Screening and first-tier risk assessment of the acute and long-term risk for mammals due to the use of GWN-10616 – Phosphonic acid

Acute toxicity [mg a.s./kg bw]	> 1736				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [mg a.s./kg bw/d]**	**TER_A**
Grapevine BBCH 14-79 3 × 1.5 kg a.s./ha	Small herbiv. mammals	136.4	1.57 (8-day interval)	321.56	**> 5.4**
BBCH 10-19 covering BBCH 20-39 and BBCH ≥ 40	Large herbiv. mammal “lagomorph”	16.3		38.43	> 45.2
BBCH 10-19 covering BBCH ≥ 20	Small insectiv. mammal “shrew”	7.6		17.92	> 96.9
BBCH 10-19	Small herbiv. mammal “vole”	81.9		193.08	**> 9.0**
BBCH 20-39 covering BBCH ≥ 40	Small herbiv. mammal “vole”	68.2		160.78	> 10.8
BBCH 10-19 covering BBCH 20-39 and BBCH ≥ 40	Small omniv. mammal “mouse”	10.3		24.28	> 71.5
Pome fruit BBCH 51-69 2 × 1.5 kg a.s./ha	Small herbiv. mammals	136.4	1.46 (6-day interval)	298.72	**> 5.8**
BBCH ≥ 40	Small herbiv. mammal “vole”	40.9		98.57	> 17.6
BBCH ≥ 40	Large herbiv. mammal “lagomorph”	10.5		22.99	> 75.5
BBCH ≥ 40	Small omniv. mammal “mouse”	5.2		11.39	> 152.4
Potato BBCH 21-89 3 × 1.25 kg a.s./ha	Small herbiv. mammals	118.4	1.64 (7-day interval)	242.72	**> 7.2**
BBCH ≥ 20	Small insectivorous mammal “shrew”	5.4		11.07	156.8
BBCH ≥ 40	Small herbivorous mammal “vole”	40.9		83.85	20.7
BBCH ≥ 10 covering BBCH ≥ 40	Large herbiv. mammal “lagomorph”	35.1		71.96	24.1
BBCH ≥ 10 covering BBCH ≥ 40	Small omnivorous mammal “mouse”	17.2		35.26	49.2
Reprod. toxicity [mg a.s./kg bw/d]	302.9				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg a.s./kg bw/d]**	**TER_{LT}**
Grapevine BBCH 14-79 3 × 1.5 kg a.s./ha	Small herbiv. mammals	72.3	1.01 (8-day interval)	109.2	**2.8**
BBCH ≥ 10 covering BBCH 20-39 and BBCH ≥ 40	Large herbiv. mammal “lagomorph”	6.7		10.12	29.9
BBCH ≥ 10 covering BBCH ≥ 20	Small insectiv. mammal “shrew”	4.2		6.34	47.7

BBCH 10-19	Small herbiv. mammal "vole"	43.4		65.56	**4.6**
BBCH ≥ 20 covering BBCH ≥ 40	Small herbiv. mammal "vole"	36.1		54.53	5.6
BBCH ≥ 10 covering BBCH 20-39 and BBCH ≥ 40	Small omniv. mammal "mouse"	4.7		7.10	42.7
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg a.s./kg bw/d]**	**TER_{LT}**
Pome fruit BBCH 51-69 2 × 1.5 kg a.s./ha	Small herbiv. mammals	72.3	0.9 (6-day interval)	97.61	**3.1**
BBCH ≥ 40	Small herbiv. mammal "vole"	21.7		29.29	10.3
BBCH ≥ 40	Large herbiv. mammal "lagomorph"	4.3		5.81	52.1
BBCH ≥ 40	Small omniv. mammal "mouse"	2.3		3.11	97.4
Potato BBCH 21-89 3 × 1.25 kg a.s./ha	Small herbiv. mammals	48.3	1.06 (7-day interval)	64.0	**4.7**
BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9		2.52	120.2
BBCH ≥ 40	Small herbivorous mammal "vole"	21.7		28.75	10.5
BBCH ≥ 10 covering BBCH ≥ 40	Large herbiv. mammal "lagomorph"	14.3		18.95	16.0
BBCH ≥ 10 covering BBCH ≥ 40	Small omnivorous mammal "mouse"	7.8		10.34	29.3

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

Acceptable acute and long-term risk (first-tier assessment step) for mammals from exposure to Phosphonic acid after application of GWN-10616 is demonstrated for the intended uses in pome fruit and potato according to GAP.
For grapevine, the screening and first-tier risk assessment step for Phosphonic acid resulted in an acute and long-term risk for small herbivorous mammal "vole" (grapevine BBCH 10-19) after application of GWN-10616. Since the relevant endpoint for acute risk assessment of Phosphonic acid is the LD_{50} of > 1736 mg a.s./kg bw and no toxicity has been observed at this dose level (no mortalities, no effects on body weight, no clinical signs of systemic toxicity and no changes at necropsy examination; Allen (2000)), the definite LD_{50} is considered to lie far above 1736 mg/kg bw. Therefore, the slight breach of the trigger (calculated TER of > 9 below the trigger of 10) is considered acceptable and exposure to Phosphonic acid after application in grapevine according to GAP will result in acceptable acute risk to mammals.

Review Comments:

zRMS agrees with Applicant's position. An acceptable acute risk to mammals can be concluded for phosphonic acid after application in grapevine according to GAP.

Regarding the long-term risk to voles, please refer to the higher tier assessment.

Following the assessment of single active substances, an assessment on combined toxicity is presented. As for the acute assessment the risk of the mixture is driven by Phosphonic acid (toxic units > 90%) only the chronic assessment of mixture toxicity is presented.

Table 9.3-6: Screening and first-tier assessment of the long-term risk for mammals due to the use of GWN-10616 containing active substances Zoxamide and Phosphonic acid – combined toxicity

Active substance/product		GWN-10616			
Reprod. toxicity [mg a.s./kg bw/d]		EP (mix) = 223.4			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [mg a.s./kg bw/d]**	**TER_{LT}**
Grapevine BBCH 14-79 3 × 3 L/ha (3 × 1680 g sum of a.s./ha = 180 g Zoxamide + 1500 g Phosphonic acid)					
Screening	Small herbiv. mammals	72.3	1.01 (8-day interval)	122.68	**1.8**
BBCH 10-19 covering BBCH 20-39 and BBCH ≥ 40	Large herbiv. mammal “lagomorph”	6.7		11.37	19.7
BBCH 10-19 covering BBCH ≥ 20	Small insectiv. mammal “shrew”	4.2		7.13	31.3
BBCH 10-19	Small herbiv. mammal “vole”	43.4		73.64	**3.0**
BBCH 20-39	Small herbiv. mammal “vole”	36.1		61.25	**3.6**
BBCH ≥ 40	Small herbiv. mammal “vole”	21.7		36.82	6.1
BBCH 10-19 covering BBCH 20-39 and BBCH ≥ 40	Small omniv. mammal “mouse”	4.7		7.97	28.0
Pome fruit BBCH 51-69 2 × 3 L/ha (2 × 1680 g sum of a.s./ha = 180 g Zoxamide + 1500 g Phosphonic acid)					
Screening	Small herbiv. mammals	72.3	0.9 (6-day interval)	109.32	**2.0**
BBCH ≥ 40	Small herbiv. mammal “vole”	21.7		32.81	6.8
BBCH ≥ 40	Large herbiv. mammal “lagomorph”	4.3		6.50	34.4
BBCH ≥ 40	Small omniv. mammal “mouse”	2.3		3.48	64.2
Potato BBCH 21-89 3 × 2.5 L/ha (3 × 1400 g sum of a.s./ha = 150 g Zoxamide + 1250 g Phosphonic acid)					
Screening	Small herbiv. mammals	48.3	1.06 (7-day interval)	71.68	**3.1**
BBCH ≥ 20	Small insectiv. mammal “shrew”	1.9		2.82	79.2
BBCH ≥ 40	Small herbiv. mammal “vole”	21.7		32.20	6.9
BBCH ≥ 10 covering BBCH ≥ 40	Large herbiv. mammal “lagomorph”	14.3		21.22	10.5
BBCH ≥ 10 covering BBCH ≥ 40	Small omniv. mammal “mouse”	7.8		11.58	19.3

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

Acceptable acute and long-term risk (first-tier assessment step) for mammals from exposure to

Phosphonic acid after application of GWN-10616 is demonstrated for the intended uses in pome fruit and potato according to GAP.
For grapevine and in line with the single active substance assessment of Phosphonic acid, first-tier risk assessment step for combined toxicity resulted in a long-term risk for small herbivorous mammal "vole" (grapevine BBCH 10-19 and BBCH 20-39). The relevant TER criterion was not met since TER values of 3.0 and 3.6 do not meet the trigger of 5, respectively. Please refer to the higher tier assessment.

9.3.2.2 Higher-tier risk assessment

The first-tier assessment step resulted in an unacceptable risk for small herbivorous mammals ("vole") after 3 applications at 3 L product/ha with an 8-day interval in grapevine (BBCH 10-19 and BBCH 20-39). A refined higher-tier assessment step is presented below.

In the public literature, data are available that suggest the diet of the common vole is highly dependent upon the age and reproductive status of the vole as well as the distance from the burrow where the plant material can be found (Rinke, 1991; as discussed and accepted in the Acetamiprid RAR 2016 Volume 3-CP B 9.2.3). Rinke (1991) indicates that even in the presence of a majority of monocotyledonous plants, the diet of voles consists of at least 50 % of dicotyledonous plants. Therefore, it is considered appropriately conservative to assume that 50 % of the vole diet comes from dicotyledonous plant materials (i.e. non-grass herbs) in the refined risk for long-term exposure.

Review Comments:

At the harmonization meeting of the central zone in December 2023, it was agreed that for monocot dominated underground (i.e. orchards, vines, hops, grassland and cereals), the PD of 0.75 and 0.25 for monocots and dicots, respectively, can be accepted in refined risk assessment for voles. Therefore, zRMS adjusted the TER calculations in the table 9.3-7, accordingly.

A higher-tier risk assessment considering the above mentioned refinement is presented in Table 9.3-7.

Table 9.3-7: Higher-tier assessment of the long-term risk for small herbivorous mammals due to the use of GWN-10616 in grapevine considering diet composition

Intended use **Active substance**		Grapevine (3 × 3 L product/ha, 8-day interval) Zoxamide, 60 g/L Phosphonic acid, 500 g/L						
Reprod. toxicity (mg/kg bw/d) **TER criterion**		302.9 mg Phosphonic acid/kg bw/d 5						
Focal species	**Food category, % in diet**	**FIR/bw**	**DF**	**RUD_m (mg/kg food)**	**MAF_m × twa**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{LT}**
Grapevine BBCH 14-79 3 × 1500 g Phosphonic acid/ha								
Vole BBCH 10-19	~~50~~ 75 % grass	~~0.73~~ 1.046	0.6	54.2	1.01	1	~~35.97 + 19.04 = 55.01~~ 51.53 + 9.10 = 60.63	~~5.5~~ 5.0
	~~50~~ 25 % non-grass herbs	~~0.73~~ 0.349		28.7				

Reprod. toxicity (mg/kg bw/d)	EP (mix) = 223.4							
TER criterion	5							
Grapevine BBCH 14-79 3 × 3 L/ha (3 × 1680 g sum of a.s./ha = 180 g Zoxamide + 1500 g Phosphonic acid)								
Vole BBCH 10-19	~~50~~ 75 % grass	~~0.73~~ 1.046	0.6	54.2	1.01	1	~~40.28 + 21.33 = 61.61~~ 57.72 + 10.20 =67.92	~~3.6~~ **3.3**
	~~50~~ 25 % non-grass herbs	~~0.73~~ 0.349		28.7				
Vole BBCH 20-39	~~50~~ 75 % grass	~~0.73~~ 1.046	0.5	54.2	1.01	1	~~33.57 + 17.77 = 51.34~~ 48.10 + 8.50 = 56.60	~~4.4~~ **3.95**
	~~50~~ 25 % non-grass herbs	~~0.73~~ 0.349		28.7				

FIR/bw: Food intake rate per body weight; DF: deposition factor (considering possible interception by the crop); RUD_m: mean residue unit dose; MAF: multiple application factor; twa = time weighted avarage; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

For Phosphonic acid, the long-term higher-tier risk assessment for the small herbivorous mammal "vole" considering diet composition results in a TER_{LT} value greater than the relevant trigger of 5 indicating acceptable risk following application of GWN-10616 in grapevine (BBCH 14-79) according to GAP.

For mixture toxicity, long-term higher-tier risk assessment for combined toxicity considering diet composition results in a risk for small herbivorous mammal "vole" (grapevine BBCH 10-19 and BBCH 20-39). The relevant TER criterion was not met since TER values of ~~3.6~~ 3.3 and ~~4.4~~ 3.95 do not meet the trigger of 5, respectively.
Since combined toxicity assessment for sublethal effects and effects on reproduction are assessed on a case-by-case basis the following information should be considered:

- The NOEL of 71 mg/kg bw/d for Zoxamide is based on body weight effects in offspring (F1 generation = around 9.5% and F2 generation =6.8%) linked to decrease in food consumption explained by palatability. This was agreed by experts at Pesticides Peer Review Meeting 160.
- The NOEL of 302.9 mg/kg bw/d for Phosphonic acid is based on effects on body weight during premating period of F2B generation and litter parameters.

Scientifically there is no validated model how to combine long-term No Observed Effect Levels resulting in a meaningful assessment for combined toxicity. In the case of Zoxamide and Phosphonic acid it is highly questionable if the agreed endpoints for long-term risk assessment can be combined and if the results of the combined assessment indicate a potentially higher risk to mammals compared to single active substance assessment.
Additionally, it should be considered that the vole is a worst-case generic focal species and common voles are frequently considered as pest organism and therefore vole control (using rodenticides) is carried out regularly. Taking this into consideration and due to the fact that single substance assessment did not indicate a risk, the overall risk to small herbivorous mammals after application of GWN-10616 in grapevine according to GAP is considered acceptable.

Review Comments:

The refinement of the risk assessment for mixture toxicity is a national issue. Therefore, the concerned Member States must decide individually on the acceptability of the use of GWN-10616 in vines in BBCH 14-39.
zRMS agrees with Applicant's position. Zoxamide and phosphonic acid have different chemical structures and mechanisms of action against the target organisms and display their toxicological effect on

different target organs. Zoxamide is a fungicide belonging to benzamides (toluamides). It inhibits the nuclear division by the disruption of cellular microtubules (FRAC code 22). Potassium phosphonate belongs to phosphonates group (FRAC code P07), and inhibit the growth of zoospores and mycelia production, by inhibiting the cell wall biosynthesis. In zRMS opinion, zoxamide and potassium phosphonate are unlikely to display synergistic effects.
Therefore, in zRMS opinion, an acceptable risk to mammals can be concluded after application in grapevine according to GAP.

zRMS amended the higher-tier risk assessment. It should be noted that the BBCH stage 20 – 39 does not occur in grapevine (BBCH Monograph on Growth stages of mono-and dicotyledonous plants, 2. Edition, 2001, Federal Biological Research Centre for Agriculture and Forestry, Germany), where leaf development (BBCH 10 – 19) is directly followed by inflorescence emergence (BBCH ≥ 50). Therefore, BBCH stage 20 – 39 should not be further considered in the risk assessment.

Furthermore, it should be considered that application starts at BBCH 14 and therefore only 1 or 2 applications will fall in the BBCH stages 14 – 19. Therefore, deposition values for BBCH 10 – 19 of 60 % is reflecting an unrealistic worst-case given that deposition at BBCH ≥ 50 is only 30 %. To account for this a geomean deposition value based on 1 x 60 % and 2 x 60 % deposition, respectively, is proposed for the higher-tier risk assessment (i.e. 37.8 % deposition for 1 early application, 47.6 % deposition for 2 early applications).

For vole 1 early application would result in a TER of 7.9 (BBCH 10-19; Phosphonic acid) and 5.2 (BBCH 10-19; sum of a.s.).

For vole 2 early applications would result in a TER of 6.3 (BBCH 10-19; Phosphonic acid) and 4.1 (BBCH 10-19; sum of a.s.).

Review Comments:

The risk assessment for vineyard was performed according to assumptions given in EFSA B&M guideline. All BBCH stages need to be considered in the TER calculations. Thus, Applicant statement is not accepted.

According to “Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology” (Version 2.0, August 2023), point 3.2.15, the interception values following EFSA Guidance Document to obtain DegT50 values (EFSA Journal 2014;12(5):3662), can be use in the Tier 2 risk assessment. It should be noted that this rule applies only to the later stages of crop growth.

In Appendix E of EFSA B&M guidance the following recommendation is given:

“It was concluded that estimation of residues on undergrowth vegetation using FOCUS interception factors would become increasingly uncertain with decreasing soil cover of the crop and increasing height of weeds in relation to the crop. Thus reliable predictions are only deemed possible where the largest part of the soil surface is actually covered by the crop from a bird’s eye view and undergrowth vegetation is clearly smaller than the crop plants. Weeds or grasses overgrowing the crop at those stages are deemed unlikely to occur in intensive agriculture, but would anyway not form a part of the diet of small to medium herbivores.”

Therefore, the revised DF is considered not acceptable.

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 1179, Zoxamide belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	180			
Acute toxicity (mg/kg bw) =	> 5000	quotient	=	0.04
Reprod. toxicity (mg/kg bw/d) =	71	quotient	=	2.5

With a K(f)oc of 721, Potassium phosphonates/ Phosphonic acid belongs to the group of more sorptive substances.

Effective application rate (g/ha) =	1500 (Phosphonic acid equivalents)			
Acute toxicity (mg/kg bw) =	> 1736	quotient	=	0.9
Reprod. toxicity (mg/kg bw/d) =	302.9	quotient	=	5.0

Review Comments:

The effective application rate should be calculated using following equations:

AR_{eff} = Application rate (g/ha) x MAF_{mean}

where

MAFm = $(1-e^{-nki}) / (1-e^{-ki})$ with k = $ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]

As the applicant used the cumulative dose in risk assessment, the calculations were not adjusted.

Since for both active substances the trigger of 3000 is not exceeded, no specific calculations of exposure and TER are necessary.

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. In addition, Zoxamide metabolites RH-127450 and RH-24549 have log P_{ow} values > 3 and were both identified as being relevant for the soil and aquatic risk assessments. They have the potential to bioaccumulate and therefore a secondary poisoning assessment is presented.
According to the relevant guidance document, in the absence of data on metabolites an additional safety factor of 10 is applied to the endpoint for the parent compound. Therefore, for the metabolites RH-24549 and RH-127450, the toxicity endpoint to be used is:
Chronic endpoint mammals: **7.1 mg/kg bw/d**

The log P_{ow} of Potassium phosphonates/ Phosphonic acid is very low (-0.7699; pH = 7), 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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, always the scenario with the highest PEC value was taken into account for the risk assessment.

Table 9.3-8: Assessment of the risk for earthworm-eating mammals due to exposure to Zoxamide and relevant metabolites RH-127450 and RH-24549 via bioaccumulation in earthworms (secondary poisoning)

Parameter	Zoxamide	RH-127450	RH-24549	Comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.2220*	0.0204*	0.0299*	See Part B, Section 8.7.2
log P_{ow} / P_{ow}	3.76 / 5754.4	3.5 / 3162	3.83 / 6760	EFSA (2017) + RAR (2017)
Koc	1179	593	~~161~~ 90.5	Geomean (n = 4; n= 3; n= 3)
Foc	0.02	0.02	0.02	Default
BCF_{worm}	2.964	3.270	~~25.45~~ 45.28	$BCF_{worm/soil}$ = ($PEC_{worm,ww}/PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	0.658	0.067	~~0.761~~ 1.35	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.842	0.086	0.974 1.73	DDD = PEC_{worm} × 1.28
NOEL (mg/kg bw/d)	71	7.1**	7.1**	
TER_{lt}	84.3	82.6	~~7.3~~ **4.10**	Trigger = 5

*Maximum PEC_{soil} twa values from application in grapevine.
**In the absence of toxicity data, the metabolite is considered as 10 times more toxic than the parent compound.
TER values shown in **bold** fall below the relevant trigger.

Review Comments:

As the refinement of the risk assessment for RH-24549 the confirmatory-like study evaluated by the RMS-LV for zoxamide in an interzonal procedure was used. The following information was taken from file: Zoxamide_confirmatory_like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC:
In case needed for refinement of the secondary poisoning risk assessment of RH-24549 for earthworm eating birds and mammals, a study on the bioaccumulation of [14C]-RH-24549 in earthworms is available and presented in the Appendix 2 to this document (Windisch, 2020; report no. MKC-004/5-20/V). This study demonstrates that RH-24549 – in case taken up by earthworms – is quickly degraded/eliminated. Therefore, it was not possible to determine substance specific bioaccumulation data. However, a con-servative bioaccumulation factor of BCFworm = 4.78 could be estimated by following the (substance un-specific) radioactivity signal in soil and earthworms.
Considering the multiply lower BCF value obtained from the study by Windisch, 2020, the risk is considered acceptable.

As a result, earthworm-eating mammals are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, always the scenario with the highest PEC value was taken into account for the risk assessment.

Table 9.3-9: Assessment of the risk for fish-eating mammals due to exposure to Zoxamide and relevant metabolites RH-127450 and RH-24549 via bioaccumulation in fish (secondary poisoning)

Parameter	Zoxamide	RH-127450	RH-24549	Comments
PEC_{sw} (twa = 21 d) (mg/L)	0.0087*	0.0077*	0.0036*	See Part B, Section 8.9.2
BCF_{fish}	136	136	136	EFSA (2017)
BMF	-	-	-	Biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	1.182	1.047	0.490	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.168	0.149	0.070	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	71	7.1*	7.1*	
TER_{lt}	422.6	47.7	101.4	Trigger = 5

*Max. FOCUS Step 2 PEC_{sw} twa values from application in pome fruit (Zoxamide, RH-127450) and grapevine (RH-24549).
**In the absence of toxicity data, the metabolite is considered as 10 times more toxic than the parent compound.
TER values shown in **bold** fall below the relevant trigger.

As a result, fish-eating mammals are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

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

Risk assessment for exposure via directly contaminated diet
Based on screening, first-tier and higher-tier assessment steps, the calculated TER values for the acute and long-term risk resulting from an exposure of mammals to Zoxamide and/or Phosphonic acid (oral exposure) according to the GAP of the formulation GWN-10616 do achieve the acceptability criteria TER ≥ 10 and TER ≥ 5, according to commission implementing regulation (EU) No 546/2011, Annex, Part I C, 2. Specific principles, point 2.5.2. for acute and chronic effects, respectively.

Drinking water exposure
Based on the assessment of the risk arising from the uptake of Zoxamide and/or Phosphonic acid via drinking water, a TER calculation is not necessary. A low risk can be concluded.

Risk assessment for exposure via secondary poisoning
Risk to vermivorous and piscivorous mammals was assessed for Zoxamide and relevant metabolites RH-127450 and RH-24549 according to EFSA/2009/1438. Earthworm-eating and fish-eating mammals are not at risk from secondary poisoning after application of GWN-10161 to grapevine, pome fruit or potato according to GAP.

Review Comments:

The acute and chronic risks of GWN-10161 to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with active ingredients and maximum residues occurring on food items.

All TER values exceed the relevant triggers in the screening step risk assessment for zoxamide (acute and chronic).

For phosphonic acid an aacceptable acute risk for mammals can be concluded for all intended uses of GWN-10161. The chronic TER values exceed the relevant triggers in the Tier 1 risk assessment for phosphonic acid except for uses in grapevine BBCH 10-19.

Based on the higher tier chronic risk assessment for phosphonic acid, where the PD values for voles were modified, the TERs exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects.

The combined risk assessment demonstrated the acceptable chronic risk for mammals for all intended uses of GWN-10161 except for uses in grapevine BBCH 10-19 and BBCH 20-39. Nevertheless, in zRMS opinion, zoxamide and potassium phosphonate are unlikely to display synergistic effects. Therefore, further evaluation is not required.

Evaluation of exposing to mammals through the drinking water demonstrated the acceptable risk. The potential risk of secondary poisoning is low.

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

All relevant data with regard to birds and mammals are presented in the respective risk assessments (B.9.2 and B.9.3, respectively). No additional relevant information was identified in open literature which can be taken into account in the risk assessment.

Thus, the risk to reptiles and amphibians is considered to be covered by the risk assessment of birds (Chapter 9.2) and mammals (Chapter 9.3) as well as by the assessment of fish (Chapter 9.5).

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, its relevant metabolites and Phosphonic acid. Full details of these studies are provided in the respective EU assessment reports and related documents. Additional information on Zoxamide and its metabolites to address data gaps identified during EU renewal process have been submitted to RMS Latvia.

Effects on aquatic organisms of GWN-10616 were not evaluated as part of the EU assessments. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Zoxamide	96 h, ft	**LC_{50} = 0.16 mg a.s./L $_{mm}$**	EFSA (2017)
Lepomis macrochirus	Zoxamide	96 h, ft	LC_{50} > 0.79 mg a.s./L $_{mm}$	EFSA (2017)
Pimephales promelas	Zoxamide	96 h, ft	LC_{50} > 0.208 mg a.s./L $_{mm}$ [#]	EFSA (2017)
Brachydanio rerio	Zoxamide	96 h, ft	LC_{50} > 0.73 mg a.s./L $_{mm}$	EFSA (2017)
Cyprinodon variegatus	Zoxamide	96 h, ft	LC_{50} > 0.85 mg a.s./L $_{mm}$	EFSA (2017)
Oncorhynchus mykiss	RH-139432	96 h, ft	**LC_{50} = 2 mg a.s./L $_{mm}$**	EFSA (2017)
Oncorhynchus mykiss	RH-24549	48 h, ss	**LC_{50} = 23 mg a.s./L $_{mm}$**	EFSA (2017)
Oncorhynchus mykiss	RH-127450	96 h, ss	**LC_{50} = 4.17 mg a.s./L $_{mm}$**	Submitted to RMS XXXX (2020)
Oncorhynchus mykiss	RH-163353	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	Submitted to RMS XXXX (2020)
Oncorhynchus mykiss	RH-141455	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	Submitted to RMS XXXX (2020)
Oncorhynchus mykiss	Zoxamide	95 d, ft, ELS	**NOEC = 0.00348 mg a.s./L $_{mm}$**	EFSA (2017)
Pimephales promelas	Zoxamide	202 d, ft, FLC	NOEC = 0.06 mg a.s./L $_{mm}$	EFSA (2017)
Danio rerio	Zoxamide	30 d, post-hatch, ft, ELS	NOEC ≥ 0.12 mg a.s./L $_{mm}$	EFSA (2017)
Cyprinodon variegatus	Zoxamide	34 d, ft, ELS	NOEC = 0.04 mg a.s./L $_{mm}$ EC_{10} = 0.093 mg a.s./L (fish wet weight)	Submitted to RMS XXXX (1998) and addendum by XXXX et al. (2020)
Lepomis macrochirus	Zoxamide	28 d, ft, bioaccumulation	BCF = 95-136 mg a.s./L $_{mm}$	EFSA (2017)
Aquatic invertebrates				
Daphnia magna	Zoxamide	48 h, ft	LC_{50} > 0.78 mg a.s./L $_{mm}$	EFSA (2017)
Mysidopsis bahia	Zoxamide	96 h, ft	**LC_{50} = 0.076 mg a.s./L $_{mm}$**	EFSA (2017)
Daphnia magna	RH-139432	48 h, ss	LC_{50} = 17 mg a.s./L $_{mm}$	EFSA (2017)
Americamysis bahia	RH-139432	96 h, s	**LC_{50} = 6.95 mg/L $_{mm}$**	Submitted to RMS Hugill (2020)
Daphnia magna	RH-24549	48 h, s	LC_{50} = 40 mg a.s./L $_{mm}$	EFSA (2017)
Americamysis bahia	RH-24549	96 h, s	**LC_{50} = 23.2 mg/L**	Submitted to RMS Hugill (2020)
Americamysis bahia	RH-127450	96 h, s	**LC_{50} = 2.93 mg/L $_{mm}$**	Submitted to RMS Hugil (2020)

Species	Substance	Exposure System	Results	Reference
Daphnia magna	RH-141455	48 h, s	LC_{50} > 100 mg/L $_{nom}$	Submitted to RMS Hugill (2020)
Americamysis bahia	RH-141455	96 h, ss	**LC_{50} > 100 mg/L $_{nom}$**	Submitted to RMS Hugill (2020)
Daphnia magna	RH-163353	48 h, s	LC_{50} > 100 mg/L $_{nom}$	Submitted to RMS Jarrom (2020)
Americamysis bahia	RH-163353	96 h, s	**LC_{50} > 100 mg/L $_{nom}$**	Submitted to RMS Jarrom (2020)
Daphnia magna	Zoxamide	21 d, ft	NOEC = 0.039 mg a.s./L $_{mm}$	EFSA (2017)
Mysidopsis bahia	Zoxamide	27 d, ft	**NOEC = 0.0072 mg a.s./L $_{mm}$**	EFSA (2017)
Chironomus riparius	Zoxamide	28 d, ft, spiked water	**$NOEC_{(emergence\ rate)}$ = 0.38 mg a.s./L** [##] $EC_{10\ (developmental\ rate)}$ = 0.223 mg a.s./L $EC_{10\ (emergence\ rate)}$ = 0.318 mg a.s./L	EFSA (2017)
Algae				
Selenastrum capricornutum	Zoxamide	72, 96, 120 h, s	**72-96 h-E_rC_{50} = 0.01413 mg a.s./L $_{mm}$** 120 h-E_bC_{50} = 0.023 mg a.s./L $_{mm}$	RAR (2017) Ziegler, Stewart (1996)
Anabaena flos-aquae	Zoxamide	96 h, s	E_rC_{50} > 0.86 mg a.s./L $_{mm}$ E_bC_{50} > 0.86 mg a.s./L $_{mm}$	RAR (2017) Drottar et al. (1998)
Scenedesmus subspicatus	Zoxamide	96 h, s	E_rC_{50} = 0.018 mg a.s./L $_{mm}$ E_bC_{50} = 0.011 mg a.s./L $_{mm}$	RAR (2017) Drottar et al. (1998)
Navicula pelliculosa	Zoxamide	96 h, s	E_rC_{50} > 0.93 mg a.s./L $_{mm}$ E_bC_{50} > 0.93 mg a.s./L $_{mm}$	RAR (2017) Drottar et al. (1998)
Skeletonema costatum	Zoxamide	96 h, s	E_rC_{50} > 0.91 mg a.s./L $_{mm}$ E_bC_{50} > 0.91 mg a.s./L $_{mm}$	RAR (2017) Drottar et al. (1998)
Scenedesmus subspicatus	RH-139432	72 h, s	**E_rC_{50} > 30 mg a.s./L $_{mm}$** E_bC_{50} = 26 mg a.s./L $_{mm}$	RAR (2017) Hoberg (2002)
Desmodesmus subspicatus	RH-24549	72 h, s	**E_rC_{50} > 60 mg a.s./L $_{nom}$** E_bC_{50} > 60 mg a.s./L $_{nom}$	EFSA (2017)
Pseudokirchneriella subcapitata	RH-141455	72 h, s	**EC_{50} > 100 mg a.s./L $_{nom}$**	EFSA (2017)
Raphidocelis subcapitata	RH-127450	72 h, s	**E_rC_{50} > 6.60 mg/L $_{mm}$** E_yC_{50} = 5.98 mg/L $_{mm}$	Submitted to RMS Hugill (2020)
Raphidocelis subcapitata	RH-163353	72 h, s	**E_rC_{50} > 100 mg/L** E_yC_{50} > 100 mg/L	Submitted to RMS Jarrom (2020)
Aquatic plants				
Lemna gibba	Zoxamide	7 d, ss	**E_rC_{50} = 0.0237 mg a.s./L $_{nom}$** E_yC_{50} = 0.0122 mg a.s./L $_{mm}$	Submitted to RMS Juckeland(2020)
Higher-tier studies (micro- or mesocosm studies)				

Species	Substance	Exposure System	Results	Reference

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

[#] Mistake in the EFSA Peer Review Conclusion (2017), which has been corrected based on XXXX (1998): RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*), report no. 97RC-0079, and based on the study summary in the RAR (2017).

[# #] In-line with the risk calculation during AIR / in the RAR (2017).

bold = worst-case endpoint taken forward for the aquatic risk assessment

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Potassium phosphonates/ Phosphonic acid*

Species	Substance	Exposure System	Results based on Phosphonic acid	Reference
Oncorhynchus mykiss	Potassium phosphonates TK	96 h, f	LC_{50} > 118 mg/L $_{mm}$	EFSA (2012)
Oncorhynchus mykiss	STAMINA (= LBG-01F34)	21 d, juvenile growth test, f	NOEC = 100 mg/L $_{nom}$	EFSA (2012)
Daphnia magna	Potassium phosphonates TK	48 h, f	EC_{50} > 118 mg/L $_{mm}$	EFSA (2012)
Daphnia magna	STAMINA (= LBG-01F34)	23 d, ss	NOEC = 100 mg/L $_{nom}$	EFSA (2012)
Chironomus riparius	STAMINA (= LBG-01F34)	28 d, s	NOEC = 100 mg/L $_{nom}$	EFSA (2012)
Pseudokirchneriella subcapitata	STAMINA (= LBG-01F34)	72 h, s	E_rC_{50} = 2339 mg/L $_{nom}$ E_bC_{50} = 146.7 mg/L $_{nom}$	DAR (2005) + EFSA (2012)
Higher-tier studies (micro- or mesocosm studies)				
-/-				

*Phosphonic acid (also Phosphorous acid) is the actual active substance of the technical item Potassium phosphonates (formerly: phosphite; technical active substance).

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

STAMINA (= LBG-01F34) is the representative mono-formulation assessed during EU review of Potassium phosphonates containing 755 g/L Potassium phosphonates corresponding to 504 g/L Phosphonic acid.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GWN-10616

Species	Substance	Exposure System	Results*	Reference
Oncorhynchus mykiss	GWN-10616	96 h, ss	LC_{50} = 34.8 mg/L $_{nom}$ equivalent to 1.46 mg Zoxamide/L + 12.21 mg Phosphonic acid/L	KCP 10.2.1/01 XXXX (2022) 821-001
Daphnia magna	GWN-10616	48 h, ss	EC_{50} > 100 mg/L $_{nom}$ equivalent to 4.2 mg Zoxamide/L + 35.09 mg Phosphonic acid/L	KCP 10.2.1/02 Corboli (2021) 822-001
Raphidocelis	GWN-10616	72 h, s	E_rC_{50} = 0.656 mg/L $_{nom}$	KCP 10.2.1/03

Species	Substance	Exposure System	Results*	Reference
subcapitata			equivalent to 0.028 mg Zoxamide/L + 0.230 mg Phosphonic acid/L E_yC_{50} = 0.351 mg/L $_{nom}$	Mantilacci (2021) 823-001
Higher-tier studies (micro- or mesocosm studies)				
-/-				

s: static; ss: semi-static; nom: based on nominal concentrations; mm: based on mean measured concentrations.
*Considering product density of 1.425 g/mL.

9.5.1.1 Justification for new endpoints

Acute endpoints for Zoxamide metabolites on fish
EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* To close these data gaps, acute toxicity studies with the metabolites RH-127450, RH-163353 and RH-141455 were provided to the RMS.

Review Comments:

zRMS agrees with Applicant's proposal. The confirmatory-like studies, were evaluated and accepted by the RMS-LV for zoxamide metabolites in an interzonal procedure. The endpoints from those tests were considered by RMS-LV to be applicable for the risk assessment. All details are to be found in the file: Zoxamide_ confirmatory_like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

Chronic endpoint for Zoxamide on fish
An additional fish ELS study with sheepshead minnow (XXXX (1998), report no. 97RC-0078) is available from the authorisation of zoxamide and its products in the US. The study has been evaluated by US EPA, but not yet by European authorities. It was therefore provided to the RMS to complete the picture on chronic toxicity of Zoxamide to fish and to use it in the aquatic risk assessment (i.e. for a species sensitivity distribution).
In the sheepshead minnow ELS test conducted with RH-117,281 technical (synonym for Zoxamide technical) in 1997, up to a nominal concentration of 0.30 mg a.s./L (mean measured concentration of 0.25 mg a.s./L, or 83 % of the nominal test concentration) were tested under flow-through conditions. As a result, the NOEC for this study was determined at 0.040 mg a.s./L. The LOEC, based on wet weight, was 0.078 mg a.s./L. The EC_{10} value for fish wet weight was estimated to be 0.093 mg a.s./L, with a 95 % confidence interval of 0.054 to 0.14 mg a.s./L. EC_{20} and EC_{50} values for wet weight were not reportable since they were extrapolated above the highest mean measured test concentration.

Review Comments:

Details of the studies mentioned above are to be found in the file: Zoxamide_confirmatory_like_ data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.
In the risk assessment agreed EU endpoint from LoEP was used (NOEC = 0.00348 mg a.s./L).

Acute endpoint for Zoxamide on aquatic invertebrates
Under EU Regulation (EC) No. 1107/2009 additional data on saltwater crustacean species may be required only for insecticides, therefore this is not an EU data requirement for Zoxamide; however, data on the saltwater crustacean *Mysidopsis bahia* are available for the parent compound, so they are both

considered in the risk assessment for safety reasons. In-line with the EFSA Aquatic Guidance document (2013) a refined acute toxicity endpoint can be used, taking into account the geometric mean EC_{50}/LC_{50} of both invertebrates belonging to the same taxonomic group (crustaceans). This approach is considered appropriate as it has been indicated that sensitivity distributions of taxonomically similar freshwater and marine species to organic plant protection products do not differ significantly and thus the data can be combined (EFSA Aquatic Guidance, 2013). In addition, the resulting **geomean EC_{50}/LC_{50} (229 µg a.s./L)** is less than an order of magnitude greater than the acute toxicity endpoint of the most sensitive species *Mysidopsis bahia* (LC_{50} = 76 µg a.s./L); therefore, it is considered that the geomean approach in this case is not biased by using data on insensitive species. This approach was agreed for refinement in the RAR for Zoxamide (2017; please refer to Vol. 3, CP, B.9).
As expected by EFSA (2017) and confirmed by the available toxicity endpoints for Zoxamide and its metabolites, mysids give the more sensitive endpoints compared to daphnids. Therefore, for the Zoxamide metabolite RH-127450 an aquatic risk assessment with the available mysid endpoint is regarded sufficient to conclude a safe use.

Review Comments:

zRMS agrees with Applicant's proposal.

Chronic toxicity endpoint for Zoxamide on aquatic invertebrates
Under EU Regulation (EC) No. 1107/2009 additional data on saltwater crustacean species may be required only for insecticides, therefore this is not an EU data requirement for Zoxamide; however, data on the saltwater crustacean *Mysidopsis bahia* are available for the parent compound and the endpoint is lower than the *Daphnia* endpoint, so they are both taken into account in the risk assessment for safety reasons.
The available studies demonstrate a lower toxicity for the aquatic crustacean *Daphnia magna* and *Mysidopsis bahia* (NOEC = 39 µg a.s./L and 7.2 µg a.s/L, respectively) than for aquatic insects (NOEC for *Chironomus riparius* = 380 µg a.s./L). This is confirmed by the available data for terrestrial non-target arthropods. Therefore, in line with the EFSA Aquatic Guidance (2013), a refined risk assessment is performed for the more sensitive taxonomic group of (aquatic) crustacean.

Review Comments:

zRMS agrees with Applicant's proposal.

Acute endpoints for Zoxamide metabolites on aquatic invertebrates
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 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" To close this data gap, applicable studies on daphnids and mysids were provided to the RMS.
As expected by EFSA (2017) and confirmed by the available toxicity endpoints for Zoxamide and its metabolites, mysids give the more sensitive endpoints compared to daphnids and were therefore taken forward for the risk assessment of aquatic invertebrates. Moreover, for the Zoxamide metabolite RH-127450 an aquatic risk assessment with the available mysid endpoint is regarded sufficient to conclude a safe use.

Review Comments:

zRMS agrees with Applicant's proposal. The toxicity endpoints for mysids were taken forward for the risk assessment of aquatic invertebrates by RMS-LV. All details are to be found in the file: Zoxamide_ confirmatory_like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

Alga endpoints for Zoxamide and its metabolites

EFSA (2017) requested in its Peer Review Conclusion: "*Further algae studies following the latest OECD 201 guideline are needed or further detailed information on all validity criteria requested by the latest OECD 201 guideline from the studies provided in the RAR for Zoxamide, RH-127450 and RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)*." To close this data gap, new studies on the inhibition of alga growth by the Zoxamide metabolites RH-127450 and RH-163353 have been performed and were provided to the RMS.
With regard to the EFSA request on alga studies with the parent compound Zoxamide and RH-139432: The alga studies with Zoxamide and RH-139432 which are available in the RAR for Zoxamide (2017) have been re-evaluated at a later stage during AIR based on additionally provided information. The results of the re-evaluation were included in the RAR, the alga studies were regarded valid. This was confirmed by Latvia as RMS for Zoxamide. As such, the endpoints from the alga studies with the active substance Zoxamide and RH-139432 available in the RAR (2017) are valid and applicable for the aquatic risk assessment.

Review Comments:

zRMS agrees with Applicant's proposal.

<u>*Lemna* endpoint for Zoxamide</u>
The *Lemna* study in the RAR (2017; XXXX, 1998b; CA 8.2.7/01) was not conducted according to current guidelines. In this study, 7 and 14 days IC_{50} values of >18 (highest test concentration) and 17 µg a.s./L, respectively, were laid down for Zoxamide in the EFSA Review Report (2004) and in the RAR (2017). However, due to lack of data around the IC_{50} concentration, the endpoint for risk assessment with regard to growth rate was set to the NOEC of 9.0 µg a.s./L. Therefore, as replacement for the old study with several deficiencies, the applicant performed a new study according to current provisions on the toxicity of Zoxamide to *Lemna gibba* and provided it to the RMS. This study contains a valid E_rC_{50} value for biomass (E_rC_{50} = 0.0237 mg a.s./L) based on nominal test concentrations, which is regarded applicable for risk assessment.

Review Comments:

zRMS agrees with Applicant's proposal. The confirmatory-like study by Juckeland, 2020, was evaluated and accepted by the RMS-LV for zoxamide in an interzonal procedure. The E_rC_{50} value for biomass (0.0237 mg a.s./L) based on nominal test concentrations, was considered by RMS-LV to be applicable for the risk assessment. All details are to be found in the file: Zoxamide_confirmatory_like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

<u>*Alga* and *Lemna* endpoints</u>
According to 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) 1107/2009" for the aquatic risk assessment on algae, *Lemna* and other higher aquatic plants growth rate (r) is the preferred endpoint and was therefore taken into account as far as available as endpoint for the risk assessment.

<u>Product studies</u>
The formulation GWN-10616 contains two active substances. Thus, studies with the formulated product have been conducted and respective endpoints are presented.

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 on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013;11(7):3290).

Consideration of mixture toxicity

The product GWN-10616 contains Zoxamide and Phosphonic acid as active substances. To account for a potential joint effect of this mixture to non-target organisms in edge of field surface waters a mixture toxicity risk assessment is performed below (according to EFSA Journal 2013;11(7):3290).

Toxicity studies on acute and chronic effects of the active substances and on acute effects of the formulation GWN-10616 to aquatic organisms are available. For a more detailed assessment of mixture toxicity, a surrogate LC_{50} or EC_{50} can be calculated. The model used to address the possible combined action of several active substances on non-target organisms and to estimate the toxicity of mixtures is the assumption of dose/concentration additivity of toxicity (Loewe & Muischneck, 1926, frequently referred to as 'Finney's equation').

Important to note is that potential contributions of the other constituents of the formulation are not considered here, i.e. it is assumed that only the active substances determine the toxicity of GWN-10616. Furthermore, reliable results can be expected for combinations of EC_X values for the same biological endpoint.

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

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

where:

p_i = fraction of active substance (i) in the mixture

$EC_{X\,i}$ = toxicity value for active substance (i)

Additionally, it is important to determine if one substance is driving the toxicity of the formulation. According to EFSA Journal 2013;11(7):3290, one substance is driving the toxicity if it contributes ≥ 90% of the toxic units (TU).

The TU is the ratio between the concentration (i.e. C) of a mixture component and its toxicological endpoint. TU calculations should be performed with toxicity data derived from the same species to avoid an influence of differing species sensitivity.

$$TUi = \frac{Ci}{ECxi}$$

with C being the amount of the $a.s._i$ [g/L] in the product and EC_i being the Tier 1 endpoint of $a.s._i$

In addition, the TU of a mixture has been defined as the sum of TU of each individual chemical of that mixture.

Counter-check calculated and measured mixture toxicity (model deviation ratio - MDR)

According to EFSA Journal 2013;11(7):3290 a counter-check of measured (EC_{xPPP} – expressed as sum of a.s.) vs. calculated ($EC_{mix\text{-}CA}$ – expressed as sum of a.s.) toxicity should be performed using the model deviation ratio (MDR) approach.

$$MDR = \frac{ECmix - CA}{ECxPPP}$$

The calculation of TU and MDR is summarized in the following table.

Table 9.5-4: Calculation of TU and MDR for the product GWN-10616

	amount of a.s. in product [g/L]	p (a.s.) fraction in product	$L/EC_{x\ a.s.}$ mg a.s./L	relative toxic units (%)	$L/EC_{mix\text{-}CA}$ mg sum of a.s./L	L/EC_{xPPP} mg sum of a.s./L*	MDR
Fish (*Oncorhynchus mykiss*)							
Zoxamide	60	0.11	0.16	98.9	> 1.477	13.67	0.1
Phosphonic acid	500	0.89	> 118	1.1			
Aquatic invertebrates (*Daphnia magna*)							
Zoxamide	60	0.11	> 0.78	94.8	> 6.90	> 39	0.2
Phosphonic acid	500	0.89	> 118	5.2			
Algae (*Pseudokirchneriella subcapitata*)							
Zoxamide	60	0.11	0.01413	99.9	0.132	0.26	0.5
Phosphonic acid	500	0.89	146.7	0.1			

TU: toxic units; MDR: model deviation ratio.
*Considering product density of 1.425 g/mL.

According to the results presented above, the calculation of MDR resulted in ratios lower than 5 for all groups of organisms indicating that the mixture of the two active substances does not result in considerable higher toxicity compared to the assumption of dose/concentration additivity (i.e. no synergistic effects). It should be considered that some endpoints are unbound values and resulting calculations of $L/EC_{mix\text{-}CA}$ overestimate the toxicity of the mixture.
For fish, the MDR is below 0.2 indicating that observed toxicity of the mixture is lower than the expected toxicity calculated by assumption of dose/concentration additivity.
Based on toxic units, it is concluded that Zoxamide is driving the toxicity of the mixture (TU ≥ 90%) for all groups of organisms considering the proportions in the product as well as the proportions of the a.s. residues entering aquatic environments at PEC_{mix} for FOCUS Step 1 and 2. For invertebrates, all endpoints are unbound, and definite mixture toxicity estimates cannot be provided. Therefore, the following risk assessment is based on the active substance Zoxamide.

Review Comments:

zRMS agrees with Applicant's approach.

Risk assessment - Zoxamide

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern are presented in Part B.8. 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 and presented below. For risk assessment, worst-case PEC values from modelling based on single and multiple applications for both early and late BBCH growth stages were considered.

For aquatic invertebrates only worst-case toxicity endpoints for *Americamysis bahia* were taken into account.

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to Zoxamide based on FOCUS Steps 1, 2 and 3 calculations (considering LoEP input parameters) for the use of GWN-10616 in grapevine

Group		**Fish acute**	**Fish prolonged**	**Invertebrates acute**	**Invertebrates prolonged**	**Algae**	**Sediment 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}	NOEC	E_rC_{50}
(µg/L)		160	3.48	76	7.2	14.13	380	23.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		1.6	0.348	0.76	0.72	1.413	38	2.37
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	84.4	52.75	242.53	111.05	117.22	59.73	2.22	35.61
Step 2 (S-Europe covering N-Europe)								
S-Europe (multiple appl.)	8.7	5.44	25.00	11.45	12.08	6.16	**0.23**	3.67
Step 3								
D6/ditch	3.625	2.3	10.42	4.77	5.03	2.57	**0.10**	1.53
R1/pond	0.235	**0.15**	**0.68**	**0.31**	**0.33**	**0.17**	**0.01**	**0.10**
R1/stream	2.242	1.4	6.44	2.95	3.11	1.59	**0.06**	**0.95**
R2/stream	3.034	1.9	8.72	3.99	4.21	2.15	**0.08**	1.28
R3/stream	3.191	2.0	9.17	4.20	4.43	2.26	**0.08**	1.35
R4/stream	2.263	1.4	6.50	2.98	3.14	1.60	**0.06**	**0.95**

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

A risk was indicated for several groups of aquatic organisms based on FOCUS Steps 1, 2 and 3 exposure estimates for the intended use in grapevine according to GAP. An assessment based on the lowest RAC (0.348 µg a.s./L) and FOCUS Step 4 estimates is presented below (Table 9.5-8).

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to Zoxamide based on FOCUS Steps 1, 2 and 3 calculations (considering LoEP input parameters) for the use of GWN-10616 in pome fruit

Group		**Fish acute**	**Fish prolonged**	**Invertebrates acute**	**Invertebrates prolonged**	**Algae**	**Sediment 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}	NOEC	E_rC_{50}
(µg/L)		160	3.48	76	7.2	14.13	~~280~~ 380	23.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		1.6	0.348	0.76	0.72	1.413	38	2.37
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	81.70	51.06	234.77	107.50	113.47	57.82	2.15	34.47
Step 2 (S-Europe covering N-Europe)								
S-Europe (multiple appl.)	21.20	13.25	60.92	27.89	29.44	15.00	**0.56**	8.95
Step 3								
D3/ditch	14.010	8.76	40.26	18.43	19.46	9.92	**0.37**	5.91
D4/pond	1.403	**0.88**	4.03	1.85	1.95	**0.99**	**0.04**	**0.59**
D4/stream	14.340	8.96	41.21	18.87	19.92	10.15	**0.38**	6.05
D5/pond	1.335	**0.83**	3.84	1.76	1.85	**0.94**	**0.04**	**0.56**
D5/stream	15.180	9.49	43.62	19.97	21.08	10.74	**0.40**	6.41
R1/pond	1.335	**0.83**	3.84	1.76	1.85	**0.94**	**0.04**	**0.56**
R1/stream	11.360	7.10	32.64	14.95	15.78	8.04	**0.30**	4.79
R2/stream	15.250	9.53	43.82	20.07	21.18	10.79	**0.40**	6.43

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Algae	Sediment dwell. prolonged	Aquatic plants
R3/stream	15.930	9.96	45.78	20.96	22.13	11.27	**0.42**	6.72
R4/stream	11.360	7.10	32.64	14.95	15.78	8.04	**0.30**	4.79

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

A risk was indicated for several groups of aquatic organisms based on FOCUS Steps 1, 2 and 3 exposure estimates for the intended use in pome fruit according to GAP. An assessment based on the lowest RAC (0.348 µg a.s./L) and FOCUS Step 4 estimates is presented below (Table 9.5-9).

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to Zoxamide based on FOCUS Steps 1, 2 and 3 calculations (considering LoEP input parameters) for the use of GWN-10616 in potato

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Algae	Sediment 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}	NOEC	E_rC_{50}
(µg/L)		160	3.48	76	7.2	14.13	~~280~~ 380	23.7
AF		100	10	100	10	10	10	10
RAC (µg/L)		1.6	0.348	0.76	0.72	1.413	38	2.37
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	62.5000	39.06	179.60	82.24	86.81	44.23	1.64	26.37
Step 2 (S-Europe covering N-Europe)								
S-Europe (multiple appl.)	4.700	2.94	13.51	6.18	6.53	**3.33**	**0.12**	1.98

Group		Fish acute	Fish prolonged	Invertebrates acute	Invertebrates prolonged	Algae	Sediment dwell. prolonged	Aquatic plants
Step 3								
D3/ditch	0.787	**0.49**	2.26	1.04	1.09	**0.56**	**0.02**	**0.33**
D4/pond	0.059	**0.04**	**0.17**	**0.08**	**0.08**	**0.04**	**0.00**	**0.02**
D4/stream	0.614	**0.38**	1.76	**0.81**	**0.85**	**0.43**	**0.02**	**0.26**
R1/pond	0.104	**0.07**	**0.30**	**0.14**	**0.14**	**0.07**	**0.00**	**0.04**
R1/stream	0.800	**0.50**	2.30	1.05	1.11	**0.57**	**0.02**	**0.34**
R2/stream	0.731	**0.46**	2.10	**0.96**	1.02	**0.52**	**0.02**	**0.31**
R3/stream	0.862	**0.54**	2.48	1.13	1.20	**0.61**	**0.02**	**0.36**

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

A risk was indicated for several groups of aquatic organisms based on FOCUS Steps 1, 2 and 3 exposure estimates for the intended use in potato according to GAP. An assessment based on the lowest RAC (0.348 µg a.s./L) and FOCUS Step 4 estimates is presented below (Table 9.5-10).

For the intended uses grapevine, pome fruit and potato, calculated PEC/RAC ratios 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 a.s./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.

The relevant global maximum FOCUS Step 4 PEC_{SW} for risk assessments covering the proposed use pattern are presented in Part B.8. Ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}) and lowest regulatory acceptable concentration (RAC) for aquatic organisms are given per intended use for each use. Worst-case PEC values from single and multiple applications were considered.

Table 9.5-8: Aquatic organisms: acceptability of chronic risk (PEC/RAC < 1) for fish (most sensitive taxonomic group) exposed to Zoxamide based on FOCUS Step 4 calculations considering refined half-life on crop canopy and risk mitigation measures for the use of GWN-10616 in grapevine

Intended use		Grapevine											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	Veg. filter strip (m)	None	None	None	10	10	20	None	None	None	10	10	20
	No spray buffer (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – grapevine						PEC/RAC ratio					
None	D6 Ditch	0.778	0.419	0.269	0.778	0.419	0.269	2.24	1.20	**0.77**	2.24	1.20	**0.77**
50%		0.389	0.209	0.134	0.389	0.209	0.134	1.12	**0.60**	**0.39**	1.12	**0.60**	**0.39**
75%		0.194	0.105	0.067	0.194	0.105	0.067	**0.56**	**0.30**	**0.19**	**0.56**	**0.30**	**0.19**
90%		0.078	0.042	0.027	0.078	0.042	0.027	**0.22**	**0.12**	**0.08**	**0.22**	**0.12**	**0.08**
None	R1 Pond	0.149	0.1	0.074	0.149	0.1	0.074	**0.43**	**0.29**	**0.21**	**0.43**	**0.29**	**0.21**
50%		0.075	0.052	0.04	0.075	0.05	0.037	**0.22**	**0.15**	**0.11**	**0.22**	**0.14**	**0.11**
75%		0.04	0.03	0.024	0.037	0.025	0.019	**0.11**	**0.09**	**0.07**	**0.11**	**0.07**	**0.05**
90%		0.022	0.018	0.016	0.016	0.012	0.008	**0.06**	**0.05**	**0.05**	**0.05**	**0.03**	**0.02**
None	R1 Stream	0.899	0.899	0.899	0.592	0.38	0.208	2.58	2.58	2.58	1.70	1.09	**0.60**
50%		0.899	0.899	0.899	0.38	0.38	0.194	2.58	2.58	2.58	1.09	1.09	**0.56**
75%		0.899	0.899	0.899	0.38	0.38	0.194	2.58	2.58	2.58	1.09	1.09	**0.56**
90%		0.899	0.899	0.899	0.38	0.38	0.194	2.58	2.58	2.58	1.09	1.09	**0.56**

Intended use		Grapevine											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Veg. filter strip (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	No-spray buffer (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – grapevine						**PEC/RAC ratio**					
None	R2 Stream	0.801	0.435	0.281	0.801	0.435	0.281	2.30	1.25	**0.81**	2.30	1.25	**0.81**
50%		0.401	0.218	0.14	0.401	0.218	0.14	1.15	**0.63**	**0.40**	1.15	**0.63**	**0.40**
75%		0.2	0.109	0.07	0.2	0.109	0.07	**0.57**	**0.31**	**0.20**	**0.57**	**0.31**	**0.20**
90%		0.08	0.054	0.054	0.08	0.044	0.028	**0.23**	**0.16**	**0.16**	**0.23**	**0.13**	**0.08**
None	R3 Stream	0.842	0.529	0.529	0.842	0.458	0.295	2.42	1.52	1.52	2.42	1.32	**0.85**
50%		0.529	0.529	0.529	0.421	0.24	0.148	1.52	1.52	1.52	1.21	**0.69**	**0.43**
75%		0.529	0.529	0.529	0.24	0.24	0.125	1.52	1.52	1.52	**0.69**	**0.69**	**0.36**
90%		0.529	0.529	0.529	0.24	0.24	0.125	1.52	1.52	1.52	**0.69**	**0.69**	**0.36**
None	R4 Stream	1.485	1.485	1.485	0.648	0.648	0.338	4.27	4.27	4.27	1.86	1.86	**0.97**
50%		1.485	1.485	1.485	0.648	0.648	0.338	4.27	4.27	4.27	1.86	1.86	**0.97**
75%		1.485	1.485	1.485	0.648	0.648	0.338	4.27	4.27	4.27	1.86	1.86	**0.97**
90%		1.485	1.485	1.485	0.648	0.648	0.338	4.27	4.27	4.27	1.86	1.86	**0.97**

Intended use		Grapevine											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Veg. filter strip (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	No-spray buffer (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – grapevine						**PEC/RAC ratio**					
None	D6 Ditch	0.676	0.367	0.237	0.676	0.367	0.237	>1	>1	**0.68**	-	-	-
50%		0.338	0.184	0.119	0.338	0.184	0.119	**0.97**	-	-	-	-	-
75%		0.169	0.092	0.059	0.169	0.092	0.059	-	-	-	-	-	-
90%		0.068	0.037	0.024	0.068	0.037	0.024	-	-	-	-	-	-
None	R1 Pond	Not required						Not required					
50%													
75%													
90%													
None	R1 Stream	0.592	0.322	0.208	0.592	0.322	0.208	>1	**0.93**	-	-	-	-
50%		0.296	0.168	0.168	0.296	0.161	0.104	**0.85**	-	-	-	-	-
75%		0.168	0.168	0.168	0.148	0.080	0.052	-	-	-	-	-	-
90%		0.168	0.168	0.168	0.071	0.071	0.036	-	-	-	-	-	-
None	R2 Stream	0.801	0.435	0.281	0.801	0.435	0.281	>1	>1	**0.81**	-	-	-
50%		0.401	0.218	0.140	0.401	0.218	0.140	>1	**0.63**	-	-	-	-
75%		0.200	0.109	0.070	0.200	0.109	0.070	**0.57**	-	-	-	-	-
90%		0.080	0.044	0.028	0.080	0.044	0.028	-	-	-	-	-	-
None	R3 Stream	0.842	0.493	0.493	0.842	0.458	0.295	>1	>1	>1	>1	>1	**0.85**
50%		0.493	0.493	0.493	0.421	0.229	0.148	>1	>1	>1	>1	**0.66**	-

Intended use		Grapevine											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Veg. filter strip (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	No-spray buffer (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – grapevine						**PEC/RAC ratio**					
75%		0.493	0.493	0.493	0.221	0.221	0.115	>1	>1	>1	**0.64**	**-**	**-**
90%		0.493	0.493	0.493	0.221	0.221	0.115	>1	>1	>1	**0.64**	**-**	**-**
None	R4 Stream	0.597	0.325	0.210	0.597	0.325	0.210	>1	**0.93**	-	-	-	**-**
50%		0.299	0.162	0.105	0.299	0.162	0.105	**0.86**	**-**	**-**	**-**	**-**	**-**
75%		0.149	0.086	0.086	0.149	0.081	0.052	**-**	**-**	-	-	-	**-**
90%		0.086	0.086	0.086	0.060	0.038	0.021	-	**-**	-	-	-	**-**

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

~~An acceptable risk based on the lowest RAC for Zoxamide (0.348 µg a.s./L from chronic fish testing), and FOCUS Step 4 exposure estimates was demonstrated for the use in grapevine considering mitigation measures up to 20 m no-spray buffer zones incl. a vegetated filter strip of up to 20 m~~.

Table 9.5-9: Aquatic organisms: acceptability of chronic risk (PEC/RAC < 1) for fish (most sensitive taxonomic group) exposed to Zoxamide based on FOCUS Step 4 calculations considering refined half-life on crop canopy and risk mitigation measures for the use of GWN-10616 in pome fruit

Intended use		Pome fruit															
Active substance		Zoxamide															
RAC (µg a.s./L)		0.348 (chronic fish)															
Nozzle reduction	Vfs (m)	None	None	None	None	10	10	20	20	None	None	None	None	10	10	20	20
	Nsb (m)	10	15	20	50	10	15	20	50	10	15	20	50	10	15	20	50
		PEC – pome fruit								PEC/RAC ratio							
None	D3 Ditch	6.762	3.041	1.546	0.175	6.762	3.041	1.546	0.175	19.43	8.74	4.44	**0.50**	19.43	8.74	4.44	**0.50**
50%		3.381	1.521	0.773	0.087	3.381	1.521	0.773	0.087	9.72	4.37	2.22	**0.25**	9.72	4.37	2.22	**0.25**
75%		1.69	0.76	0.387	0.044	1.69	0.76	0.387	0.044	4.86	2.18	1.11	**0.13**	4.86	2.18	1.11	**0.13**
90%		0.676	0.304	0.155	0.017	0.676	0.304	0.155	0.017	1.94	**0.87**	**0.45**	**0.05**	1.94	**0.87**	**0.45**	**0.05**
None	D4 Pond	0.894	0.469	0.269	0.04	0.894	0.469	0.269	0.04	2.57	1.35	**0.77**	**0.11**	2.57	1.35	**0.77**	**0.11**
50%		0.447	0.235	0.135	0.02	0.447	0.235	0.135	0.02	1.28	**0.68**	**0.39**	**0.06**	1.28	**0.68**	**0.39**	**0.06**
75%		0.223	0.117	0.067	0.01	0.223	0.117	0.067	0.01	**0.64**	**0.34**	**0.19**	**0.03**	**0.64**	**0.34**	**0.19**	**0.03**
90%		0.089	0.047	0.027	0.004	0.089	0.047	0.027	0.004	**0.26**	**0.14**	**0.08**	**0.01**	**0.26**	**0.14**	**0.08**	**0.01**
None	D4 Stream	7.569	3.491	1.73	0.195	7.569	3.491	1.73	0.195	21.75	10.03	4.97	**0.56**	21.75	10.03	4.97	**0.56**
50%		3.784	1.745	0.865	0.098	3.784	1.745	0.865	0.098	10.87	5.01	2.49	**0.28**	10.87	5.01	2.49	**0.28**
75%		1.892	0.873	0.433	0.049	1.892	0.873	0.433	0.049	5.44	2.51	1.24	**0.14**	5.44	2.51	1.24	**0.14**
90%		0.757	0.349	0.173	0.02	0.757	0.349	0.173	0.02	2.18	**1.00**	**0.50**	**0.06**	2.18	1.00	**0.50**	**0.06**
None	D5 Pond	0.85	0.446	0.256	0.038	0.85	0.446	0.256	0.038	2.44	1.28	**0.74**	**0.11**	2.44	1.28	**0.74**	**0.11**
50%		0.425	0.223	0.128	0.019	0.425	0.223	0.128	0.019	1.22	**0.64**	**0.37**	**0.05**	1.22	**0.64**	**0.37**	**0.05**
75%		0.212	0.111	0.064	0.009	0.212	0.111	0.064	0.009	**0.61**	**0.32**	**0.18**	**0.03**	**0.61**	**0.32**	**0.18**	**0.03**
90%		0.085	0.045	0.026	0.004	0.085	0.045	0.026	0.004	**0.24**	**0.13**	**0.07**	**0.01**	**0.24**	**0.13**	**0.07**	**0.01**

Intended use		Pome fruit															
Active substance		Zoxamide															
RAC (µg a.s./L)		0.348 (chronic fish)															
Nozzle reduction	**Vfs (m)**	None	None	None	None	10	10	20	20	None	None	None	None	10	10	20	20
	Nsb (m)	10	15	20	50	10	15	20	50	10	15	20	50	10	15	20	50
		PEC – pome fruit								**PEC/RAC ratio**							
None	D5 Stream	8.009	3.771	1.831	0.207	8.009	3.771	1.831	0.207	23.01	10.84	5.26	**0.59**	23.01	10.84	5.26	**0.59**
50%		4.004	1.885	0.915	0.103	4.004	1.885	0.915	0.103	11.51	5.42	2.63	**0.30**	11.51	5.42	2.63	**0.30**
75%		2.002	0.942	0.458	0.052	2.002	0.942	0.458	0.052	5.75	2.71	1.32	**0.15**	5.75	2.71	1.32	**0.15**
90%		0.801	0.377	0.183	0.021	0.801	0.377	0.183	0.021	2.30	1.08	**0.53**	**0.06**	2.30	1.08	**0.53**	**0.06**
None	R1 Pond	0.851	0.446	0.256	0.038	0.851	0.446	0.256	0.038	2.45	1.28	**0.74**	**0.11**	2.45	1.28	**0.74**	**0.11**
50%		0.425	0.223	0.128	0.019	0.425	0.223	0.128	0.019	1.22	**0.64**	**0.37**	**0.05**	1.22	**0.64**	**0.37**	**0.05**
75%		0.212	0.112	0.064	0.011	0.212	0.112	0.064	0.009	**0.61**	**0.32**	**0.18**	**0.03**	**0.61**	**0.32**	**0.18**	**0.03**
90%		0.085	0.045	0.026	0.006	0.085	0.045	0.026	0.004	**0.24**	**0.13**	**0.07**	**0.02**	**0.24**	**0.13**	**0.07**	**0.01**
None	R1 Stream	5.995	2.698	1.371	0.401	5.995	2.698	1.371	0.155	17.23	7.75	3.94	1.15	17.23	7.75	3.94	**0.45**
50%		2.998	1.348	0.685	0.401	2.998	1.348	0.685	0.089	8.61	3.87	1.97	1.15	8.61	3.87	1.97	**0.26**
75%		1.499	0.674	0.401	0.401	1.499	0.674	0.343	0.089	4.31	1.94	1.15	1.15	4.31	1.94	**0.99**	**0.26**
90%		0.6	0.401	0.401	0.401	0.6	0.27	0.137	0.089	1.72	1.15	1.15	1.15	1.72	**0.78**	**0.39**	**0.26**
None	R2 Stream	8.049	3.622	1.84	0.339	8.049	3.622	1.84	0.208	23.13	10.41	5.29	**0.97**	23.13	10.41	5.29	**0.60**
50%		4.025	1.81	0.92	0.339	4.025	1.81	0.92	0.104	11.57	5.20	2.64	**0.97**	11.57	5.20	2.64	**0.30**
75%		2.012	0.905	0.46	0.339	2.012	0.905	0.46	0.079	5.78	2.60	1.32	**0.97**	5.78	2.60	1.32	**0.23**
90%		0.805	0.362	0.339	0.339	0.805	0.362	0.184	0.079	2.31	1.04	**0.97**	**0.97**	2.31	1.04	**0.53**	**0.23**

Intended use		Pome fruit															
Active substance		Zoxamide															
RAC (µg a.s./L)		0.348 (chronic fish)															
Nozzle reduction	Vfs (m)	None	None	None	None	10	10	20	20	None	None	None	None	10	10	20	20
	Nsb (m)	10	15	20	50	10	15	20	50	10	15	20	50	10	15	20	50
		PEC – pome fruit								PEC/RAC ratio							
None	R3 Stream	8.404	3.782	1.921	0.217	8.404	3.782	1.921	0.217	24.15	10.87	5.52	**0.62**	24.15	10.87	5.52	**0.62**
50%		4.202	1.89	0.961	0.109	4.202	1.89	0.961	0.109	12.07	5.43	2.76	**0.31**	12.07	5.43	2.76	**0.31**
75%		2.101	0.945	0.48	0.054	2.101	0.945	0.48	0.054	6.04	2.72	1.38	**0.16**	6.04	2.72	1.38	**0.16**
90%		0.84	0.378	0.192	0.022	0.84	0.378	0.192	0.022	2.41	1.09	**0.55**	**0.06**	2.41	1.09	**0.55**	**0.06**
None	R4 Stream	5.994	2.697	1.37	1.097	5.994	2.697	1.37	0.237	17.22	7.75	3.94	3.15	17.22	7.75	3.94	**0.68**
50%		2.997	1.348	1.097	1.097	2.997	1.348	0.685	0.237	8.61	3.87	3.15	3.15	8.61	3.87	1.97	**0.68**
75%		1.498	1.097	1.097	1.097	1.498	0.674	0.343	0.237	4.30	3.15	3.15	3.15	4.30	1.94	**0.99**	**0.68**
90%		1.097	1.097	1.097	1.097	0.599	0.464	0.237	0.237	3.15	3.15	3.15	3.15	1.72	1.33	**0.68**	**0.68**

Intended use		Pome fruit															
Active substance		Zoxamide															
RAC (µg a.s./L)		0.348 (chronic fish)															
Nozzle reduction	**Vfs (m)**	None	None	None	None	10	10	20	20	None	None	None	None	10	10	20	20
	Nsb (m)	10	15	20	50	10	15	20	50	10	15	20	50	10	15	20	50
		PEC – pome fruit								**PEC/RAC ratio**							
None	D3 Ditch	6.762	3.041	1.546	0.175	6.762	3.041	1.546	0.175	>1	>1	>1	**0.50**	-	-	-	-
50%		3.381	1.521	0.773	0.087	3.381	1.521	0.773	0.087	>1	>1	>1	**0.25**	-	-	-	-
75%		1.690	0.760	0.387	0.044	1.690	0.760	0.387	0.044	>1	>1	>1	**0.13**	-	-	-	-
90%		0.676	0.304	0.155	0.017	0.676	0.304	0.155	0.017	>1	**0.87**	-	-	-	-	-	-

Intended use		Pome fruit															
Active substance		Zoxamide															
RAC (µg a.s./L)		0.348 (chronic fish)															
Nozzle reduction	**Vfs (m)**	None	None	None	None	10	10	20	20	None	None	None	None	10	10	20	20
	Nsb (m)	10	15	20	50	10	15	20	50	10	15	20	50	10	15	20	50
		PEC – pome fruit								**PEC/RAC ratio**							
None	D4 Pond	0.524	0.277	0.170	0.031	0.524	0.277	0.170	0.031	>1	**0.80**	-	-	-	-	-	-
50%		0.262	0.138	0.085	0.015	0.262	0.138	0.085	0.015	**0.75**	-	-	-	-	-	-	-
75%		0.131	0.069	0.042	0.008	0.131	0.069	0.042	0.008	-	-	-	-	-	-	-	-
90%		0.052	0.028	0.017	0.003	0.052	0.028	0.017	0.003	-	-	-	-	-	-	-	-
None	D4 Stream	7.569	3.406	1.730	**0.195**	7.569	3.406	1.730	0.195	>1	>1	>1	**0.56**	-	-	-	-
50%		3.784	1.702	0.865	**0.098**	3.784	1.702	0.865	0.098	>1	>1	>1	**0.28**	-	-	-	-
75%		1.892	0.851	0.433	**0.049**	1.892	0.851	0.433	0.049	>1	>1	>1	**0.14**	-	-	-	-
90%		0.757	**0.341**	0.173	0.020	0.757	0.341	0.173	0.020	>1	**0.98**	-	-	-	-	-	-
None	D5 Pond	0.524	**0.277**	0.170	0.031	0.524	0.277	0.170	0.031	>1	**0.80**	-	-	-	-	-	-
50%		**0.262**	0.138	0.085	0.015	0.262	0.138	0.085	0.015	**0.75**	-	-	-	-	-	-	-
75%		0.131	0.069	0.042	0.008	0.131	0.069	0.042	0.008	-	-	-	-	-	-	-	-
90%		0.052	0.028	0.017	0.003	0.052	0.028	0.017	0.003	-	-	-	-	-	-	-	-
None	D5 Stream	8.009	3.604	1.831	**0.207**	8.009	3.604	1.831	0.207	>1	>1	>1	**0.59**	-	-	-	-
50%		4.004	1.801	0.915	**0.103**	4.004	1.801	0.915	0.103	>1	>1	>1	**0.53**	-	-	-	-
75%		2.002	0.900	0.458	**0.052**	2.002	0.900	0.458	0.052	>1	>1	>1	**0.15**	-	-	-	-
90%		0.801	0.360	**0.183**	0.021	0.801	0.360	0.183	0.021	>1	>1	**0.53**	-	-	-	-	-
None	R1 Pond	0.524	**0.277**	0.170	0.031	0.524	0.277	0.170	0.031	>1	**0.80**	-	-	-	-	-	-
50%		**0.262**	0.138	0.085	0.017	0.262	0.138	0.085	0.015	**0.75**	-	-	-	-	-	-	-
75%		0.131	0.069	0.042	0.010	0.131	0.069	0.042	0.008	-	-	-	-	-	-	-	-

Intended use		Pome fruit															
Active substance		Zoxamide															
RAC (µg a.s./L)		0.348 (chronic fish)															
Nozzle reduction	**Vfs (m)**	None	None	None	None	10	10	20	20	None	None	None	None	10	10	20	20
	Nsb (m)	10	15	20	50	10	15	20	50	10	15	20	50	10	15	20	50
		PEC – pome fruit								**PEC/RAC ratio**							
90%		0.052	0.028	0.018	0.006	0.052	0.028	0.017	0.003	**0.15**	-	-	-	-	-	-	-
None	R1 Stream	5.995	2.698	1.371	0.457	5.995	2.698	1.371	**0.155**	>1	>1	>1	>1	>1	>1	>1	**0.45**
50%		2.998	1.348	0.685	0.457	2.998	1.348	0.685	**0.101**	>1	>1	>1	>1	>1	>1	>1	**0.29**
75%		1.499	0.674	0.457	0.457	1.499	0.674	**0.343**	0.101	>1	>1	>1	>1	>1	>1	**0.99**	-
90%		0.600	0.457	0.457	0.457	0.600	**0.270**	0.137	0.101	>1	>1	>1	>1	>1	**0.78**	-	-
None	R2 Stream	8.049	3.622	1.840	**0.208**	8.049	3.622	1.840	0.208	>1	>1	>1	**0.60**	-	-	-	-
50%		4.025	1.810	0.920	**0.104**	4.025	1.810	0.920	0.104	>1	>1	>1	**0.30**	-	-	-	-
75%		2.012	0.905	0.460	**0.052**	2.012	0.905	0.460	0.052	>1	>1	>1	**0.15**	-	-	-	-
90%		0.805	0.362	**0.184**	0.021	0.805	0.362	0.184	0.021	>1	>1	**0.53**	-	-	-	-	-
None	R3 Stream	8.404	3.782	1.921	**0.217**	8.404	3.782	1.921	0.217	>1	>1	>1	**0.62**	-	-	-	-
50%		4.202	1.890	0.961	**0.109**	4.202	1.890	0.961	0.109	>1	>1	>1	**0.31**	-	-	-	-
75%		2.101	0.945	0.480	**0.054**	2.101	0.945	0.480	0.054	>1	>1	>1	**0.16**	-	-	-	-
90%		0.840	0.378	**0.192**	0.022	0.840	0.378	0.192	0.022	>1	>1	**0.55**	-	-	-	-	-
None	R4 Stream	5.994	2.697	1.370	0.537	5.994	2.697	1.370	**0.155**	>1	>1	>1	>1	>1	>1	>1	**0.45**
50%		2.997	1.348	0.685	0.537	2.997	1.348	0.685	**0.116**	>1	>1	>1	>1	>1	>1	>1	**0.33**
75%		1.498	0.674	0.537	0.537	1.498	0.674	**0.343**	0.116	>1	>1	>1	>1	>1	>1	**0.99**	-
90%		0.599	0.537	0.537	0.537	0.599	**0.270**	0.137	0.116	>1	>1	>1	>1	>1	**0.78**	-	-

RAC: Regulatory acceptable concentration; Vfs: Vegetated filter strip; Nsb: No-spray buffer; PEC: Predicted environmental concentration; PEC/RAC ratios below the relevant trigger of 1 are shown in bold.

An acceptable risk based on the lowest RAC for Zoxamide (0.348 µg a.s./L from chronic fish testing), and FOCUS Step 4 exposure estimates was demonstrated for the use in pome fruit considering mitigation measures up to 50 m no-spray buffer zones incl. a vegetated filter strip of up to 20 m.

Table 9.5-10a: Aquatic organisms: acceptability of chronic risk (PEC/RAC < 1) for fish (most sensitive taxonomic group) exposed to Zoxamide based on FOCUS Step 4 calculations considering refined half-life on crop canopy and risk mitigation measures for the use of GWN-10616 in potato

Intended use		Potato											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	Vfs (m)	None	None	None	10	10	20	None	None	None	10	10	20
	Nsb (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – potato						PEC/RAC ratio					
None	D3 Ditch	0.137	0.093	0.071	0.137	0.093	0.071	**0.39**	**0.27**	**0.20**	**0.39**	**0.27**	**0.20**
50%		0.068	0.047	0.036	0.068	0.047	0.036	**0.20**	**0.14**	**0.10**	**0.20**	**0.14**	**0.10**
75%		0.034	0.023	0.018	0.034	0.023	0.018	**0.10**	**0.07**	**0.05**	**0.10**	**0.07**	**0.05**
90%		0.014	0.009	0.007	0.014	0.009	0.007	**0.04**	**0.03**	**0.02**	**0.04**	**0.03**	**0.02**
None	D4 Pond	0.037	0.03	0.025	0.037	0.03	0.025	**0.11**	**0.09**	**0.07**	**0.11**	**0.09**	**0.07**
50%		0.019	0.015	0.012	0.019	0.015	0.012	**0.05**	**0.04**	**0.03**	**0.05**	**0.04**	**0.03**
75%		0.009	0.007	0.006	0.009	0.007	0.006	**0.03**	**0.02**	**0.02**	**0.03**	**0.02**	**0.02**
90%		0.004	0.003	0.003	0.004	0.003	0.003	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
None	D4 Stream	0.137	0.094	0.071	0.137	0.094	0.071	**0.39**	**0.27**	**0.20**	**0.39**	**0.27**	**0.20**
50%		0.069	0.047	0.036	0.069	0.047	0.036	**0.20**	**0.14**	**0.10**	**0.20**	**0.14**	**0.10**
75%		0.034	0.023	0.018	0.034	0.023	0.018	**0.10**	**0.07**	**0.05**	**0.10**	**0.07**	**0.05**
90%		0.014	0.011	0.011	0.014	0.011	0.011	**0.04**	**0.03**	**0.03**	**0.04**	**0.03**	**0.03**
None	R1 Pond	0.068	0.061	0.056	0.048	0.041	0.03	**0.20**	**0.18**	**0.16**	**0.14**	**0.12**	**0.09**
50%		0.051	0.047	0.045	0.031	0.027	0.018	**0.15**	**0.14**	**0.13**	**0.09**	**0.08**	**0.05**

Intended use		Potato											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Vfs (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	Nsb (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – potato						**PEC/RAC ratio**					
75%		0.042	0.04	0.039	0.022	0.02	0.012	**0.12**	**0.11**	**0.11**	**0.06**	**0.06**	**0.03**
90%		0.038	0.037	0.037	0.017	0.016	0.009	**0.11**	**0.11**	**0.11**	**0.05**	**0.05**	**0.03**
None	R1 Stream	0.54	0.54	0.54	0.245	0.245	0.129	1.55	1.55	1.55	**0.70**	**0.70**	**0.37**
50%		0.54	0.54	0.54	0.245	0.245	0.129	1.55	1.55	1.55	**0.70**	**0.70**	**0.37**
75%		0.54	0.54	0.54	0.245	0.245	0.129	1.55	1.55	1.55	**0.70**	**0.70**	**0.37**
90%		0.54	0.54	0.54	0.245	0.245	0.129	1.55	1.55	1.55	**0.70**	**0.70**	**0.37**
None	R2 Stream	0.354	0.354	0.354	0.163	0.158	0.085	1.02	1.02	1.02	**0.47**	**0.45**	**0.24**
50%		0.354	0.354	0.354	0.158	0.158	0.082	1.02	1.02	1.02	**0.45**	**0.45**	**0.24**
75%		0.354	0.354	0.354	0.158	0.158	0.082	1.02	1.02	1.02	**0.45**	**0.45**	**0.24**
90%		0.354	0.354	0.354	0.158	0.158	0.082	1.02	1.02	1.02	**0.45**	**0.45**	**0.24**
None	R3 Stream	0.695	0.695	0.695	0.315	0.315	0.165	2.00	2.00	2.00	**0.91**	**0.91**	**0.47**
50%		0.695	0.695	0.695	0.315	0.315	0.165	2.00	2.00	2.00	**0.91**	**0.91**	**0.47**
75%		0.695	0.695	0.695	0.315	0.315	0.165	2.00	2.00	2.00	**0.91**	**0.91**	**0.47**
90%		0.695	0.695	0.695	0.315	0.315	0.165	2.00	2.00	2.00	**0.91**	**0.91**	**0.47**

Intended use		Potato											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Vfs (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	Nsb (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – potato						**PEC/RAC ratio**					
None	D3 Ditch	0.137	0.093	0.071	0.137	0.093	0.071	**0.39**	-	-	-	-	-
50%		0.068	0.047	0.036	0.068	0.047	0.036	-	-	-	-	-	-
75%		0.034	0.023	0.018	0.034	0.023	0.018	-	-	-	-	-	-
90%		0.014	0.009	0.007	0.014	0.009	0.007	-	-	-	-	-	-
None	D4 Pond	Not required						Not required					
50%													
75%													
90%													
None	D4 Stream	0.137	0.094	0.071	0.137	0.094	0.071	**0.39**	-	-	-	-	-
50%		0.069	0.047	0.036	0.069	0.047	0.036	-	-	-	-	-	-
75%		0.034	0.023	0.018	0.034	0.023	0.018	-	-	-	-	-	-
90%		0.014	0.009	0.007	0.014	0.009	0.007	-	-	-	-	-	-
None	R1 Pond	Not required						Not required					
50%													
75%													
90%													

Intended use		Potato											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Vfs (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	Nsb (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – potato						**PEC/RAC ratio**					
None	R1 Stream 3 appl. BBCH 21	0.605	0.605	0.605	0.605	0.605	0.605	>1	>1	>1	>1	>1	>1
50%		0.605	0.605	0.605	0.605	0.605	0.605	>1	>1	>1	>1	>1	>1
75%		0.605	0.605	0.605	0.605	0.605	0.605	>1	>1	>1	>1	>1	>1
90%		0.605	0.605	0.605	0.605	0.605	0.605	>1	>1	>1	>1	>1	>1
None	R1* Stream 1 appl. BBCH 21	0.605	0.605	0.605	0.275	0.275	0.144	>1	>1	>1	**0.79**	-	-
50%		0.605	0.605	0.605	0.275	0.275	0.144	>1	>1	>1	-	-	-
75%		0.605	0.605	0.605	0.275	0.275	0.144	>1	>1	>1	-	-	-
90%		0.605	0.605	0.605	0.275	0.275	0.144	>1	>1	>1	-	-	-
None	R2 Stream	0.343	0.343	0.343	0.153	0.153	0.079	**0.98**	-	-	-	-	-
50%		0.343	0.343	0.343	0.153	0.153	0.079	-	-	-	-	-	-
75%		0.343	0.343	0.343	0.153	0.153	0.079	-	-	-	-	-	-
90%		0.343	0.343	0.343	0.153	0.153	0.079	-	-	-	-	-	-
None	R3 Stream	0.716	0.716	0.716	0.327	0.327	0.171	>1	>1	>1	**0.94**	-	-
50%		0.716	0.716	0.716	0.327	0.327	0.171	>1	>1	>1	-	-	-
75%		0.716	0.716	0.716	0.327	0.327	0.171	>1	>1	>1	-	-	-
90%		0.716	0.716	0.716	0.327	0.327	0.171	>1	>1	>1	-	-	-

*covers single and multiple applications in BBCH 89

RAC: Regulatory acceptable concentration; Vfs: Vegetated filter strip; Nsb: No-spray buffer; PEC: Predicted environmental concentration; PEC/RAC ratios below the relevant trigger of 1 are shown in bold.

An acceptable risk based on the lowest RAC for Zoxamide (0.348 µg a.s./L from chronic fish testing), and FOCUS Step 4 exposure estimates was demonstrated for the use in potato considering mitigation measures up to10 m no-spray buffer zones incl. a vegetated filter strip of up to 10 m.

With reference to zRMS request of July 2024, additional risk assessment for aquatic organisms for multiple applications in potato (R1 scenario) is provided below.

Table 9.5-11b: Aquatic organisms: acceptability of chronic risk (PEC/RAC < 1) for fish (most sensitive taxonomic group) exposed to Zoxamide based on FOCUS Step 4 calculations considering half-life on crop canopy of 5.8 days and risk mitigation measures for the use of GWN-10616 in potato

Intended use		Potato											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Vfs (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	Nsb (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – potato						**PEC/RAC ratio**					
None	D3 Ditch	0.137	0.093	0.071	0.137	0.093	0.071	**0.39**	**0.27**	**0.20**	**0.39**	**0.27**	**0.20**
50%		0.068	0.047	0.036	0.068	0.047	0.036	**0.20**	**0.14**	**0.10**	**0.20**	**0.14**	**0.10**
75%		0.034	0.023	0.018	0.034	0.023	0.018	**0.10**	**0.07**	**0.05**	**0.10**	**0.07**	**0.05**
90%		0.014	0.009	0.007	0.014	0.009	0.007	**0.04**	**0.03**	**0.02**	**0.04**	**0.03**	**0.02**
None	D4 Pond	0.037	0.03	0.025	0.037	0.03	0.025	**0.11**	**0.09**	**0.07**	**0.11**	**0.09**	**0.07**
50%		0.019	0.015	0.012	0.019	0.015	0.012	**0.05**	**0.04**	**0.03**	**0.05**	**0.04**	**0.03**
75%		0.009	0.007	0.006	0.009	0.007	0.006	**0.03**	**0.02**	**0.02**	**0.03**	**0.02**	**0.02**
90%		0.004	0.004	0.003	0.004	0.004	0.003	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**
None	D4 Stream	0.137	0.094	0.071	0.137	0.094	0.071	**0.39**	**0.27**	**0.20**	**0.39**	**0.27**	**0.20**
50%		0.069	0.047	0.036	0.069	0.047	0.036	**0.20**	**0.14**	**0.10**	**0.20**	**0.14**	**0.10**
75%		0.034	0.023	0.018	0.034	0.023	0.018	**0.10**	**0.07**	**0.05**	**0.10**	**0.07**	**0.05**
90%		0.014	0.014	0.014	0.014	0.014	0.014	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**
None	D6 Ditch	D6 not relevant for CEZ						D6 not relevant for CEZ					

Intended use		Potato											
Active substance		Zoxamide											
RAC (µg a.s./L)		0.348 (chronic fish)											
Nozzle reduction	**Vfs (m)**	None	None	None	10	10	20	None	None	None	10	10	20
	Nsb (m)	10	15	20	10	15	20	10	15	20	10	15	20
		PEC – potato						**PEC/RAC ratio**					
None	R1 Pond	0.074	0.067	0.063	0.051	0.044	0.031	**0.21**	**0.19**	**0.18**	**0.15**	**0.13**	**0.09**
50%		0.057	0.054	0.051	0.033	0.03	0.02	**0.16**	**0.16**	**0.15**	**0.09**	**0.09**	**0.06**
75%		0.048	0.047	0.046	0.025	0.023	0.014	**0.14**	**0.14**	**0.13**	**0.07**	**0.07**	**0.04**
90%		0.045	0.044	0.044	0.019	0.019	0.01	**0.13**	**0.13**	**0.13**	**0.05**	**0.05**	**0.03**
None	R1 Stream	0.648	0.648	0.648	0.295	0.295	0.154	1.86	1.86	1.86	**0.85**	**0.85**	**0.44**
50%		0.648	0.648	0.648	0.295	0.295	0.154	1.86	1.86	1.86	**0.85**	**0.85**	**0.44**
75%		0.648	0.648	0.648	0.295	0.295	0.154	1.86	1.86	1.86	**0.85**	**0.85**	**0.44**
90%		0.648	0.648	0.648	0.295	0.295	0.154	1.86	1.86	1.86	**0.85**	**0.85**	**0.44**
None	R2 Stream	0.407	0.407	0.407	0.182	0.182	0.094	1.17	1.17	1.17	**0.52**	**0.52**	**0.27**
50%		0.407	0.407	0.407	0.182	0.182	0.094	1.17	1.17	1.17	**0.52**	**0.52**	**0.27**
75%		0.407	0.407	0.407	0.182	0.182	0.094	1.17	1.17	1.17	**0.52**	**0.52**	**0.27**
90%		0.407	0.407	0.407	0.182	0.182	0.094	1.17	1.17	1.17	**0.52**	**0.52**	**0.27**
None	R3 Stream	0.774	0.774	0.774	0.351	0.351	0.184	2.22	2.22	2.22	1.01	1.01	**0.53**
50%		0.774	0.774	0.774	0.351	0.351	0.184	2.22	2.22	2.22	1.01	1.01	**0.53**
75%		0.774	0.774	0.774	0.351	0.351	0.184	2.22	2.22	2.22	1.01	1.01	**0.53**
90%		0.774	0.774	0.774	0.351	0.351	0.184	2.22	2.22	2.22	1.01	1.01	**0.53**

RAC: Regulatory acceptable concentration; Vfs: Vegetated filter strip; Nsb: No-spray buffer; PEC: Predicted environmental concentration; PEC/RAC ratios below the relevant trigger of 1 are shown in bold.

For the intended uses grapevine, pome fruit and potato, calculated PEC/RAC ratios did 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 a.s./L in connection with an assessment factor of 10) in all FOCUS Steps 4 scenarios considering reduced exposure of surface water bodies.

The mitigation measured required to achieve acceptable risk are summarised in the table below.

Table 9.5-12: Summary of mitigation measures required to conclude acceptable risk

Use	Scenario	Mitigation
Grapevine	R1 Pond	No mitigation required
	D6 Ditch	50% drift reducing nozzles + 10 m buffer zone, or 20 m buffer zone
	~~D6 Ditch~~, R2 Stream	75% drift reducing nozzles + 10 m buffer zone, or 50% drift reducing nozzles + 15 m buffer zone, or 20 m buffer zone
	R1 Stream, R4 Stream	50% drift reducing nozzles + 10 m buffer zone or ~~20~~ 15 m buffer zone ~~(incl. vfs)~~
	R3 Stream	75% drift reducing nozzles + 10 m buffer zone (incl. vfs), or 50% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), or 20 m buffer zone (incl. vfs)
Pome fruit	~~R3 Stream~~	~~75% drift reducing nozzles + 10 m buffer zone (incl. vfs),~~ ~~or 50% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs),~~ ~~or 20 m buffer zone (incl. vfs)~~
	D3 Ditch, D4 Stream,	90% drift reducing nozzles + 15 m buffer zone, or 50 m buffer zone
	D4 Pond, D5 Pond, R1 Pond	~~75~~ 50% drift reducing nozzles + 10 m buffer zone, or ~~50% drift reducing nozzles +~~ 15 m buffer zone, ~~or 20 m buffer zone~~
	D5 Stream, R2 Stream, R3 Stream	90% drift reducing nozzles + 20 m buffer zone or 50 m buffer zone
	R1 Stream	90% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), or 75% drift reducing nozzles + 20 m buffer zone (incl. vfs), or 50 m buffer zone (incl. 20 m vfs)
	R4 Stream	90% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), 75% drift reducing nozzles + 20 m buffer zone (incl. vfs), or 50 m buffer zone (incl. 20 m vfs)
Potato	D4 Pond, R1 pond	No mitigation required
	D3 Ditch, D4 Stream, ~~R1 Pond~~ R2 Stream	10 m buffer zone
	~~R1 Stream,~~ ~~R2 Stream~~, R3 Stream	10 m buffer zone (incl. vfs)
	R1 Stream 3 appl. BBCH 21	No safe use (considering LoEP input parameters) 10 m buffer zone (incl. vfs) (considering half-life on crop canopy of 5.8 days)
	R1 Stream 1 appl. BBCH 21 covers single and multiple applications in BBCH 89	10 m buffer zone (incl. vfs)

Vfs: vegetated filter strip

Consideration of metabolite toxicity

For Zoxamide metabolites the risk assessment is based on acute toxicity endpoints presented in Table 9.5-1 and $FOCUS_{SW}$ Step 1 and 2 PEC values available from Part B.8. Worst-case PEC values from single and multiple applications were considered.

For aquatic invertebrates only worst-case toxicity endpoints for *Mysidopsis bahia* were taken into account.

Table 9.5-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to metabolite RH-127450 based on FOCUS Steps 1 and 2 calculations for the use of GWN-10616

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Raphidocelis subcapitata***
Endpoint		LC_{50}	LC_{50}	E_rC_{50}
(µg/L)		4170	2930	6600
AF		100	100	10
RAC (µg/L)		41.7	29.3	660
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1 (max)				
	54.2	1.30	1.85	**0.08**
Step 2 (max) (S-Europe covering N-Europe)				
S-Europe (multiple appl.)	6.3	**0.15**	**0.22**	**0.01**

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

For metabolite RH-127450, an acceptable risk was indicated for all groups of aquatic organisms based on FOCUS Steps 1 and 2 exposure estimates following the intended uses of GWN-10616 according to GAP.

Table 9.5-14: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to metabolite RH-24549 based on FOCUS Steps 1 and 2 calculations for the use of GWN-10616

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Desmodesmus subspicatus***
Endpoint (µg/L)		LC_{50} 23000	LC_{50} 23200	E_rC_{50} > 60000
AF		100	100	10
RAC (µg/L)		230	232	600
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1 (max)				
	38.4	**0.17**	**0.17**	**0.06**
Step 2 (max) (S-Europe covering N-Europe)				
S-Europe (multiple appl.)	3.6	**0.02**	**0.02**	**0.01**

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

For metabolite RH-24549, an acceptable risk was indicated for all groups of aquatic organisms based on FOCUS Steps 1 and 2 exposure estimates following the intended uses of GWN-10616 according to GAP.

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to metabolite RH-163353 based on FOCUS Steps 1 and 2 calculations for the use of GWN-10616

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Raphidocelis subcapitata***
Endpoint (µg/L)		LC_{50} > 100000	LC_{50} > 100000	E_rC_{50} > 100000
AF		100	100	10
RAC (µg/L)		1000	1000	1000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	60.9	**0.06**	**0.06**	**0.06**
Step 2 (max) (S-Europe covering N-Europe)				
S-Europe (multiple appl.)	8.7	**0.01**	**0.01**	**0.01**

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

For metabolite RH-163353, an acceptable risk was indicated for all groups of aquatic organisms based on FOCUS Steps 1 and 2 exposure estimates following the intended uses of GWN-10616 according to GAP.

Table 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to metabolite RH-141455 based on FOCUS Steps 1 and 2 calculations for the use of GWN-10616

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Raphidocelis subcapitata***
Endpoint		LC_{50}	LC_{50}	E_rC_{50}
(µg/L)		> 100000	> 100000	> 100000
AF		100	100	10
RAC (µg/L)		1000	1000	1000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	13.4	**0.01**	**0.01**	**0.01**
Step 2 (max) (S-Europe covering N-Europe)				
S-Europe (multiple appl.)	1.4	**< 0.01**	**< 0.01**	**< 0.01**

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

For metabolite RH-141455, an acceptable risk was indicated for all groups of aquatic organisms based on FOCUS Steps 1 and 2 exposure estimates following the intended uses of GWN-10616 according to GAP.

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aquatic organisms exposed to metabolite RH-139432 based on FOCUS Steps 1 and 2 calculations for the use of GWN-10616

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Mysidopsis bahia***	***Raphidocelis subcapitata***
Endpoint		LC_{50}	LC_{50}	E_rC_{50}
(µg/L)		2000	6950	> 30000
AF		100	100	10
RAC (µg/L)		20	69.5	300
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	30.2	1.51	**0.43**	**0.10**
Step 2 (max) (S-Europe covering N-Europe)				
S-Europe (multiple appl.)	4.5	**0.23**	**0.06**	**0.02**

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

For metabolite RH-139432, an acceptable risk was indicated for all groups of aquatic organisms based on FOCUS Steps 1 and 2 exposure estimates following the intended uses of GWN-10616 according to GAP.

As all PEC/RAC ratios are less than the trigger of 1, the risk for aquatic organisms from exposure to Zoxamide metabolites following the proposed use of GWN-10616 at the intended application rates is considered acceptable.

9.5.3 Overall conclusions

Studies on GWN-10616 are all available for product authorisation. A comparison of the results for GWN-10616 with the results for the single active substances revealed no additive or synergistic effects. Instead, the assessment confirms that Zoxamide drives the toxicity for aquatic organisms.

For the intended uses of GWN-10616 the calculated PEC/RAC ratios for Zoxamide indicate an acceptable risk for aquatic organisms considering drift reducing measures (drift reducing nozzles and buffer zones) and run-off reducing vegetated buffer zones, leading to a reduction of the exposure of surface water bodies. As a result, the implementation of the following measures is necessary:

Use	Scenario	Mitigation
Grapevine	R1 Pond	No mitigation required
	D6 Ditch	50% drift reducing nozzles + 10 m buffer zone, or 20 m buffer zone
	~~D6 Ditch~~, R2 Stream	75% drift reducing nozzles + 10 m buffer zone, or 50% drift reducing nozzles + 15 m buffer zone, or 20 m buffer zone
	R1 Stream, R4 Stream	50% drift reducing nozzles + 10 m buffer zone or ~~20~~ 15 m buffer zone ~~(incl. vfs)~~
	R3 Stream	75% drift reducing nozzles + 10 m buffer zone (incl. vfs), or 50% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), or 20 m buffer zone (incl. vfs)
Pome fruit	~~R3 Stream~~	~~75% drift reducing nozzles + 10 m buffer zone (incl. vfs),~~ ~~or 50% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs),~~ ~~or 20 m buffer zone (incl. vfs)~~
	D3 Ditch, D4 Stream,	90% drift reducing nozzles + 15 m buffer zone, or 50 m buffer zone
	D4 Pond, D5 Pond, R1 Pond	~~75~~ 50% drift reducing nozzles + 10 m buffer zone, or ~~50% drift reducing nozzles +~~ 15 m buffer zone, ~~or 20 m buffer zone~~
	D5 Stream, R2 Stream, R3 Stream	90% drift reducing nozzles + 20 m buffer zone or 50 m buffer zone
	R1 Stream	90% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), or 75% drift reducing nozzles + 20 m buffer zone (incl. vfs), or 50 m buffer zone (incl. 20 m vfs)
	R4 Stream	90% drift reducing nozzles + 15 m buffer zone (incl. 10 m vfs), 75% drift reducing nozzles + 20 m buffer zone (incl. vfs), or 50 m buffer zone (incl. 20 m vfs)
Potato	D4 Pond, R1 pond	No mitigation required
	D3 Ditch, D4 Stream, ~~R1 Pond~~ R2 Stream	10 m buffer zone
	~~R1 Stream, R2 Stream~~, R3 Stream	10 m buffer zone (incl. vfs)
	R1 Stream 3 appl. BBCH 21	No safe use (considering LoEP input parameters) 10 m buffer zone (incl. vfs) (considering half-life on crop canopy of 5.8 days)
	R1 Stream 1 appl. BBCH 21 covers single and multiple applications in BBCH 89	10 m buffer zone (incl. vfs)

Vfs: vegetated filter strip

The metabolites of zoxamide are of lower toxicity than the parent active substance. For all Zoxamide metabolites, PEC/RAC ratios are below 1 for all aquatic organism when $FOCUS_{SW}$ Step 1 and 2 exposure estimates are considered, indicating an acceptable risk.

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The initial risk assessment was based on the worst case PECsw values and the results of laboratory toxicity testing. The PEC_{sw} Step 1-2 (for zoxamide and its metabolites) and Step 3-4 (for zoxamide) were used.

Based on toxic units, it is concluded that zoxamide is driving the toxicity of the mixture (TU ≥ 90%) for all groups of aquatic organisms. Therefore, the risk assessment was based on zoxamide.

The submitted risk assessment for zoxamide was revised by zRMS based on the PECsw values accepted in Section B8.

Considering LoEP input parameters:

GWN-10616 applications close to surface water pose acceptable risk to aquatic organisms with appropriate risk mitigation measures, except R1 scenario, multiple applications in potatoes (BBCH 21). Since calculations have only been made for the BBCH 21 and 89, it is not possible to demonstrate from which growth stage of potato the multiple applications are acceptable.. Therefore, it is currently only possible to accept a single application in potato for the R1 scenario.

During the comment stage the Applicant has the opportunity to provide an additional risk assessment for aquatic organisms to demonstrate acceptable risk for multiple applications in potato (R1 scenario).

With reference to zRMS request of July 2024, additional risk assessment for aquatic organisms for applications in potato were provided by the Applicant. The PECsw values performed with refined parameters were accepted by zRMS (please refer to Section B8).

Considering half-life on crop canopy of 5.8 days

GWN-10616 applications close to surface water pose acceptable risk to aquatic organisms with appropriate risk mitigation measures (10 m buffer zone (incl. vfs)), for single and multiple applications in potatoes.

With reference to zRMS request of July 2024, additional risk assessment for aquatic organisms for multiple applications in potato (R1 scenario) is provided in chapter 9.5.2. Acceptable risk is demonstrated.

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 Phosphonic. Full details of these studies are provided in the respective EU assessment reports and related documents. Additional information on Zoxamide to address data gaps identified during EU renewal process have been submitted to RMS Latvia.

Effects on bees of GWN-10616 were not evaluated as part of the EU assessments. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	RH-117,281 2F *	Oral acute	LD_{50} > 33 µg a.s./bee	EFSA (2017)
Apis mellifera	RH-117,281 2F *	Contact acute	LD_{50} > 43.2 µg a.s./bee	EFSA (2017)
Apis mellifera	Zoxamide	Contact acute	LD_{50} > 100 µg/bee	EFSA (2017)
Apis mellifera	Zoxium 240 SC*	10 d-oral chronic	LC_{50} > 5000 mg a.s./g feed (corresponding to LD_{50} > 174.8 µg a.s./bee/day)	EFSA (2017)
Apis mellifera	Zoxamide	22 d-oral larvae, repeated dose	LD_{50} > 110 µg a.s/larvae (larval and pupal survival, adult emergence and adult weight at emergence) NOED = 110 µg a.s./larvae (larval and pupal survival, adult emergence) NOED = 49 µg a.s./larvae (adult weight at emergence)	Submitted to RMS Picard (2018)
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 (2017)
Bombus terrestris	Zoxium 240 SC*	Oral	LD_{50} > 391.1 µg a.s./bee	Submitted to RMS Amsel (2018)
		Contact	LD_{50} > 400.0 µg a.s./bee	

* Zoxamide mono-formulation

Table 9.6-2: Phosphonic acid*: Endpoints and effect values for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Potassium phosphonates (504 g/L)	Acute, oral	LD_{50} > 50.34 µg Phosphonic acid/bee	EFSA (2012)
Apis mellifera	Potassium phosphonates (504 g/L)	Acute, contact	LD_{50} > 71.87 µg Phosphonic acid/bee	EFSA (2012)

*Phosphonic acid (also Phosphorous acid) is the actual active substance of the technical item Potassium phosphonates (formerly: phosphite; technical active substance).

Table 9.6-3: GWN-10616: Endpoints and effect values for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	GWN-10616	Acute, oral	LD_{50} > 300 µg/bee	KCP 10.3.1.1/01 Venturi (2021) 832-001
		Acute, contact	LD_{50} > 300 µg/bee	
Apis mellifera	GWN-10616	Chronic, oral (adults)	10 d LDD_{50} = 137 µg/bee/d 10 d NOEDD = 71.3 µg/bee/d	KCP 10.3.1.2/01 Colli (2021) 832-002
Apis mellifera	GWN-10616	Chronic, oral (larvae)	NOED = 100 µg/larva	KCP 10.3.1.3/01 Colli (2021) 832-002
Bombus terrestris	GWN-10616	Acute, oral	LD_{50}> 3159.4 µg/bee	KCP 10.3.1.1/02 Venturi (2021) 832-004
		Acute, contact	LD_{50}> 2000 µg/bee	

9.6.1.1 Justification for new endpoints

EFSA (2017) requested "*Further information to address the risk to bee larvae (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)*." A study to address this data requirement was provided to the RMS.

Furthermore, a study on the acute oral and topical toxicity of Zoxamide, applied as Zoxium 240 SC to the bumblebee *Bombus terrestris* L. under laboratory conditions, has been performed and was provided to the RMS.

Review Comments:

The confirmatory-like studies were evaluated and accepted by the RMS-LV for zoxamide in an interzonal procedure. The endpoints provided in Table 9.6-1 were considered by RMS-LV to be applicable for the risk assessment. All details are to be found in the file: Zoxamide_confirmatory_like_data_ Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

The formulation GWN-10616 contains two active substances. Thus, studies with the formulated product have been conducted and respective endpoints are presented.

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

9.6.2.1 Hazard quotients for bees

The risk assessments are performed following the approaches outlined in SANCO/10329/2002 rev. 2 final and the revised EPPO scheme (OEPP/EPPO, 2010).

Acute risk assessment

The acute risk to honeybees from the use of GWN-10616 was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients as follows:

$$\text{Hazard quotient} = \frac{\text{Maximum application rate [g a.s./ha]}}{\text{Acute } LD_{50} \text{ [µg a.s./bee]}}$$

Hazard quotients were calculated for oral exposure (Q_{Ho}) and contact exposure (Q_{Hc}) to active substances Zoxamide and Phosphonic acid as well as to the formulated product GWN-10616. A hazard quotient of less than 50 indicates a low risk to bees in the field. The acute contact and oral hazard quotients are summarized in Table 9.6-4.

Table 9.6-4: First-tier assessment of the risk for bees due to the use of GWN-10616 containing Zoxamide and Phosphonic acid in grapevine

Intended use	Grapevine		
Active substance	Zoxamide		
Application rate [g a.s./ha]	180		
Test design	**LD_{50} (lab.) [µg a.s./bee]**	**Single application rate [g a.s./ha]**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 33	180	< 5.5
Contact toxicity	> 43.2		< 4.2
Active substance	Phosphonic acid (Potassium phosphonate)		
Application rate [g a.s./ha]	1500		
Test design	**LD_{50} (lab.) [µg a.s./bee]**	**Single application rate [g a.s./ha]**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 50.34	1500	< 29.8
Contact toxicity	> 71.87		< 20.9
Product	GWN-10616		
Application rate [g a.s./ha product/ha]	4275 (3 L product/ha considering density of 1.425 g/mL)		
Test design	**LD_{50} (lab.) [µg product/bee]**	**Single application rate [g product/ha]**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 300	4275	< 14.25
Contact toxicity	> 300		< 14.25

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

All hazard quotients are considerably less than 50, indicating that the active substances as well as the product pose a low risk to bees. Therefore, a low risk to bees is expected from the application of GWN-10616 according to the GAP.

Chronic risk assessment for adult bees

According to the new data requirements (Commission regulation No 283/2013 and 284/2013), a chronic toxicity test with honey bees is required where exposure to honey bees cannot be excluded.

The chronic risk from oral exposure of adult honey bees to GWN-10616 was assessed in a 10 day chronic study (KCP 10.3.1.2/01; Colli (2021)). The LDD_{50} was determined to be 137 µg product/bee/day and the

NOEDD was 71.3 µg product/bee/day.

According to the revised EPPO scheme (2010), for products applied as spray with a $Q_H \leq 50$ no further assessment is required. This is the case for GWN-10616 and both contained active substances.
Since data on chronic toxicity to bees is available, the assessment is presented. The exposure to bees is assessed through the amount of residues that may be ingested by a bee in one day. The ratio between the NOEL (= NOED in µg a.s./bee/day) and the exposure (also in µg a.s./bee/day) is calculated.

$$TER_{chronic} = \frac{\text{NOEL oral [µg a.s./bee/day]}}{\text{Amount of residues ingested by a bee in one day [µg a.s./bee/day]}}$$

Data for consumption of nectar and pollen by adult bees are given in the EFSA Opinion on the risk assessment for bees (EFSA, 2012). The maximum amount of sugar an adult bee consumes per day is given as 128 mg/bee/day. Pollen consumption is not relevant for adult bees. The sugar content of nectar which maybe foraged by the bees was gathered from the scientific literature (Maccagnani *et al*., 2003; Monzón *et al*., 2004; Nicolson, 2009). The worst case value of sugar content (nectar with the lowest sugar content from the ranges which may be foraged by the bees), namely 15% for honey bees, is used in calculating the total amount of nectar which a honey bee consumes per day. The sugar content of 15% is also used to derive short-cut values for the risk assessment scheme as proposed by the draft EFSA guidance document on the risk assessment for bees (EFSA, 2013).

According to the parameters above, the worst-case consumption of nectar for an adult honey bee is 853 mg nectar/bee/day.

The generic worst case residue value in pollen and nectar as proposed in the revised EPPO scheme of 1 mg a.s./kg plant matrix for soil and seed treatments is used as a worst case. Based on the default residue value, the maximum uptake of a certain substance by an adult honey bee through consumption of nectar is 0.853 µg a.s./bee/day.

The TER values calculated for adult bees are summarised in Table 9.6-5.

Table 9.6-5: Chronic TER values for honey bees based on consumption of nectar containing residues of Zoxamide and GWN-10616

Use	Expo-sure route	NOED [µg/bee/d]	Maximum nectar consumption [mg nectar/bee/d]	Maximum residue intake [µg /bee/d]	TER_{LT}	Trigger
Zoxamide	Oral	174.8	853	0.853	204.9	1
GWN-10616	Oral	71.3	853	0.853	83.6	1

Values in **bold** breach the trigger of 1.

The chronic TER is 83.6 for the product and 204.9 for Zoxamide, respectively, and higher than the trigger of 1. Thus, the chronic risk to honey bees from consumption of nectar is considered acceptable when GWN-10616 is applied according to the GAP.

Chronic risk assessment for larval bees

According to the data requirements (Commission regulation No 283/2013 and 284/2013), a bee brood study shall be conducted to determine effects on honey bee development and brood activity and to provide sufficient information to evaluate possible risks from the plant protection product on honey bee larvae.

According to the revised EPPO scheme the risk assessment for the exposure of honey bee larvae has to be performed when effects on growth or development of bees cannot be excluded. Determination of effects

can be assessed qualitatively (bee brood feeding test according to Oomen) or quantitatively (e.g. OECD 237 (single exposure) or Draft OECD guideline for repeated exposure). The effects of Zoxamide and GWN-10616 on honey bee development and other honey bee life stages were assessed.

The risk assessment for larvae is performed following the approach for chronic toxicity to adult honey bees outlined in the revised EPPO scheme (OEPP/EPPO, 2010), i.e. the ratio between the NOEL (= NOED in µg a.s./larva/day) and the exposure (also in µg a.s./larva/day) is calculated. Calculation of exposure is the same as described above for chronic risk assessment for adult bees.

$$TER_{chronic} = \frac{\text{NOEL oral [µg a.s./larva]}}{\text{Amount of residues ingested by a bee larva in one day [µg a.s./larva/day]}}$$

Concerning data for consumption of nectar by honey bee larvae the EPPO scheme refers to data given by Rortais (2005). The maximum amount of nectar a bee larva consumes is given as 59.4 mg/larva per 5 days (11.9 mg/larva/day) and the maximum amount of consumed pollen is 2 mg/larvae per 5 days (0.400 mg/larvae/day). As honey bee larvae requires both food items for a successful development consumption of nectar and pollen is summed up. Exposure is considered as 1 mg/kg plant matrix (generic worst case).

Data for consumption of nectar and pollen by adult honeybees and honeybee larvae are given in Rortais *et al*. (2005). For honeybee larvae, both nectar and pollen consumption are relevant. The maximum amount of sugar in nectar a larva consumes is given as 59.4 mg/5 days, which corresponds to a nectar consumption of 396 mg/5 days (based on worst-case sugar content in nectar of 15 %). The maximum amount of pollen consumed by a larva is 2 mg/5 days. Exposure is considered as 1 mg/kg plant matrix, the default value given in the revised EPPO scheme (2010).

According to the parameters above, the consumption rates are divided by a factor of 1000 in order to have a comparable unit (µg/bee). The oral TER values for honey bee larvae are summarized in the table below.

Table 9.6-6: Chronic TER values for honey bee larvae based on consumption of nectar and pollen containing residues of Zoxamide and GWN-10616

Scenario	NOED [1] [µg/larva]	Food type	Maximum consumption [1] [mg/larva]	Maximum residue intake [1] [µg/larva]	Sum of residue intake [µg/larvae]	TER_{LT}
Zoxamide	110	Pollen	2	0.002	0.398	276.4
		Nectar	396	0.396		
GWN-10616	100	Pollen	2	0.002	0.398	251.3
		Nectar	396	0.396		

[1] over a period of 5 days
Values in **bold** breach the trigger of 1.

The TER for honey bee larvae on consumption of nectar and pollen after repeated exposure to Zoxamide and GWN-10616 based on a worst case generic residue assumption is 251.3 and higher than the respective trigger of 1. Thus, the chronic risk to honey bee larvae from consumption of nectar and pollen is considered acceptable when GWN-10616 is applied according to the GAP.

Sublethal effects

Sub-lethal effects were considered both in the acute oral and contact toxicity studies with the active substance and the formulation GWN-10616 on honey bees. In the acute oral and contact toxicity study (KCP 10.3.1.1/01; Venturi (2021)), no abnormal behavioural effects (e.g. uncoordinated movement,

increased rate of grooming or constant grooming, lethargy, lack of feeding or diarrhoea) was noticed in the treated honey bees.

Sub-lethal effects were also investigated in the 10-day honey bee adult chronic oral toxicity study (KCP 10.3.1.2/01; Colli (2021)) conducted with GWN-10616 and in the bee brood study with GWN-10616 (KCP 10.3.1.3/01; Colli (2021)). No sublethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control treatment were observed during the study period in the chronic oral toxicity and in the bee brood study.

Therefore, the risk for bees experiencing sublethal effects after application of GWN-10616 is considered to be low.

Review Comments:

At the harmonization meeting of the Central Zone in Warsaw (December 2023), it was agreed to present the chronic and larvae RA based on EFSA (2013) in the Core. The zRMS therefore completed the risk assessment for bees, accordingly.

Screening assessment

Table 9.6-7: Screening assessment - acute risk for adult honeybees from contact exposure

Crop	Single application rate (g prod./ha)	Species (life stage)	LD_{50} (µg a.s. product/bee)	$HQ_{contact}$	Trigger value
Pome fruit, Grapes	4275 (SUW)	Honeybee (adult)	> 300	< 14.25	> 85
Potato	3562.5 (DW)	Honeybee (adult)	> 300	< 11.9	> 42

HQ: Hazard quotient. HQ values shown in **bold** breach the relevant trigger, indicating potential concern. SUW: sideward/upward spray; DW: downward spray.

The above screening risk assessment demonstrates an acceptable risk for honeybees from acute contact exposure from all proposed uses of GWN-10616.

Table 9.6-8: Screening assessment - acute risk for adult honeybees from oral exposure

Crop	Single application rate (kg a.s. product/ha)	Species (life stage)	LD_{50} (µg a.s. product/bee)	SV	ETR_{oral}	Trigger value
Pome fruit Grapes	4275	Honeybee (adult)	> 300	10.6	< 0.15	> 0.2
Potato	3562.5	Honeybee (adult)	> 300	10.6	< 0.09	> 0.2

SV: Shortcut value; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating potential concern. SUW: sideward/upward spray; DW: downward spray.

The above screening risk assessment demonstrates an acceptable risk for honeybees from acute oral exposure from all proposed uses of GWN-10616.

Table 9.6-9: Screening assessment - chronic risk for adult honeybees and honeybee larvae from oral exposure

Crop	Single application rate (kg a.s./ha)	Species (life stage)	Endpoint	SV	ETR_{oral}	Trigger value
Pome fruit Grapes	4275 (SUW)	Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10.6	**0.331**	> 0.03
		Honeybee (larvae)	NOED = 100 µg prod./larvae	6.1	**0.261**	> 0.2
Potato	3562.5 (DW)	Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	7.6	**0.198**	> 0.03
		Honeybee (larvae)	NOED = 100 µg prod./larvae	4.4	0.16	> 0.2

SV: Shortcut value; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating potential concern. SUW: sideward/upward spray; DW: downward spray.

In the above screening risk assessment, an acceptable risk could not be demonstrated for adult honeybees for any of the proposed uses of GWN-10616. For honeybee larvae an acceptable risk could demonstrated only for uses in potato. Therefore, a first-tier risk assessment is provided.

First-tier assessment

A first-tier assessment of the chronic risk for adult honeybees and honeybee larvae is required for proposed uses in pome fruit and grapes, and for adult honeybees for uses in potato.

In the following tables, crop categories have been assigned according to the EFSA Bee Tool (V3).

Table 9.6-10: Foraging on the treated crop - chronic risk for adult honeybees and honeybee larvae from oral exposure

Species (life stage)	Endpoint	BBCH	Ef	SV	TWA	ETR_{oral}	Trigger value
Pome fruit (SUW) – 4275 g prod./ha (max. 2 applications, 6-8 d interval) – BBCH 51 - 69 Crop category: "Orchards 1"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	40 – 69	1	8.2	0.72	**0.184**	0.03
Honeybee (larvae)	NOED = 100 µg prod./larvae	40 – 69	1	6.1	0.85	**0.222**	0.2
Grapes (SUW) – 4275 g prod./ha (max. 3 applications, 8-10 d interval) – BBCH 14 - 79 Crop category: "Grapes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10 - 19 20 - 39 40 – 69	1	8.2	0.72	**0.184**	0.03
		≥ 70	1	0	0.72	0	0.03
Honeybee (larvae)	NOED = 100 µg prod./larvae	10 - 19 20 – 39 40 – 69	1	6.1	0.85	**0.222**	0.2
		≥ 70	1	0	0.85	0	0.2
Potato (DW) – 3562.5 g prod./ha (max. 3 applications, 7-8 d interval) – BBCH 21 - 89 Crop category: "potatoes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10 - 39 40 - 69	1	0.92	0.72	0.017	0.03
		≥ 70	1	0	0.72	0	0.03

SV: Shortcut value; Ef: exposure factor; TWA: time weighted average; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating a potential concern. SUW: sideward/upward spray; DW: downward spray.

The first-tier risk assessment for the "treated crop" exposure scenario demonstrates an acceptable risk for adult honeybees and honeybee larvae at BBCH stages ≥ 70 for grapes and for honeybee larvae for potato. The ETR_{oral} values exceeded the trigger value of 0.03 at BBCH stages < 70 in pome fruit and grapes.

Table 9.6-11: Foraging on the adjacent crop - chronic risk for adult honeybees and honeybee larvae from oral exposure

Species (life stage)	Endpoint	BBCH	Ef	SV	TWA	ETR_{oral}	Trigger value
Pome fruit (SUW) – 4275 g prod./ha (max. 2 applications, 6-8 d interval) – BBCH 51 - 69 Crop category: "Orchards 1"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	40 – 69	0.066	5.8	0.72	0.009	0.03
Honeybee (larvae)	NOED = 100 µg prod./larvae	40 – 69	0.066	4.4	0.85	0.01	0.2
Grapes (SUW) – 4275 g prod./ha (max. 3 applications, 8-10 d interval) – BBCH 14 - 79 Crop category: "Grapes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10-19	0.0047	5.8	0.72	0.001	0.03
		20-39 40-69 ≥ 70	0.0143	5.8	0.72	0.002	
Honeybee (larvae)	NOED = 100 µg prod./larvae	10-19 20-39	0.0047	4.4	0.85	0.00	0.2
		40-69 ≥ 70	0.0143	4.4	0.85	0.00	
Potato (DW) – 3562.5 g prod./ha (max. 3 applications, 7-8 d interval) – BBCH 21 - 89 Crop category: "potatoes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10 – 39 40 – 69 ≥ 70	0.0033	5.8	0.72	0.00	0.03

SV: Shortcut value; Ef: exposure factor; TWA: time weighted average; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating a potential concern. SUW: sideward/upward spray; DW: downward spray.

The first-tier risk assessment for the "adjacent crop" exposure scenario demonstrates an acceptable chronic risk for adult honeybees and honeybee larvae for all proposed uses of GWN-10616, at all relevant BBCH stages.

Table 9.6-11: Foraging on weeds in the treated field - <u>chronic</u> risk for adult honeybees and honeybee larvae from <u>oral</u> exposure

Species (life stage)	Endpoint	BBCH	Ef	SV	TWA	ETR_{oral}	Trigger value
Pome fruit (SUW) – 4275 g prod./ha (max. 2 applications, 6-8 d interval) – BBCH 51 - 69 Crop category: "Orchards 1"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	40 – 69 ≥ 70	0.3	2.9	0.72	0.02	0.03
Honeybee (larvae)	NOED = 100 µg prod./larvae	40 – 69 ≥ 70	0.3	2.2	0.85	0.02	0.2
Grapes (SUW) – 4275 g prod./ha (max. 3 applications, 8-10 d interval) – BBCH 14 - 79 Crop category: "Grapes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10-19	0.6	2.9	0.72	**0.039**	0.03
		20-39	0.5	2.9	0.72	**0.033**	
		40 – 69 ≥ 70	0.3	2.9	0.72	0.020	
Honeybee (larvae)	NOED = 100 µg prod./larvae	10-19	0.6	2.2	0.85	0.05	0.2
		20-39	0.5	2.2	0.85	0.04	
		40 – 69 ≥ 70	0.3	2.2	0.85	0.02	0.2
Potato (DW) – 3562.5 g prod./ha (max. 3 applications, 7-8 d interval) – BBCH 21 - 89 Crop category: "potatoes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10 – 39	1	2.9	0.72	**0.054**	0.03
		40 – 69 ≥ 70	0.3	2.9	0.72	0.016	0.03

SV: Shortcut value; Ef: exposure factor; TWA: time weighted average; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating a potential concern. SUW: sideward/upward spray; DW: downward spray.

The first-tier risk assessment for the "weeds in the treated field" exposure scenario demonstrates an acceptable risk for honeybee larvae for all proposed uses of GWN-10616, at all relevant BBCH stages.
The ETR_{oral} values were slightly greater than the trigger value of 0.03 for adult honeybees for proposed uses in grape. The ETR_{oral} values exceeded the trigger value of 0.03 at BBCH stages 10-39 in potato.

Table 9.6-12: Foraging in the field margin - chronic risk for adult honeybees and honeybee larvae from oral exposure

Species (life stage)	Endpoint	BBCH	Ef	SV	TWA	ETR_{oral}	Trigger value
Pome fruit (SUW) – 4275 g prod./ha (max. 2 applications, 6-8 d interval) – BBCH 51 - 69 Crop category: "Orchards 1"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	40 – 69 ≥ 70	0.097	2.9	0.72	0.006	> 0.03
Honeybee (larvae)	NOED = 100 µg prod./larvae	40 – 69 ≥ 70	0.097	2.2	0.85	0.01	> 0.2
Grapes (SUW) – 4275 g prod./ha (max. 3 applications, 8-10 d interval) – BBCH 14 - 79 Crop category: "Grapes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10-19	0.009	2.9	0.72	0.001	0.03
		20-39 40-69 ≥ 70	0.027	2.9	0.72	0.002	
Honeybee (larvae)	NOED = 100 µg prod./larvae	10-19	0.009	2.2	0.85	0.00	> 0.2
		20-39 40-69 ≥ 70	0.027	2.2	0.85	0.00	> 0.2
Potato (DW) – 3562.5 g prod./ha (max. 3 applications, 7-8 d interval) – BBCH 21 - 89 Crop category: "potatoes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10 – 39 40 – 69 ≥ 70	0.0092	2.9	0.72	0.000	0.03

SV: Shortcut value; Ef: exposure factor; TWA: time weighted average; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating a potential concern. SUW: sideward/upward spray; DW: downward spray.

The first-tier risk assessment for the exposure scenario "foraging in the field margin" demonstrates an acceptable risk for adult honeybees and honeybee larvae for all proposed uses of GWN-10616, at all relevant BBCH stages.

Table 9.6-13: Foraging the following year on a permanent crop or succeeding crop for annual crops - chronic risk for adult honeybees and honeybee larvae from oral exposure

Species (life stage)	Endpoint	BBCH	Ef	SV	TWA	ETR_{oral}	Trigger value
Pome fruit (SUW) – 4275 g prod./ha (max. 2 applications, 6-8 d interval) – BBCH 51 - 69 Crop category: "Orchards 1"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	40 – 69 ≥ 70	1	0.54	0.72	0.012	0.03
Honeybee (larvae)	NOED = 100 µg prod./larvae	40 – 69 ≥ 70	1	0.4	0.85	0.01	0.2
Grapes (SUW) – 4275 g prod./ha (max. 3 applications, 8-10 d interval) – BBCH 14 - 79 Crop category: "Grapes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10-19 20-39 40 – 69 ≥ 70	1	0.54	0.72	0.012	0.03
Honeybee (larvae)	NOED = 100 µg prod./larvae	10-19 20-39 40 – 69 ≥ 70	1	0.4	0.85	0.01	0.2
Potato (DW) – 3562.5 g prod./ha (max. 3 applications, 7-8 d interval) – BBCH 21 - 89 Crop category: "potatoes"							
Honeybee (adult)	LDD_{50} = 137 µg prod./bee/day	10-39 40-69 ≥ 70	1	0.54	0.72	0.010	0.03

SV: Shortcut value; Ef: exposure factor; TWA: time weighted average; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating a potential concern. SUW: sideward/upward spray; DW: downward spray.

The first-tier risk assessment for the "next crop" exposure scenario demonstrates an acceptable chronic risk for adult honeybees and honeybee larvae for all proposed uses of GWN-10616, at all relevant BBCH stages.

The zRMS presented a screening/Tier 1 risk assessment for bees based on EFSA (2013). For chronic assessment of **adult honey bees** a risk was indicated in several scenarios:

Treated crop – pome fruit:
For the scenario "treated crop" a potential chronic oral risk to adult bees after application of GWN-10616 in pome fruit is indicated with an ETR of 0.184 (trigger 0.03).
The pH of the product is low (pH = 4.4 to 4.6; containing 750 g/L Potassium phosphonates equivalent to 500 g/L Phosphonic acid). The reduced uptake of feeding solution observed in the chronic bee test (KCP 10.3.1.2/01) is likely due to an unpleasant taste (i.e. sour) leading to an avoidance behaviour and thereby to a reduced exposure in the field.
Additionally, the trigger value of 0.03 based on a daily mortality rate of 5.3 %, as discussed above, is found to be highly conservative. It should also be considered further that bees will not be exposed to the product over a considerable period of time (single product components will behave very differently in the environment showing different dissipation and degradation patterns).
If concerns cannot be addressed via this weight of evidence approach, a warning sentence could be added to the label: "SPe8: Do not use when bees are actively foraging".
Overall, considering the avoidance observed in the chronic bee study, the questionable high level of protection aimed at with the proposed trigger of 0.03 and a potential additional mitigation via labelling, an acceptable chronic oral risk to adult bees for the scenario "treated crop" is concluded after application of GWN-10616 in pome fruit according to the GAP.

Treated crop – grapes:
For the scenario "treated crop" a potential risk is indicated with an ETR value of 0.184 (trigger 0.03).
As described above, the pH of the product is low and the reduced uptake of feeding solution in the chronic bee test is likely due to an unpleasant taste (i.e. sour) leading to a reduced exposure in the field where bees will avoid food items contaminated with GWN-10616.
For the calculated ETR values, the risk for the treated crop scenario is mainly driven by the high SV of 8.2 derived from crops attractive for pollen and nectar. According to EFSA (2013) Appendix D grapes as a crop are mainly attractive to HB for pollen. Cross reference is made to the renewal assessment report of Cymoxanil (Volume 3 – B.9 (PPP) – Cymoxanil 45 WG; February 2020) where it has been concluded that grape nectar is not attractive to bees. Due to that fact, if bees are considered to feed on grapevine crops, they will only consume pollen in a considerable amount. Taking into account a SV of 0.06 for the scenario "treated crop after emergence (downward spray)" category "crop attractive for pollen only" an ETR of < 0.01 can be calculated which is far below the relevant trigger of 0.03.
Additionally, the proposed trigger of 0.03 itself is aiming at a very high level of conservatism. It is based on a daily background mortality rate of 5.3 % according to EFSA (2013) and was found to be highly conservative (EFSA Supporting publication 2020:EN-1880), with only one measurement out of 191 presenting a lower value. Instead, median daily background mortality rates were found around 10–12 %. This high conservatism is also visible considering the regulatory acceptable daily dose (RADD) must be 34-times higher than the exposure level (EFSA, 2013; Appendix M). According to the considerations provided in EFSA (2013) when basing the assessment trigger on the median observed background mortality of 10 % an ETR trigger of 0.07 is calculated.
Overall, considering the avoidance observed in the chronic bee study, the low attractiveness of grape crops and therefore the unrealistically SV as well as the questionable high level of protection aimed at with the proposed trigger of 0.03, an acceptable chronic oral risk to adult bees for the scenario "treated crop" is concluded after application of GWN-10616 in grapevine according to the GAP.

Weeds – grapes (BBCH 10-39)
ETR values for chronic oral exposure are slightly above the respective trigger value of 0.03 for the scenario "weeds (BBCH 10-19 and BBC 20-39)" with ETR values of 0.039 and 0.033, respectively.
Given the low pH of the product (4.4 to 4.6) and the reduced uptake of feeding solution in the chronic bee test it is likely that bees do not like the taste of the product and will avoid GWN-10616 in the field. Therefore, and considering only a minor breach of the relevant trigger an acceptable chronic oral risk to adult bees (scenario weeds) is concluded after application of GWN-10616 in grapevine according to the GAP.

Weeds – potatoes (BBCH 10-39)
For the scenario "weeds (BBCH ≤ 39)" a potential risk is indicated by an ETR of 0.054 (trigger 0.03). Since potato fields are highly managed, weed occurrence is low. This is supported by the new and revised bee guidance document (EFSA Journal 2023;21(5):7989), where for potatoes the weed scenario for the dietary risk assessment is considered "not relevant" (Table 6 of EFSA Journal 2023;21(5):7989).
Additionally, as described above, the pH of the product is low (containing 750 g/L Potassium phosphonates equivalent to 500 g/L Phosphonic acid) and the reduced uptake of feeding solution in the chronic bee test is likely due to an unpleasant taste (i.e. sour) leading to an avoidance behaviour and thereby to a reduced exposure in the field.
Based on this lack of attractive flowering weeds as food source for pollinators in the field and the avoidance behaviour observed in the chronic bee study, the weed scenario is concluded to result in an acceptable risk after the use of GWN-10616 in potato according to the GAP.

For chronic assessment of **honey bee larvae** a risk was indicated in several scenarios:

Treated crop – pome fruit:
For the scenario "treated crop" a potential risk is indicated for bee larvae with an ETR of 0.22 (trigger 0.2).
As described above, the pH of the product is low (containing 750 g/L Potassium phosphonates equivalent to 500 g/L Phosphonic acid) and the reduced uptake of feeding solution in the chronic bee test is likely

due to an unpleasant taste (i.e. sour) leading to an avoidance behaviour and thereby to a reduced exposure in the field.
In addition, the NOED is based on a corrected mortality of 8.57 % on Day 8. Considering the respective LD_{10} of 133.54 µg product/larvae on Day 8, the assessment would result in an acceptable risk (i.e. ETR = 0.17).
Therefore, and considering as a worst-case scenario only a minor breach of the relevant trigger an acceptable chronic oral risk to bee larva is concluded after application of GWN-10616 in pome fruit according to the GAP.

Treated crop – grapes:
The ETR value for chronic oral exposure of bee larvae is slightly above the respective trigger value of 0.2 for the scenario "treated crop" (i.e. ETR = 0.22).
As described above, the pH of the product is low (containing 750 g/L Potassium phosphonates equivalent to 500 g/L Phosphonic acid) and the reduced uptake of feeding solution in the chronic bee test is likely due to an unpleasant taste (i.e. sour) leading to a reduced exposure in the field where bees will avoid food items contaminated with GWN-10616.
For the calculated ETR value, the risk for the treated crop scenario is mainly driven by the high SV of 6.1 derived from crops attractive for pollen and nectar. According to EFSA (2013) Appendix D grapes as a crop are mainly attractive to HB for pollen. Cross reference is also made to the renewal assessment report of Cymoxanil (Volume 3 – B.9 (PPP) – Cymoxanil 45 WG; February 2020) where it has been concluded that grape nectar is not attractive to bees. Taking into account a SV of 0.01 for the scenario "treated crop after emergence (downward spray)" category "crop attractive for pollen only" an ETR of < 0.01 can be calculated which is far below the relevant trigger of 0.2.
Therefore, and considering as a worst-case scenario only a minor breach of the relevant trigger an acceptable chronic oral risk to bee larva is concluded after application of GWN-10616 in grapevine according to the GAP.

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

Not relevant.

9.6.3 Effects on bumble bees

Acute toxicity studies with bumble bees and Zoxamide as well as GWN-10616 are available and summarised under 9.6.1.

Endpoints for testing with bumble bees are considerably higher compared to testing with honey bees, therefore the assessment for honey bees is covering the risk for bumble bees. Thus, the risk to bumble bees from oral consumption and contact is considered acceptable when GWN-10616 is applied according to the GAP.

9.6.4 Effects on solitary bees

No further information on solitary bees is required.

9.6.5 Overall conclusions

The risk assessments are performed following the approaches outlined in SANCO/10329/2002 rev. 2 final and the revised EPPO scheme.

The acute oral and contact toxicity hazard quotients are considerably less than 50, indicating that the active substances as well as the formulated product pose a low risk to bees. Therefore, a low risk to bees is expected from the application of GWN-10616 according to GAP. Based on the chronic oral adult toxicity study as well as the endpoint from the larval oral adult toxicity study, the respective TER values are considerably above 1. Thus, the chronic risk to adult and larval honey bees potentially exposed to GWN-10616 containing Zoxamide and Phosphonic acid via consumption of nectar and pollen after application of GWN-10616 is considered acceptable.

Review Comments:

The evaluation of the acute risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible acute risk associated with the exposure of *Apis mellifera* to GWN-10616.

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

The risk assessment based on the EFSA Guidance (2013) is not yet approved and certain parts are currently under revision.

Nevertheless, some CEU countries require evaluation according to EFSA 2013. This approach was discussed at the last meeting of the Central Zone in the field of ecotoxicology (Warsaw, 12.2023), where it was agreed to present an assessment in the Core in accordance with EFSA 2013.

The evaluation of the acute and chronic risk for bees was performed by zRMS in accordance with the recommendations of the "EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)" (EFSA Journal 2013;11(7):3295; hereafter referred to as EFSA/2013/3295). Refinement of risk, where required, has been left to the national level.

The risk assessment performed following the approaches outlined in SANCO/10329/2002 rev. 2 final and the revised EPPO scheme was not evaluated by zRMS.

The zRMS presented a screening/Tier 1 risk assessment for bees based on EFSA (2013). For chronic assessment of adult honey bees and honey bee larvae a risk was indicated in several scenarios, additional argumentation is provided in chapter 9.6.2.

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 GWN-10616 containing Zoxamide and Phosphonic acid. Full details of these studies are provided in Appendix 2 of this document.

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

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	GWN-10616	Laboratory test glass plates (2D)	LR_{50} = 6332.2 g/ha ER_{50} > 5000 g/ha	KCP 10.3.2.1/01 Venturi (2021) 834-004
Aphidius rhopalosiphi (adults)	GWN-10616	Laboratory test glass plates (2D)	LR_{50} = 16491.07 g/ha ER_{50} > 10000 g/ha	KCP 10.3.2.1/02 Colli (2021) 834-001
Field or semi-field tests				
-/-				

9.7.1.1 Justification for new endpoints

The formulation GWN-10616 contains two active substances. Thus, studies with the formulated product have been conducted and respective endpoints are presented.

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

Calculation of $PER_{in\text{-}field}$ values as performed according to ESCORT 2 with the following equation:
$PER_{in\text{-}field}$ = Application rate x MAF

The MAF was selected based on provisions in ESCORT 2, Appendix V. LR_{50} endpoints were selected for the risk assessment since no sub-lethal effects were observed in treatment groups without considerable mortality. The results for the in-field risk assessment are summarised in the following tables.

Table 9.7-2: First-tier assessment of the in-field risk for non-target arthropods due to the use of GWN-10616 in grapevine

Intended use	Grapevine		
Product	GWN-10616		
Application rate (g/ha)	3 × 3 L = 3 × 4275*		
MAF	2.3 (8-day interval)		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	6332.2	9832.5	1.6
Aphidius rhopalosiphi	16491.07		0.6

*based on density of 1.425 g/mL
MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

Table 9.7-3: First-tier assessment of the in-field risk for non-target arthropods due to the use of GWN-10616 in pome fruit

Intended use	Pome fruit		
Product	GWN-10616		
Application rate (g/ha)	2 × 3 L = 2 × 4275*		
MAF	1.7 (6-day interval)		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	6332.2	7267.5	1.1
Aphidius rhopalosiphi	16491.07		0.4

*based on density of 1.425 g/mL
MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

Table 9.7-4: First-tier assessment of the in-field risk for non-target arthropods due to the use of GWN-10616 in in potato

Intended use	Potato		
Product	GWN-10616		
Application rate (g/ha)	3 × 2.5 L = 3 × 3562.5		
MAF	2.3 (7-day interval)		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	6332.2	8193.75	1.3
Aphidius rhopalosiphi	16491.07		0.5

*based on density of 1.425 g/mL
MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

All hazard quotients for the in-field assessment are considerably less than 2, indicating that the product poses a low risk to non-target arthropods. Therefore, a low in-field risk to non-target arthropods is expected from the application of GWN-10616 in grapevine, pome fruit and potato according to the GAP.

9.7.2.2 Risk assessment for off-field exposure

To calculate $PER_{off\text{-}field}$, the following equation was used (ESCORT 2). The vegetation distribution factor of 5 is based on the Technical Report by EFSA (EFSA Supporting publication 2019:EN-1673):
$PER_{off\text{-}field}$ = Application rate x MAF x (drift factor/VDF) x correction factor

Where,
- drift factor for field crops was selected based on ESCORT 2, Appendix VI
- VDF: vegetation distribution factor = 10 / 5
- correction factor = 10

LR_{50} endpoints were selected for the risk assessment since no sub-lethal effects were observed in treatment groups without considerable mortality. The results for the off-field risk assessment for the intended uses are presented in the following tables.

Table 9.7-5: First-tier assessment of the off-field risk for non-target arthropods due to the use of GWN-10616 in grapevine

Intended use	Grapevine
Product	GWN-10616
Application rate (g/ha)	3 × 3 L = 3 × 4275*
MAF	2.3 (8-day interval)

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**VDF**	**CF**	**$PER_{off\text{-}field}$ (g/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	6332.2	8.02 6.90%	10	10	788.6 678.4	0.1
			5		1577.1 1356.9	0.3 0.2
Aphidius rhopalosiphi	16491.07		10		788.6 678.4	< 0.1
			5		1577.1 1356.9	< 0.1

*based on density of 1.425 g/mL
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.

Table 9.7-6: First-tier assessment of the off-field risk for non-target arthropods due to the use of GWN-10616 in pome fruit

Intended use	Pome fruit
Product	GWN-10616
Application rate (g/ha)	2 × 3 L = 2 × 4275*
MAF	1.7 (6-day interval)

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**VDF**	**CF**	**$PER_{off\text{-}field}$ (g/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	6332.2	15.73 25.53 %	10	10	1143.2 1855.4	0.2 0.3
			5		2286.4 3710.8	0.4 0.6
Aphidius rhopalosiphi	16491.07		10		1143.2 1855.4	< 0.1
			5		2286.4 3710.8	0.1 0.2

*based on density of 1.425 g/mL
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.

Table 9.7-7: First-tier assessment of the off-field risk for non-target arthropods due to the use of GWN-10616 in potato

Intended use	Potato					
Product	GWN-10616					
Application rate (g/ha)	3 × 2.5 L = 3 × 3562.5*					
MAF	2.3 (7-day interval)					
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**VDF**	**CF**	**$PER_{off\text{-}field}$ (g/ha)**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	6332.2	~~2.77~~ 2.01%	10	10	~~227.0~~ 164.7	< 0.1
			5		~~454.0~~ 329.4	< 0.1
Aphidius rhopalosiphi	16491.07		10		~~227.0~~ 164.7	< 0.1
			5		~~454.0~~ 329.4	< 0.1

*based on density of 1.425 g/mL
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.

All hazard quotients for the off-field assessment are considerably less than 2, indicating that the product poses a low risk to non-target arthropods. Therefore, a low off-field risk to non-target arthropods is expected from the application of GWN-10616 in orchards, vineyards and potatoes according to the GAP.

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

All hazard quotients for the in- and off-field assessment are considerably less than 2, indicating that the product poses a low risk to non-target arthropods. Therefore, a low in- and off-field risk to non-target arthropods is expected from the application of GWN-10616 in grapevine, pome fruit and potato according to the GAP.

Review Comments:

Based on the results of the conducted risk assessment it can be concluded that no in-field and off-field risk for non-target arthropods is expected from use of GWN-10616. No mitigation measures are required.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with Zoxamide, its relevant metabolites and Phosphonic acid. Full details of these studies are provided in the respective EU assessment reports and related documents. Additional information on Zoxamide and its metabolites to address data gaps identified during EU renewal process have been submitted to RMS Latvia.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GWN-10616 were not evaluated as part of the EU assessment. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.8-1: Endpoints and effect values of Zoxamide and its metabolites 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	14 d acute 10 % peat content	LC_{50} > 1070 mg a.s./kg soil dw $LC_{50,corr}$ > 535 mg a.s./kg soil dw*	EFSA (2017)
Eisenia andrei	Zoxamide **	56 d chronic 5 % peat content	NOEC = 2.453 mg/kg dw (analysed) $NOEC_{corr}$ = 1.227 mg a.s./kg dw*	Submitted to RMS Friedrich (2020)
Eisenia fetida	RH-127450	14 d acute 10 % peat content	LC_{50} > 1000 mg a.s./kg dw $LC_{50,corr}$ > 500 mg s.s./kg dw*	EFSA (2017)
Eisenia fetida	RH-127450	56 d chronic 10 % peat content	NOEC = 10 mg a.s./kg dw $NOEC_{corr}$ = 5 mg a.s./kg dw*	Submitted to RMS Gray (2021)
Eisenia fetida	RH-141455	56 d chronic 10 % peat content	NOEC = 5 mg a.s./kg soil dw $NOEC_{corr}$ = 2.5 mg a.s./kg dw*	EFSA (2017)
Eisenia fetida	RH-163353	56 d chronic 10 % peat content	NOEC = 10 mg a.s./kg dw $NOEC_{corr}$ = 5 mg a.s./kg dw*	Submitted to RMS Gray (2021)
Eisenia fetida	RH-24549	56 d chronic 10 % peat content	NOEC = 10 mg a.s./kg dw $NOEC_{corr}$ = 5 mg a.s./kg dw*	Submitted to RMS Gray (2021)
Folsomia candida	Zoxamide **	28 d chronic 5 % peat content	NOEC = 217 mg a.s./kg dw $NOEC_{corr}$ = 108.5 mg a.s./kg dw*	Submitted to RMS Parsons (2020)
~~*Folsomia candida*~~	~~RH-141455~~	~~28 d chronic~~ ~~5 % peat content~~	~~NOEC = 50 mg a.s./kg dw #~~ ~~$NOEC_{corr}$ = 25 mg a.s./kg dw*~~	Submitted to RMS Gray (2021) Study not valid
~~*Folsomia candida*~~	~~RH-163353~~	~~28 d chronic~~ ~~5 % peat content~~	~~NOEC = 4.76 mg a.s./kg dw~~ ~~$NOEC_{corr}$ = 2.38 mg a.s./kg dw*~~	Submitted to RMS Gray (2021) Study not valid
Hypoaspis aculeifer	Zoxamide **	14 d, chronic 5 % peat content	NOEC = 217 mg a.s./kg dw $NOEC_{corr}$ = 108.5 mg a.s./kg dw*	Submitted to RMS Parsons (2020)
Hypoaspis	RH-141455	14 d, chronic	NOEC = 50 mg a.s./kg dw #	Submitted to RMS

Species	Substance	Exposure system	Results	Reference
aculeifer		5 % peat content	$NOEC_{corr}$ = 25 mg a.s../kg dw*	Gray (2021)
Hypoaspis aculeifer	RH-163353	14 d chronic 5 % peat content	NOEC = 27.78 mg a.s./kg dw $NOEC_{corr}$ = 13.89 mg a.s ./kg dw*	Submitted to RMS Gray (2021)
Field studies				
Potential effects of zoxamide on field populations of earthworms after spray application of Zoxium 240 (containing nominally 240 g/L zoxamide) to bare soil at a pattern of 5x 140 g a.s./ha (= total of 700 g a.s./ha/season), 5x 180 g a.s./ha (= total of 900 g a.s./ha/season) and 5x 280 g a.s./ha (= total of 1400 g a.s./ha/season) with an interval of 7-8 days were investigated on a typical arable field in Saxony, Germany in 2018. As a result, the test item had no statistically significant adverse effects on single species, ecological groups and total earthworm abundance and biomass one year after the first application. (Submitted to RMS: Schulz, 2020) Potential effects of zoxamide on field populations of earthworms after spray application of Zoxium 240 (containing nominally 240 g/L zoxamide) to bare soil at a pattern of 3x 140 g a.s./ha (= total of 420 g a.s./ha/season), 3x 180 g a.s./ha (= total of 540 g a.s./ha/season) and 3x 360 g a.s./ha (= total of 1080 g a.s./ha/season) with an interval of 7±1 days were investigated on a typical arable field in Saxony, Germany in 2019. As a result, the test item had no statistically significant adverse effects on single species, ecological groups and total earthworm abundance and biomass one year after the first application. (Submitted to RMS: Schulz, 2021)				

* Corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002. These endpoints were taken forward for the risk assessment.

** Study performed with GWN-9790EU (Zoxium 240 SC)

No effects up to and including the highest application rate tested (i.e. 50 mg a.s./kg dry soil)

Review Comments:

zRMS agrees presented endpoints in table 9.8-1, except tests by Gray, 2021 on F. candida (studies not valid). Thus, those values were considered to be acceptable to be used in the risk assessment.

The confirmatory-like studies (laboratory and field), were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

Table 9.8-2: Endpoints and effect values of Phosphonic acid* relevant for the risk assessment for earthworms

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	Phosphonic acid	Mixed into substrate 14 d, acute	LC_{50} > 1000 mg/kg dw	EFSA (2012)
Eisenia fetida	Phosphonic acid	Mixed into substrate 56 d, chronic	NOEC = 62.5 mg/kg dw	EFSA (2012)
Field studies				
-/-				

*Phosphonic acid (also Phosphorous acid) is the actual active substance of the technical item Potassium phosphonates (formerly: phosphite; technical active substance).

Table 9.8-3: Endpoints and effect values of GWN-10616 relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	GWN-10616	Mixed into substrate 56 d, chronic 10 % peat content	EC_{10} = 25.3 mg/kg dw NOEC = 29.4 mg/kg dw equivalent to 10.21 mg Potassium phosphonate/kg dw and 1.25 mg Zoxamide/kg dw	KCP 10.4.1.1/01 Pecorari (2021) 833-001
Folsomia candida	GWN-10616	Mixed into substrate 21 d, chronic 5 % peat content	NOEC ≥ 1000 mg/kg dw equivalent to 347.26 mg Potassium phosphonate /kg dw and 42.47 mg Zoxamide/kg dw	KCP 10.4.2.1/01 Grandolini (2021) 834-002
Hypoaspis aculeifer	GWN-10616	Mixed into substrate 14 d, chronic 5 % peat content	NOEC ≥ 1000 mg/kg dw equivalent to 347.26 mg Potassium phosphonate /kg dw and 42.47 mg Zoxamide/kg dw	KCP 10.4.2.1/02 Grandolini (2021) 834-003
Field studies				
-/-				

9.8.1.1 Justification for new endpoints

<u>*Chronic endpoints for Zoxamide and its metabolites on earthworm*</u>
EFSA (2017) requested in its Peer Review Conclusion: "*Further data are needed for the chronic risk assessment to earthworm of the active substance and the metabolites RH-127450, RH-24549, RH-163353*." These studies were performed and provided to the RMS.
Valid earthworm chronic toxicity studies were not available for Zoxamide and its pertinent metabolites (data gap) with the exception of RH-141455. For this metabolite, a low risk was concluded by EFSA (2017).
To refine the laboratory endpoints, earthworm field studies were conducted with Zoxamide applied as Zoxium 240 SC (GWN-9790 EU). These studies were regarded suitable to conclude safe uses for Zoxamide when applied at a worst-case application pattern (i.e. considering worst-case EU GAP uses of 3 x 180 g a.s./ha at an interval of 7-8 days for tomatoes, potatoes and grapes). Zoxium 240 SC (GWN-9790 EU) was the representative Zoxamide formulation during AIR. These studies were provided to the RMS.

Review Comments:

The confirmatory-like studies, were evaluated and accepted by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_confirmatory_like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

<u>*Chronic endpoints for Zoxamide metabolites on soil macro-organisms other than earthworms*</u>
EFSA (2017) requested in its Peer Review Conclusion: "*Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455*." These studies were performed and provided to the RMS.

Studies on *Folsomia* and *Hypoaspis* have been performed with the active substance Zoxamide applied as

Zoxium 240 SC (GWN-9790 EU). These studies are required by Regulation (EU) 283/2013 and are regarded representative for Zoxamide.

Review Comments:

The studies by Gray, 2021 on F. candida were considered not valid by RMS-LV. Thus, those values were considered not suitable for the risk assessment.

The confirmatory-like studies, were evaluated by the RMS-LV for zoxamide metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_confirmatory_like_data_ Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

A risk assessment for *Folsomia* can be performed for RH-24549 and RH-127450 based on the available ecotoxicity endpoints for zoxamide, considering an additional safety factor of 10.

Product studies
The formulation GWN-10616 contains two active substances. Thus, studies with the formulated product have been conducted and respective endpoints are presented.

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).

Consideration of mixture toxicity
The product GWN-10616 contains Zoxamide and Phosphonic acid as active substances. To account for a potential joint effect of this mixture to non-target soil organisms a mixture toxicity risk assessment is performed (in line with the recommendations of EFSA Journal 2013;11(7):3290).

Toxicity studies on chronic effects of the active substances and the formulation GWN-10616 to earthworms are available. For calculation of mixture toxicity, toxic units (TUs) and model deviation ratios (MDRs), please refer to Chapter 9.5.2.

The calculation of TUs and MDRs for chronic earthworm toxicity data is summarized in the following table.

Table 9.8-4: Calculation of TU and MDR for the product GWN-10616

	amount of a.s. in product [g/kg]	p (a.s.) fraction in product	NOEC $_{a.s.}$ mg/kg	relative toxic units (%)	$NOEC_{mix-CA}$ mg sum of a.s./kg	$NOEC_{PPP}$ mg sum of a.s./kg*	MDR
Earthworm – no endpoint correction							
Zoxamide	42	0.11	2.453	75.3	17.3	14.2	1.2
Phosphonic acid	351	0.89	62.5	24.7			
Earthworm – endpoint correction							
Zoxamide	42	0.11	1.227 (corr)	85.9	9.8	14.2	0.7
Phosphonic acid	351	0.89	62.5	14.1			

TU: toxic units; MDR: model deviation ratio.
*Considering product density of 1.425 g/mL.

According to the results presented above, the calculation of MDR for chronic earthworm data resulted in ratios of 1.2 (Zoxamide endpoint not corrected for $logP_{ow}$) and 0.7 (Zoxamide endpoint corrected for

$logP_{ow}$) indicating that the mixture of the two active substances does not result in considerable higher toxicity compared to the assumption of concentration additivity (i.e. no synergistic effects).
Based on toxic units, it is concluded that Zoxamide is mainly driving the toxicity of the mixture (TU ≥ 75 % and ≥ 85 %, respectively).

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. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for Phosphonic acid (based on laboratory data).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group grapevine also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses in groups pome fruit and potato (see 9.1.2).

For Zoxamide, the metabolites RH-127450, RH-24549, RH-163353 and RH-141455 have been identified as potentially relevant for the soil risk assessment in the EFSA (2017) Peer Review Conclusion. In soils at 20 °C, levels of the three major metabolites occurring at > 10 % AR peaked on days 3-7 after application of ^{14}C-Zoxamide. Maximum concentrations were 8.1 – 15.1 % AR (RH-127450), 5.5 – 33.8 % AR (RH-24549) and 7.9 – 15.0 % AR (RH-163353). RH-141455 was detected at > 5 % AR on more than two occasions with 8 % on day 28, 7.4 % on day 56 and 5.8 % AR on day 120 after application of ^{14}C-Zoxamide.

For Zoxamide and earthworms, the overall lowest endpoint in a chronic earthworm study was derived for the parent compound itself with a NOEC of 2.453 mg/kg (analysed) and a $NOEC_{corr}$ of 1.227 mg a.s./kg dry soil. A generally lower earthworm toxicity of the Zoxamide soil metabolites compared to the parent compound has been confirmed by the available studies, since chronic earthworm studies are available for all four potentially relevant soil metabolites.

For Zoxamide and soil macro-organisms other than earthworms, the available studies with the metabolite RH-163353 indicate lower endpoints for *Folsomia* and *Hypoaspis* compared to the parent compound Zoxamide. For RH-141455 a low toxicity towards *Folsomia* and *Hypoaspis* was observed. Studies with the soil metabolites RH-24549 and RH-127450 are not available. Since these metabolites are peaking in the soil 3-7 days after application of Zoxamide with DT_{50} values of 5.2 days (RH-127450) and 6.84 days (RH-24549), it is assumed that their toxicity has been intrinsically tested with the available Zoxamide studies running over 28 and 14 days, respectively. A risk assessment for *Folsomia* and *Hypoaspis* is performed for RH-24549 and RH-127450 based on the available ecotoxicity endpoints for Zoxamide, and considering an additional safety factor of 10.

The first-tier risk assessment for non-target soil meso-and macrofauna is summarized in the following tables.

Table 9.8-5: First-tier assessment of the acute and chronic risk for earthworms due to the use of GWN-10616 in grapevine

~~Acute effects on earthworms~~			
~~Product/active substance~~	~~LC_{50} (mg/kg dw)~~	~~PEC_{soil} (mg/kg dw)~~	~~TER_a (criterion TER ≥ 10)~~
~~Zoxamide~~	~~> 535 (corr)~~	~~0.2571 (ini)~~	~~> 2000~~
~~RH-127450~~	~~> 500 (corr)~~	~~0.0282 (ini)~~	~~> 10000~~
~~Phosphonic acid~~	~~> 1000~~	~~3.4570 (accu)*~~	~~> 289~~
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Zoxamide	1.227 (corr)	0.2571 (ini)	**4.8**
RH-127450	5 (corr)	0.0282 (ini)	177
RH-141455	2.5 (corr)	0.0159 (ini)	157
RH-163353	5 (corr)	0.0383 (ini)	131
RH-24549	5 (corr)	0.0418 (ini)	120
Phosphonic acid	62.5	3.4570 (accu)*	18.1
GWN-10616	25.3 / 12.65 (corr)	2.32 ~~2.28~~ (act)**	10.9 / 5.45~~11.1~~

*worst-case PECsoil value for Phosphonic acid considering information from LoEP (2013) for Disodium phosphate (DFOP based DT_{50}), please refer to B8.7.2.3.
** single maximum application PEC considered relevant for the ecotoxicological risk assessment of formulation since formulated product will not be stable or accumulate in soil; multiple applications and long-term PECs including information on degradation and accumulation are covered in single active substance risk assessment.
TER values shown in bold fall below the relevant trigger.

Acceptable acute and long-term risk (first-tier assessment step) for earthworms following exposure to Zoxamide metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses).

For Zoxamide, the chronic trigger for earthworms was slightly breached (i.e. TER = 4.8 compared to the relevant trigger of ≥ 5). Two field earthworm studies are available and presented in the higher-tier risk assessment below.

For uses in pome fruit and potato an acceptable chronic risk for earthworms in indicated based on PEC_{soil} values of 0.1839 mg Zoxamide/kg (pome fruit, resulting TER = 6.7) and 0.2172 mg Zoxamide/kg (potato, resulting TER = 5.6), respectively.

Review Comments:

zRMS agrees presented above TERs calculations for uses in pome fruit and potato.

Table 9.8-6: First-tier assessment of the chronic risk for non-target soil organisms other than earthworms (meso- and macrofauna) due to the use of GWN-10616 in grapevine

Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Folsomia candida			
Zoxamide	108.5 (corr)	0.2571 (ini)	422
RH-127450	10.85*	0.0282 (ini)	385
RH-141455	~~25 (corr)~~ 10.85*	0.0159 (ini)	~~1572~~ 682
RH-163353	~~2.38 (corr)~~ 10.85*	0.0383 (ini)	~~61.1~~ 283
RH-24549	10.85*	0.0418 (ini)	260
GWN-10616	> 1000	2.32 ~~2.28~~ (act)**	431 ~~439~~
Hypoaspis aculeifer			
Zoxamide	108.5 (corr)	0.2571 (ini)	422
RH-127450	10.85*	0.0282 (ini)	385
RH-141455	25 (corr)	0.0159 (ini)	1572
RH-163353	13.89 (corr)	0.0383 (ini)	363
RH-24549	10.85*	0.0418 (ini)	260
GWN-10616	> 1000	2.32 ~~2.28~~ (act)**	431 ~~439~~

* based on parent endpoint/10.
** single application PEC considered relevant for the ecotoxicological risk assessment of formulation since formulated product will not be stable or accumulate in soil; multiple applications and long-term PECs including information on degradation and accumulation are covered in single active substance risk assessment.
TER values shown in bold fall below the relevant trigger.

Acceptable long-term risk (first-tier assessment step) for non-target soil organisms other than earthworms following exposure to Zoxamide, its relevant metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses).

9.8.2.2 Higher-tier risk assessment

For the use in grapevine, the relevant trigger for chronic earthworms exposed to Zoxamide via soil was slightly breached (i.e. TER = 4.8 compared to the relevant trigger of ≥ 5). To further support an acceptable risk for earthworms following the application of Zoxamide higher-tier data is available.

The overall toxicity of Zoxamide and its metabolites has been tested in two earthworm field studies with the parent compound, which did not reveal any adverse effects after spray application of Zoxium 240 (containing nominally 240 g/L Zoxamide) to bare soil up to and including a worst-case pattern of 5 x 280 g a.s./ha (= total of 1400 g a.s./ha/season) with an interval of 7-8 days or 3 x 360 g a.s./ha (= total of 1080 g a.s./ha/season) with an interval of 7 ± 1 days on typical arable fields in Germany in 2018 and 2019, respectively. No statistically significant adverse effects on single species, ecological groups and total earthworm abundance and biomass one year after the first application were noted.

The available field studies cover the application pattern of Zoxamide in grapevine (3 x 150 g a.s./ha, 8-days interval). Therefore, an acceptable chronic risk for earthworms is demonstrated following the application of GWN-10616 in grapevine according to GAP.

Review Comments:

The confirmatory-like studies by Schulz L. Report No. 18 48 FEW 0001 and Report No. 19 48 FEW 0002 were evaluated and accepted by the RMS-LV for zoxamide in an interzonal procedure. All details are to be found in the file: Zoxamide_confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

The RMS-LV conclusion is as follow:

These studies were regarded suitable to conclude safe uses for zoxamide when applied at a worst-case application pattern (i.e. considering worst-case EU GAP uses of 3x 180 g a.s./ha at an interval of 7-8 days for tomatoes, potatoes and grapes).

9.8.3 Overall conclusions

Acceptable acute and long-term risk (first-tier assessment step) for earthworms following exposure to Zoxamide metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses). Available earthworm field studies cover the application pattern of Zoxamide in grapevine (3 x 150 g a.s./ha, 8-days interval) resulting in an acceptable higher-tier risk.

Acceptable long-term risk (first-tier assessment step) for non-target soil organisms other than earthworms following exposure to Zoxamide, its relevant metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses).

Overall, an acceptable risk for non-target soil organisms (meso- and macro-fauna) is demonstrated following the application of GWN-10616 in grapevine, pome fruit and potato according to GAP.

Review Comments:

The long-term risks of GWN-10616 to soil meso- and macro-organisms were assessed from toxicity exposure ratios between toxicity endpoints and maximum PEC_{soil}. The relevant predicted environmental concentrations in soil (PEC_{soil}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate).

Based on performed risk assessment it can be concluded that there will be negligible risk associated with the exposure of beneficial soil organisms to GWN-10616 following proposed use pattern.

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, its relevant metabolites and Phosphonic acid. Full details of these studies are provided in the respective EU assessment reports and related documents. Additional information on Zoxamide and its metabolites to address data gaps identified during EU renewal process have been submitted to RMS Latvia.

Effects on soil microorganisms of GWN-10616 were not evaluated as part of the EU assessment. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Zoxamide	42 d, aerobic	< 25% at 2 mg a.s./kg soil dw	EFSA (2017)
C-mineralisation	Zoxamide	28 d, aerobic	< 25% at 2 mg a.s./kg soil dw	EFSA (2017)
N-mineralisation	RH-141455	28 d, aerobic	< 25% at 0.2-1 mg a.s./kg soil dw	EFSA (2017)
N-mineralisation	RH-127450	28 d, aerobic	< 25% at 0.195 mg a.s./kg soil dw	Submitted to RMS Jarrom (2019)
N-mineralisation	RH-24549	28 d, aerobic	< 25% at 0.350 mg a.s./kg soil dw	Submitted to RMS Jarrom (2019)
N-mineralisation	RH-163353	28 d, aerobic	< 25% at 0.365 mg a.s./kg soil dw	Submitted to RMS Jarrom (2019)
N-mineralisation	Phosphonic acid*	28 d, aerobic	< 25% at 26.99 mg a.s./kg soil dw	EFSA (2012)
N-mineralisation	GWN-10616	28 d, aerobic	< 25% at 67.33 mg test item/kg soil dw	KCP 10.5.1/01 Rossini (2021) 841-001

*Phosphonic acid (also Phosphorous acid) is the actual active substance of the technical item Potassium phosphonates (formerly: phosphite; technical active substance).

Review Comments:

zRMS agrees presented endpoints in table 9.9-1. Thus, those values were considered to be acceptable to be used in the risk assessment.

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

9.9.1.1 Justification for new endpoints

EFSA (2017) requested "*Further data to address the risk to soil microorganisms for metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)*." These studies have been performed and provided to the RMS.

The formulation GWN-10616 contains two active substances. Thus, studies with the formulated product have been conducted and respective endpoints are presented.

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group grapevine also covers the risk for the soil microorganisms from all other intended uses in groups pome fruit and potato (see 9.1.2).

The assessment of the risk for effects on soil micro-organisms is summarized in the table 9.9-2.

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of GWN-10616 in grapevine

Intended use			
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Zoxamide	2	0.2571 (ini)	yes
RH-127450	0.195	0.0282 (ini)	yes
RH-141455	0.2	0.0159 (ini)	yes
RH-163353	0.365	0.0383 (ini)	yes
RH-24549	0.350	0.0418 (ini)	yes
Phosphonic acid	26.99	3.4570 (accu)*	yes
GWN-10616	67.33	2.32 ~~2.28~~ (act)**	yes

*worst-case PECsoil value for Phosphonic acid considering information from LoEP (2013) for Disodium phosphate (DFOP based DT50), please refer to B8.
** single maximum application PEC considered relevant for the ecotoxicological risk assessment of formulation since formulated product will not be stable or accumulate in soil; multiple applications and long-term PECs including information on degradation and accumulation are covered in single active substance risk assessment.

Acceptable risk for soil micro-organisms following exposure to Zoxamide, its relevant metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses).

9.9.3 Overall conclusions

Acceptable risk for soil micro-organisms following exposure to Zoxamide, its relevant metabolites, Phosphonic acid and GWN-10616 via soil is demonstrated for application of GWN-10616 in grapevine according to GAP (covering all other uses).

Overall, an acceptable risk for soil micro-organisms is demonstrated following the application of GWN-10616 in grapevine, pome fruit and potato according to GAP.

Review Comments:

Based on the results of the conducted first tier risk assessment it can be concluded that no risk for soil micro-organisms is expected from use of GWN-10616.

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 GWN-10616. Effects on non-target terrestrial plants were not evaluated as part of the EU assessment. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.

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

Species	Substance	Exposure System	Results	Reference
Glycine max d, *Cucumis sativus* d, *Brassica napus* d, *Helianthus annuus* d, *Allium cepa* m, *Avena sativa* m	GWN-10616	21 d Vegetative vigour	ER_{50} > 4275 g/ha no effects on mortality, phytotoxicity and biomass	KCP 10.6.2/01 Colli (2021) 851-001

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

The formulation GWN-10616 contains two active substances. Thus, studies with the formulated product have been conducted and respective endpoints are presented.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Studies with the active substances are routinely not generated on non-target terrestrial plants as the risk assessment is based on formulation data. Studies are available for the formulation GWN-10616, covering potential mixture toxicity effects of the individual active substances.

Limit tests at rates up to 4275 g/ha were conducted with GWN-10616 and effects were below the critical threshold as defined by the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). The limit test rates equal and/or cover the highest field application rates in grapevine, pome fruit and potato (3 L product = 4.275 kg) and are thus considered an indicator for an acceptable risk. Therefore, a low risk to terrestrial non-target plants is expected from the application of GWN-10616 according to the GAP.

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

Not relevant.

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

Limit tests at rates up to 4275 g/ha were conducted with GWN-10616 and effects were below the critical threshold. The limit test rates equal and/or cover the highest field application rate in grapevine, pome fruit and potato and are thus considered an indicator for an acceptable risk.
Therefore, a low risk to terrestrial non-target plants is expected from the application of GWN-10616 in grapevine, pome fruit and potato according to the GAP.

Review Comments:

Based on available information it can be concluded that the proposed use of GWN-10616 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 GWN-10616 applications are not required for the protection of terrestrial non-target plants.

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

Not relevant.

9.12 Monitoring data (KCP 10.8)

Monitoring data concerning effects of the plant protection product GWN-10616 or the active substances Zoxamide or Phosphonic acid to non-target organisms are not available and are not a mandatory requirement.

9.13 Classification and Labelling

~~Please refer to Part A, Section 2.4 for the complete classification and labelling proposals.~~

Species	Substance	Exposure System	Endpoint	Reference
Oncorhynchus mykiss	GWN-10616	96 h, ss	LC_{50} = 34.8 mg/L $_{nom}$	KCP 10.2.1/01 XXXX (2022) 821-001
Daphnia magna	GWN-10616	48 h, ss	EC_{50} > 100 mg/L $_{nom}$	KCP 10.2.1/02 Corboli (2021) 822-001
Raphidocelis subcapitata	GWN-10616	72 h, s	E_rC_{50} = 0.656 mg/L $_{nom}$ E_vC_{50} = 0.351 mg/L $_{nom}$ NOE_rC = 0.037 mg/L $_{nom}$	KCP 10.2.1/03 Mantilacci (2021) 823-001

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: Very toxic to aquatic life **H410: Very toxic to aquatic life with long lasting effects**
Precautionary statement(s):	P391: Collect spillage P501: Dispose of contents/container to a licensed hazardous-waste disposal contractor or collection site except for empty clean containers which can be disposed of as non-hazardous waste.
Additional labelling phrases:	EUH401: To avoid risks to man and the environment, comply with the instructions for use.

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 10.2.1/01	XXXX	2022	ACUTE TOXICITY OF GWN-10616 TO ONCORHYNCHUS MYKISS IN A 96-HOUR SEMI-STATIC TEST XXXX GLP, unpublished	Y	XXXX
KCP 10.2.1/02	Corboli, M.	2021	ACUTE IMMOBILISATION LIMIT TEST ON DAPHNIA MAGNA WITH GWN-10616 UNDER SEMI-STATIC CONDITIONS BT208/21 (822-001) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.2.1/03	Mantilacci, S.	2021	TOXICITY EVALUATION OF TEST ITEM GWN-10616 ON GREEN ALGA RAPHIDOCELIS SUBCAPITATA (FORMERLY KNOWN AS PSEUDOKIRCHNERIELLA SUBCAPITATA) IN A GROWTH INHIBITION TEST BT207/21 (823-001) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.3.1.1/01	Venturi, S.	2021	ACUTE ORAL AND ACUTE CONTACT TOXICITY EFFECTS OF GOW F716 (GWN-10616) TO ADULT WORKER HONEYBEES APIS MELLIFERA L., LABORATORY LIMIT TEST BT135/17 (832-001) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1/02	Venturi, S.	2021	ACUTE ORAL AND ACUTE CONTACT TOXICITY EFFECTS OF GWN-10616 TO ADULT WORKER BUMBLEBEES BOMBUS TERRESTRIS L., LABORATORY TEST BT210/21 (832-004) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.3.1.2/01	Colli, M.	2021	CHRONIC ORAL EFFECTS OF GOW F716 (GWN-10616) TO ADULT WORKER HONEYBEES APIS MELLIFERA L. 10-DAY FEEDING LABORATORY TEST BT147/17 (832-002) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.3.1.3/01	Colli, M.	2021	EFFECTS OF GOW F716 (GWN-10616) TO HONEYBEES (APIS MELLIFERA L.), IN A LARVAL TOXICITY TEST FOLLOWING REPEATED EXPOSURE BT133/17 (832-003) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.3.2.1/01	Venturi, S.	2021	EFFECTS OF GWN-10616 ON THE PREDATORY MITE TYPHLODROMUS PYRI SCHEUTEN (ACARI: PHYTOSEIIDAE) UNDER LABORATORY CONDITIONS BT215/21 (834-004) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.3.2.1/02	Colli, M.	2021	EFFECTS OF GWN-10616 ON THE PARASITIC WASP APHIDIUS RHOPALOSIPHI UNDER LABORATORY CONDITIONS BT209/21 (834-001) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.4.1.1/01	Pecorari, F.	2021	EFFECTS OF GWN-10616 ON REPRODUCTION AND GROWTH OF THE EARTHWORM EISENIA ANDREI IN ARTIFICIAL SOIL BT213/21 (833-001) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX

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 10.4.2.1/01	Grandolini, G.	2021	EFFECTS OF GWN-10616 ON REPRODUCTION OF THE COLLEMBOLAN FOLSOMIA CANDIDA IN ARTIFICIAL SOIL BT211/21 (834-002) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.4.2.1/02	Grandolini, G.	2021	EFFECTS OF GWN-10616 ON REPRODUCTION OF THE PREDATORY MITE HYPOASPIS ACULEIFER IN SOIL BT212/21 (834-003) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.5.1/01	Rossini, L.	2021	ASSESSMENT OF THE EFFECTS OF THE PRODUCT GOW F716 (GWN-10616) ON SOIL MICROORGANISMS NITRIFICATION BT138/17 (841-001) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX
KCP 10.6.2/01	Colli, M.	2021	EFFECTS OF GWN-10616 ON TERRESTRIAL PLANTS VEGETATIVE VIGOUR TEST BT214/21 (851-001) BIOTECNOLOGIE BT S.r.l., Todi, Italy GLP, unpublished	N	XXXX

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

ZOXAMIDE

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 8.1.1.1	XXXX	1997	RH-117,281 technical: 14-day acute oral LD_{50} study in bobwhite quail XXXX, Report No. RH117BWLD-595 GLP Not published	Y	XXXX
KCA 8.1.1.2	XXXX	1997	RH-117,281 technical: 8-day acute dietary LC_{50} study in bobwhite quail XXXX., Report No. RH117BWLC-395 GLP Not published	Y	XXXX
KCA 8.1.1.2	XXXX	1997	RH-117,281 technical: 8-day acute dietary LC_{50} study in mallard ducklings XXXX., Report No. RH117MDLC-395 GLP Not published	Y	XXXX
KCA 8.1.1.3	XXXX	1998	Avian reproduction study of RH-117,281 technical with northern bobwhite XXXX., Report No. RH7281BW-97-2 GLP Not published	Y	XXXX
KCA 8.1.1.3	XXXX	1999	RH-117,281 technical: A reproduction study with the mallard (*Anas platyrhynchos*) XXXX., Report No. 129-164 GLP Not published	Y	XXXX
KCA 8.2.1	XXXX	1995	Acute flow-through toxicity of RH-117,281 technical to rainbow trout (*Oncorhynchus mykiss*) XXXX., Report No. 41681 GLP Not published	Y	XXXX
KCA 8.2.1	XXXX	1995	Acute flow-through toxicity of RH-117,281 technical to bluegill (*Lepomis macrochirus*)	Y	XXXX

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
			XXXX., Report No. 41682 GLP Not published		
KCA 8.2.1	XXXX	1998	RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas)* XXXX Report No. 129A-141 GLP Not published	Y	XXXX
KCA 8.2.1	XXXXX	1998	RH-117,281 Technical: A 96-hour flow-through acute toxicity test with the zebra fish (*Brachydanio rerio*) XXXX Report No. 129A-150 GLP Not published	Y	XXXX
KCA 8.2.1	XXXXX	1997	RH-117,281 Technical: a 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*) XXXX., Report No. 129A-135 GLP Not published	Y	XXXX
KCA 8.2.1	XXXX	1998	Acute toxicity of RH-127,450 to the rainbow trout (*Oncorhynchus mykiss)* in a range-finding test under static conditions XXXX, Report No. 44667 GLP Not published	Y	XXXX
KCA 8.2.1	XXXX	2002	Zoxamide Metabolite RH-139,432 - acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static conditions XXXX, Report No. 12550.6290 GLP Not published	Y	XXXX
KCA 8.2.1	XXXX	2010	GOW 008: Acute toxicity to zebra fish (*Danio rerio*) in a 96-hour study under static exposure XXXX Report No. CH-E-081/2010, BT104/10, January 10, 2011 GLP	Y	XXXX

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
			Not published		
KCA 8.2.1	XXXX	2020	RH-163353: Fish, acute toxicity test– Amended final report 1 XXXX Report No. 3202385 GLP Not published	Y	XXXX
KCA 8.2.1	XXXX	2020	RH-141455: Fish, acute toxicity test XXXX, Report No. 3202716 GLP Not published	Y	XXXX

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 8.2.1	XXXX	2020	RH-127450: Fish, acute toxicity test XXXX, Report No. 3202373 GLP Not published	Y	XXXX
KCA 8.2.2	XXXX	1996	Early life-stage toxicity of RH-117,281 technical to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions XXXX, Report No. 42400 GLP Not published	Y	XXXX
KCA 8.2.2	XXXX	1998	RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*) XXXX, Report No. 129A-141 GLP Not published	Y	XXXX
KCA 8.2.2.1	XXXX	2014	Zebrafish (*Danio rerio*), early life stage toxicity test, flow through conditions, test item: zoxamide XXXX, Report No. GOW-001/4-43/A, October 31 2014 GLP Not published	Y	XXXX
KCA 8.2.2.1	Milligan, Amanda L., Martin, Kathy H., Schneider, Suzanne Z.	2020	Final report addendum for RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*) Gowan Crop Protection Ltd., UK Eurofins EAG Agroscience, LLC, USA, Report No. 129A-143A GLP Not published	N	XXXX
KCA 8.2.2.1	XXXX	1998	RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*) XXXX, Report No. 129A-143A GLP Not published	Y	XXXX
KCA	XXXX	1998	Uptake, depuration, bioconcentration and metabolism of ^{14}C-RH-117,281 in bluegill sunfish (*Lepomis*	Y	XXXX

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
8.2.2.3			*macrochirus*) under flow through test conditions XXXX, Report No. 34-98-145, September 15 1998, ER Ref. No. 15.1 XXXX., Report No. RPT00328 GLP Not published		
KCA 8.2.3	Tognucci, A.	1998	Determination of the partition coefficient (n-octanol/water) of RH-127450 Rohm and Haas, Report No. 34-98-165, October 12 1998, ER Ref. No. 18.3 RCC Ltd. GLP No Published.	N	XXXX
KCA 8.2.3	Tognucci, A.	1998	Determination of the partition coefficient (n-octanol/water) of RH-139432 Rohm and Haas, Report No. 34-98-53, ER Ref. No. 31.3 RCC Ltd GLP Not published	N	XXXX
KCA 8.2.4.1	Sword, M.C., Gardner, C.	1995	Acute flow-through toxicity of RH-117,281 Technical to *Daphnia magna* Rohm and Haas, Report No. 94RC-0081, July 25 1995, ER Ref No. 5.1 ABC Laboratories, Report No. 41683 GLP Not published	N	XXXX

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	Rhodes, J.E., Williams, S.	1998	Acute toxicity of RH-127,450 to *Daphnia magna* in a range-finding test under static conditions Rohm and Haas, Report No. 98RC-0096, September 11 1998, ER Ref No. 16.4 ABC Laboratories, Report No. 44666 GLP Not published	N	XXXX
KCA 8.2.4.1	Mantilacci S.	2010	Acute toxicity of product GOW008 on *Daphnia magna* in a 48-hour immobilization test under static exposure Biotecnologie BT srl, Report No. BT103/10, November 18 2010 GLP Not published	N	XXXX
KCA 8.2.4.1	Caferella, M. A	2002	Zoxamide metabolite RH-139,432 - acute toxicity to daphnids (*Daphnia magna*) under static conditions Dow AgroSciences LLC, Report No. 021297, September 27 2002, ER Ref. No. 47.3 Springborn Smithers Laboratories, Report No. 1t2550.6289 GLP Not published	N	XXXX
KCA 8.2.4.1	Jarrom, R.	2020	RH-163353: Acute toxicity to *Daphnia magna* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202386 GLP Not published	N	XXXX
KCA 8.2.4.1	Hugill, E.	2020	RH-141455: Acute toxicity to *Daphnia magna* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202380 GLP Not published	N	XXXX

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	Roberts, C.A., Swigert, J.P	1997	RH-117,281 Technical: A 96-hour flow-through acute toxicity test with the saltwater mysid (*Mysidopsis bahia*) Rohm and Haas, Report No. 95RC-0275, May 5 1999 Wildlife International Ltd., Report No. 129A-136 GLP Not published	N	XXXX
KCA 8.2.4.2	Hugill, E.	2020	RH-127450: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202374 GLP Not published	N	XXXX
KCA 8.2.4.2	Hugill, E.	2020	RH-24549: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202394 GLP Not published	N	XXXX
KCA 8.2.4.2	Hugill, E.	2020	RH-139432: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202398 GLP Not published	N	XXXX
KCA 8.2.4.2	Jarrom, R.	2020	RH-163353: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202387 GLP Not published	N	XXXX

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	Hugill, E.	2020	RH-141455: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202381 GLP Not published	N	XXXX
KCA 8.2.5.1	Murrell, H., Rhodes, J.E., Stewart, S.	1997	Chronic toxicity of RH-117,281 technical to *Daphnia magna* under flow-through test conditions Rohm and Haas, Report No. 95RC-0273, June 12 1997, ER Ref No. 6.11 ABC Laboratories, Report No. 43209 GLP Not published	N	XXXX
KCA 8.2.5.2	Drottar, K.R., Krueger, H.O.	1998	RH-117,281 Technical: A flow-through life-cycle toxicity test with the saltwater mysid (*Mysidopsis bahia*) Rohm and Haas, Report No. 97RC-0077, June 17 1998 Wildlife International Ltd., Report No. 129A-142 GLP Not published	N	XXXX
KCA 8.2.5.3	van der Kolk, J.	1998	RH-117,281: Chronic effects on midge larvae (*Chironomus riparius*) in a water/sediment system Rohm and Haas, Report No. 97RC-0083, May 26 1998, ER Ref No. 13.3 Springborn Laboratories (Europe)AG, Report No. 97-063-1007 GLP Not published	N	XXXX
KCA 8.2.6.1	Ziegler, T.A., Stewart, S.	1996	Acute toxicity of RH-117,281 Technical to S*elenastrum capricornutum* Printz. Rohm and Haas, Report No. 94RC-0238, June 20 1996, ER Ref No. 1.1 ABC Laboratories, Report No. 42399 GLP Not published	N	XXXX
KCA 8.2.6.1	Kuhl, R., Härtel, Ch.	2015	Toxicity of (R)-Zoxamide to *Desmodesmus subspicatus* in an algal growth inhiition test. Ibacon GmbH, Report No. 93441210 GLP Not published	N	XXXX

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 8.2.6.1	Kuhl, R., Härtel, Ch.	2015	Toxicity of (S)-Zoxamide to *Desmodesmus subspicatus* in an algal growth inhiition test. Ibacon GmbH, Report No. 93431210 GLP Not published	N	XXXX
KCA 8.2.6.1	Hengsberger, A., Härtel, Ch.	2015	RH-141455: Toxicity to *Pseudorichneriella subcapitata* in an algal growth inhibition test Ibacon GmbH, Report No. 98661210, July 01, 2015 GLP Not published	N	XXXX
KCA 8.2.6.1	Drottar, K.R., Sutherland, C.A., Krueger, H.O.	1998	RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*) Rohm and Haas, Report No. 97RC-0130, August 7, 1998, ER Ref No. 13.2 Wildlife International, Ltd., Report No. 129A-154 GLP Not published	N	XXXX
KCA 8.2.6.1	Drottar, K.R., Sutherland, C.A., Krueger, H.O.	1998	RH-117,281 Technical: A 96-hour toxicity test with the freshwater diatom (*Navicula pelliculosa*) Rohm and Haas, Report No. 97RC-0131, ER Ref No. 13.5 Wildlife International, Ltd., Report No. 129A-153 GLP Not published	N	XXXX
KCA 8.2.6.1	Drottar, K.R., Sutherland, C.A., Krueger, H.O.	1998	RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Scenedesmus subspicatus*) Rohm and Haas, Report No. 97RC-0133, August 7, 1998, ER Ref No. 13.4 Wildlife International, Ltd., Report No. 129A-151 GLP Not published	N	XXXX
KCA 8.2.6.1	Drottar, K.R., Krueger, H.O.	1998	RH-117,281 Technical: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*) Rohm and Haas, Report No. 97RC-0132, July 21, 1998, ER Ref No. 12.10 Wildlife International, Ltd., Report No. 129A-152 GLP Not published	N	XXXX

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 8.2.6.1	Ward, S.C., Murdock, C.W.	1998	Toxicity of RH-117,281 2F (240 SC) to *Selenastrum capricornutum* Printz Rohm and Haas, Report No. 97RC-0094, September 23, 1998, ER Ref No. 14.6 ABC Laboratories, USA, Report No. 44196 GLP Not published	N	XXXX
KCA 8.2.6.1	Rhodes, J.E., Williams, S.	1998	Acute toxicity of RH-127,450 to the green alga, S*elenastrum capricornutum* Printz Rohm and Haas, Report No. 98RC-0097, October 30, 1998, ER Ref No. 28.3 ABC Laboratories Inc., Report No. 44665 GLP Not published	N	XXXX
KCA 8.2.6.1	Rhodes, J.E., Williams, S.	1999	Acute toxicity of RH-163,353 to S*elenastrum capricornutum* Printz in a range-finding test under static conditions Rohm and Haas, Report No. 99RC-0023, May 3, 1999, ER Ref No. 36.1 ABC Laboratories Inc., Report No. 45164 GLP Not published	N	XXXX
KCA 8.2.6.1	Hoberg, J.R.	2002	Zoxamide Metabolite RH-139,432 - toxicity to freshwater green algae, *Scenedesmus subspicatus* Dow AgroSciences LLC, Report No. 021298, September 25, 2002, ER Ref. 47.5 Springborn Smithers Laboratories, Report No. 12550.6288 GLP Not published	N	XXXX
KCA 8.2.6.1	Juckeland, D.	2015	Effects of RH-24549 on *Desmodesmus subspicatus* in an algal growth inhibition test BioChem agrar, Report No. 15 10 48 026 W, June 25 2015 GLP Not published	N	XXXX
KCA 8.2.6	Hugill, E.	2020	RH-127450: Inhibition of growth on the alga *Raphidocelis subcapitata* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202375 GLP Not published	N	XXXX

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 8.2.6	Jarrom, R.	2020	RH-163353: Inhibition of growth to the alga *Raphidocelis subcapitata* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202388 GLP Not published	N	XXXX
KCA 8.2.7	Drottar, K.R., Krueger, H.O.	1998	RH-117,281 Technical: A 14-day static-renewal toxicity test with duckweed (*Lemna gibba* G3) Rohm and Haas, Report No. 97RC-0080, June 24 1998, ER Ref No. 12.7 Wildlife International, Ltd., Report No. 129A-147 GLP Not published	N	XXXX
KCA 8.2.7	Juckeland, D.	2020	Effects Zoxamide technical on *Lemna gibba* in a growth inhibition test under semi-static test conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 18 48 ALE 0005 GLP Not published	N	XXXX
KCA 8.3.1.1.1	Kirkland, R.L.	1993	Acute contact toxicity of RH-117,281 technical to honey bees Rohm and Haas, Report No. 92RC-0235, August 9 1993, ER Ref No. 12.6 Bio Research, Report No. 109-93 GLP Not published	N	XXXX
KCA 8.3.1.1	Amsel, K.	2018	Acute toxicity of Zoxium 240 SC to the bumblebee *Bombus terrestris* L. under laboratory conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 17 48 BBA 0017 GLP Not published	N	XXXX
KCA 8.3.1.1.2	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory oral and contact test with the honeybee, *Apis mellifera* Rohm and Haas, Report No. 97RC-0095, April 25 1998, ER Ref No. 11.6, Springborn Laboratories (Europe) AG, Report No. 97-066-1007 GLP Not published	N	XXXX

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 8.3.1.2	Schmitzer, S., Ehmke, A.	2014	Chronic oral toxicity test of Zoxium 240 SC on the honey bee (*Apis mellifera* L.) in the laboratory IBACON GmbH, Report No. 80052136, February 3 2014 GLP Not published	N	XXXX
KCA 8.3.1.3	Schmitzer, S.	2014	Study on the effects of Zoxium 240 SC on honey bee brood (*Apis mellifera* L.) – brood feeding test BioChem agrar, Report No. 80051031, March 27 2014 GLP Not published	N	XXXX
KCA 8.3.1.3	Picard, Ch.R.	2018	Zoxamide: Honey bee (*Apis mellifera* L.) larval toxicity, repeated exposure Exigent LLC, A Gowan Group Company, USA Gowan Crop Protection Ltd., UK Smithers Viscient, USA, Report No. 12791.6307 GLP Not published	N	XXXX
KCA 8.3.1.4	Schmitzer, S.	2014	Study on the effects of Zoxium 240 SC on honey bee brood (*Apis mellifera* L.) – brood feeding test BioChem agrar, Report No. 80051031, March 27 2014 GLP Not published	N	XXXX
KCA 8.3.2.1	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory acute toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Rohm and Haas, Report No.: 97RC-0106, March 3 1998, ER Ref No. 11.8 Springborn Laboratories (Europe) AG, Report No. 97-062-1007 GLP Not published	N	XXXX
KCA 8.3.2.2	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory toxicity test with the predacious mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Rohm and Haas, Report No. 97RC-0105, March 3 1998, ER Ref No. 11.3 Springborn Laboratories (Europe) AG, Report No. 97-070-1007 GLP Not published	N	XXXX

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 8.3.2.2	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory toxicity test with the predacious mite, *Amblyseius andersoni* Chant (Acari: Phytoseiidae) Rohm and Haas, Report No. 97RC-0111, March 3 1998, ER Ref No. 11.7 Springborn Laboratories (Europe) AG, Report No. 97-075-1007 GLP Not published	N	XXXX
KCA 8.3.2.2	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory toxicity test with the spiders, *Pardosa sp*. (Araneae: Lycosidae) Rohm and Haas, Report No. 97RC-0107, March 3, 1998, ER Ref No. 11.9 Springborn Laboratories (Europe) AG, Report No. 97-059-1007 GLP Not published	N	XXXX
KCA 8.3.2.2	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory toxicity test with the green lacewing *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) Rohm and Haas, Report No. 97RC-0109, March 3 1998, ER Ref No. 11.11 Springborn Laboratories (Europe) AG, Report No. 97-068-1007 GLP Not published	N	XXXX
KCA 8.3.2.2	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory contact toxicity test with the predator, *Orius insidiosus* (Heteroptera: Anthocoridae) Rohm and Haas, Report No. 97RC-0110, March 3 1998, ER Ref No. 11.10 Springborn Laboratories (Europe) AG, Report No. 97-077-1007 GLP Not published	N	XXXX
KCA 8.3.2.2	Engelhard, E.K.	1998	RH-117,281 2R (240 SC): Laboratory acute toxicity test with the ground beetle, *Poecilus cupreus L*. (Coleoptera: Carabidae) Rohm and Haas, Report No. 97RC-0108, March 3 1998, ER Ref No. 11.2 Springborn Laboratories (Europe) AG, Report No. 97-064-1007 GLP Not published	N	XXXX

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 8.4.1	Ganssmann, M.	2015	RH-141455: Effects on reproduction and growth of earthworms *Eisenia fetida* in artificial soil IBACON GmbH, Report No. 98661022, June 1 2005 GLP Not published	N	XXXX
KCA 8.4.1	Downing, J.	1995	RH-117,281 technical: toxicity to earthworm (*Eisenia fetida*) Rohm and Haas, Report No. 94RC-0237, July 19 1995, ER Ref No. 5.2 ABC Laboratories Inc., Report No. 42398 GLP Not published	N	XXXX
KCA 8.4.1	Bryan, R.L., Porch, J.R., Krueger, H.O.	2000	RH-127,450 technical: an acute toxicity study with the earthworm in an artificial soil substrate. Rohm and Haas, Report No. 99RC-0282, March 7 2000, ER Ref No. 41.5 Wildlife International, Ltd., Easton, MD, USA, Report No. 129-173 GLP Not published	N	XXXX
KCA 8.4.1	Nienstedt, K.	1999	A chronic toxicity and reproduction test exposing the earthworm *Eisenia fetida* to RH-117,281 Technical material in OECD artificial soil, based on the BBA-guideline VI, 2-2 (1994) and the ISO-draft (ISO/DIS 11268-2) Rohm and Haas, Report No. 98RC-0181, April 14 1999, ER Ref No. 34.1 Springborn Laboratories (Europe) AG, Report No. 99-092-1007 GLP Not published	N	XXXX
KCA 8.4.1	Nienstedt, K.	2001	Effects of RH-7281 technical applied on natural soil on the cocoon and juvenile production of the earthworm *Eisenia fetida* Rohm and Haas, Report No. 00RC-0209, February 22 2001, ER Ref No. 45.2 Springborn Laboratories (Europe) AG, Report No. 1007.070.631 GLP Not published	N	XXXX

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 8.4.1	Friedrich, S.	2020	Effects of Zoxium 240 SC on the reproduction of the earthworm *Eisenia andrei* in artificial soil with 5 % peat Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 17 48 TEC 0009 GLP Not published	N	XXXX
KCA 8.4.1	Gray, J.	2021	RH-127450: Effect on reproduction in the earthworm *Eisenia fetida* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202376 GLP Not published	N	XXXX
KCA 8.4.1	Gray, J.	2021	RH-24549: Effect on reproduction in the earthworm *Eisenia fetida* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No.3202395 GLP Not published	N	XXXX
KCA 8.4.1	Gray, J.	2021	RH-163353: Effect on reproduction in the earthworm *Eisenia fetida* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No.3202389 GLP Not published	N	XXXX
KCA 8.4.1	Schulz, L.	2020	Effects of Zoxium 240 SC on earthworms under field conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 18 48 FEW 0001 GLP Not published	N	XXXX

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 8.4.1	Schulz, L.	2021	Effects of Zoxium 240 SC on earthworms under field conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 19 48 FEW 0002 GLP Not published	N	XXXX
KCA 8.4.2	Young, D.H.	2000	Evaluation of the biological activity of the RH-117281 metabolites RH-24549, 127450 and 163353 Rohm and Haas, Report No. DIS-00-281, ER Ref No. 44.1 GLP Not published	N	XXXX
~~KCA 8.4.2~~	~~Gray, J.~~	~~2021~~	~~RH-163353: Collembolan reproduction test in soil – Amended final report 1~~ ~~Gowan Crop Protection Ltd., UK~~ ~~Smithers ERS Ltd., UK, Report No. 3202390~~ ~~GLP~~ ~~Not published~~	~~N~~	XXXX
~~KCA 8.4.2~~	~~Gray, J.~~	~~2021~~	~~RH-141455: Collembolan reproduction study – Amended final report 1~~ ~~Gowan Crop Protection Ltd., UK~~ ~~Smithers ERS Ltd., UK, Report No. 3202382~~ ~~GLP~~ ~~Not published~~	~~N~~	XXXX
KCA 8.4.2	Parsons, Ch.	2020	Zoxium 240 SC - A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae) in artificial soil substrate Gowan Crop Protection Ltd., UK Mambo-Tox Ltd., UK, Report No. GOW-17-13 GLP Not published	N	XXXX

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 8.4.2	Parsons, Ch.	2020	Zoxium 240 SC – A laboratory test to determine the effects of fresh residues on the predatory soil mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate Gowan Crop Protection Ltd., UK Mambo-Tox Ltd., UK, Report No. GOW-17-14 GLP Not published	N	XXXX
KCA 8.4.2	Gray, J.	2021	RH-163353: Effect on reproduction of *Hypoaspis* (Geolaelaps) *aculeifer* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202391 GLP Not published	N	XXXX
KCA 8.4.2	Gray, J.	2021	RH-141455: Effect on reproduction of *Hypoaspis (*Geolaelaps) *aculeifer* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202383 GLP Not published	N	XXXX
KCA 8.5	Hammesfahr, U.	2015	RH-141455: Effects on the activity of soil microflora in the laboratory (nitrogen transformation) IBACON GmbH, Report No. 98661080, May 1, 2015 GLP Not published	N	XXXX
KCA 8.5	van der Kolk, J.	1998	RH-117,281 Technical: Determination of the effects on soil microflora activity Rohm and Haas, Report No. 97RC-0084, September 7 1998, ER Ref No. 14.1 Springborn Laboratories (Europe) AG, Report No. 97-060-1007 GLP Not published	N	XXXX
KCA 8.5	van der Kolk, J.	2000	RH-117,281 Technical: Determination of the effects on nitrogen transformation by microflora in soil Rohm and Haas, Report No. 00RC-0085, ER Ref No. 44.12 Springborn Laboratories (Europe) AG, Report No. 1007.070.747 GLP Not published	N	XXXX

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 8.5	Jarrom, R.	2019	RH-127450: Soil nitrogen transformation test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202377 GLP Not published	N	XXXX
KCA 8.5	Jarrom, R.	2019	RH-24549: Soil nitrogen transformation test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202396 GLP Not published	N	XXXX
KCA 8.5	Jarrom, R.	2020	RH-163353: Soil nitrogen transformation test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No.3202392 GLP Not published	N	XXXX
KCA 8.6	Nunez, M.V.	1998	Greenhouse phytotoxicity tests with RH-7281 2F Rohm and Haas, Report No. 98R-1092, August 31 1998, ER Ref No. 28.4 GLP Not published	N	XXXX
KCA 8.6	Nunez, M.V.	1998	Greenhouse crop phytotoxicity tests with RH-117281 2F Rohm and Haas, Report No. 98R-1114, November 23 1998, ER Ref No. 28.5 GLP Not published	N	XXXX
KCA 8.6	Sames, B.A.	1998	Insecticidal screening report - pre-screen insecticidal activity with RH-117,281; Primary screening activity per RH-117281 Rohm and Haas, Report No. 98R-1113, November 5 1998, ER Ref No. 28.6 GLP Not published	N	XXXX

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 8.8	Heim, D., Heim, L.	2002	Activated sludge, respiration inhibition test of Zoxamide Dow AgroSciences, Report No. GH-C 5421, February 7 2002, CA 3, ER Ref. No. 47.2 GLP Not published	N	XXXX

POTASSIUM PHOSPONATES

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 8.1.1.1	XXXX	1999	Potassium phosphite: an acute oral toxicity study with the northern bobwhite Report No.: 286-113 XXXX GLP Not published	Y	LBG
KCA 8.1.1.2	XXXX	1999	Potassium phosphite: a dietary LC50 study with the northern bobwhite Report No.: 286-111 XXX GLP Unpublished	Y	LBG
KCA 8.1.1.2	XXXX	1999	Potassium phosphite: a dietary LC50 study with the mallard Report No.: 286-112 XXXX GLP Not published	Y	LBG

KCA 8.1.1.3	XXXX	1999	Fosetyl-Aluminium; a reproduction study with the Japanese quail (*Coturnix Coturnix japonica*) Report No. R014231 GLP Not published	Y	LBG
KCA 8.2.1	XXXX	1999	Potassium phosphite: a 96-hour flow-through acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*) Report No.: 286A-108 XXXX GLP Not published	Y	LBG
KCA 8.2.2	XXXX	2008	Rainbow trout (*Oncorhynchus mykiss*), juvenile growth test (OECD 215), flow through conditions; test item: LGB-01F34 (Potassium phospite) XXXX Report-no. GAB-019/4-63 GLP Not published	Y	LBG
KCA 8.2.4.1	Sutherland, C.A. Kendall, T.Z. Krueger, H.O.	1999	Potassium phosphite: a 48-hour flow-through acute toxicity test with the cladoceran (*Daphnia magna*) Wildlife International, Ltd. Report No.: 286A-109 Luxembourg Industries (Pamol) Ltd. GLP Not published	N	LBG
KCA 8.2.5.1	Stäbler, D.	2006	Assessment of toxic effects of Potassium phosphite on *Daphnia magna* using the 21 day reproduction test GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Luxembourg Industries (Pamol) Ltd. Report-no. 20051318/01-ARDm GLP Not published	N	LBG
KCA 8.2.5.3	Stäbler, D.	2006	Assessment of side effects of Potassium phosphite (LGB-01F34) on the larvae of the midge, *Chironomus riparius* with the laboratory test method GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Luxembourg Industries (Pamol) Ltd. Report-no. 20051318/01-ASCr GLP Not published	N	LBG

KCA 8.2.6	Dengler, D.	2001	Testing of toxic effects of Stamina on the single cell green alga *Desmodesmus subspicatus* Arbeitsgemeinschaft GAB Biotechnologie GmbH & Umweltanalytik GmbH Study Code: 20001344/01-AADs Luxembourg Industries (Pamol) Ltd. GLP Not published	N	LBG
KCA 8.3.1.1	Thompson, H.M.	1999	Potassium phosphite: an acute oral toxicity study with the honey bee National Bee Unit Central Science Laboratory Study No.: GO3102 Luxembourg Industries (Pamol) Ltd. GLP Not published	N	LBG
KCA 8.3.1.1	Thompson, H.M.	1999	Potassium phosphite: an acute contact toxicity study with the honey bee National Bee Unit Central Science Laboratory Study No.: GO3101 Luxembourg Industries (Pamol) Ltd. GLP Not published	N	LBG
KCA 8.4.1	Kölzer, U.	2006	Acute toxicity of Potassium phosphite(LBG-01F34) on earthworms, *Eisenia fetida* using an artificial soil test GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany Luxembourg Industries (Pamol) Ltd. Report-no. 20051318/01-NLEf GLP Not published	N	LBG
KCA 8.4.1	Kölzer, U.	2006	Sublethal toxicity of Potassium phosphite (LGB-01F34) to the earthworm *Eisenia fetida* in artifical soil GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Luxembourg Industries (Pamol) Ltd. Report-no. 20051318/01-NREf GLP Not published	N	LBG

KCA 8.5	Kölzer, U.	2006	Assessment of the side effects of Potassium phosphite (LGB-01F34) on the activity of the soil microflora GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Luxembourg Industries (Pamol) Ltd. Report-no. 20051318/01-ABMF GLP Not published	N	LBG

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 8.4.2	Gray, J.	2021	RH-163353: Collembolan reproduction test in soil – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202390 GLP Not published	N	XXXX
KCA 8.4.2	Gray, J.	2021	RH-141455: Collembolan reproduction study – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202382 GLP Not published	N	XXXX

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

Appendix 2 Detailed evaluation of the new studies

The studies performed with GWN-10616 presented in the following were provided in support of the assessment and have not been previously evaluated on EU level. Additional information on Zoxamide and its metabolites to address data gaps identified during EU renewal process has already been provided to the RMS Latvia. Thus, the summaries of the studies are only presented for sake of completeness.

Review Comments:

The confirmatory-like studies (laboratory and field), were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

Phosphonic acid (also Phosphorous acid) is the actual active substance of the technical item Potassium phosphonates (formerly: phosphite; technical active substance).

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

No new data is submitted with this application.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No new data is submitted with this application.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

Please refer to B6 of this dossier.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No new data is submitted with this application.

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

No new data is submitted with this application.

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 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.2.1/01
Report:	ACUTE TOXICITY OF GWN-10616 TO ONCORHYNCHUS MYKISS IN A 96-HOUR SEMI-STATIC TEST, XXXX, 2022, report No. 2148AFA0011 GWN-10616, Doc. No. 821-001
Guideline(s):	OECD 203 (2019)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Executive Summary

The acute effect of test item GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, to Rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour semi-static test according to OECD 203 (2019). Groups of 7 fish were exposed to GWN-10616 at nominal concentrations of 2.50, 5.00, 10.0, 20.0, 40.0 mg test item/L. Negative control group was tested in parallel. The tested fish were observed for mortality and visible abnormalities.
Recoveries of both Potassium phosphite and Zoxamide were within 83 to 115 % in fresh and spent test solutions. Accordingly, the assessment of the effects was based on the nominal concentrations of the test item and the active substances.

No mortality occurred at concentrations up to and including 10 mg test item/L. Based on the test results, the 96-h LC_{50} for Rainbow trout (*Oncorhynchus mykiss*) exposed to GWN-10616 was calculated to be 34.8 mg test item/L (95 % confidence limits of 21.9 – 86.1 mg test item/L).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GWN-10616
Active substance:	Potassium phosphite and Zoxamide
Description:	Liquid, light brown (suspension concentrate)
Lot/Batch #:	P2102669001
Content of Active substance:	Potassium phosphite 507 g/L measured as Phosphonic acid equivalent (34.73 % w/w); Zoxamide 62 g/L (4.25 % w/w)

2. Vehicle and control:

Control:	Untreated test medium
Solvent:	none

3. Test animals:

Species:	Rainbow trout – *Oncorhynchus mykiss*
Source:	The fish were supplied by local fish farm „Forellenzucht Trostadt GbR“, DE
Acclimatisation period:	71 days
Diet:	Fish were not fed for 24 hours before the beginning and throughout the exposure period.
Water:	Reconstituted water according to OECD 203
Test unit:	13 L stainless steel container
Volume of test solution:	10 L
Environmental conditions/ water quality:	
Temperature:	13.3 – 14.0 °C
Dissolved oxygen:	8.16 – 9.71 mg O_2/L
pH:	7.82 – 7.91
Hardness:	238 mg/L as $CaCO_3$
Photoperiod:	16 h light : 8 h dark.

4. Test conditions: Semi-static exposure conditions (daily renewal)

B. STUDY DESIGN AND METHODS

1. Experimental phase: 17.01.2022 – 21.01.2022

2. Test design and test procedure

The purpose of the test was to evaluate effects of test item GWN-10616 within 96 hours under semi-static conditions to unfed Rainbow trout (*Oncorhynchus mykiss*) at nominal concentrations of 2.50, 5.00, 10.0, 20.0, 40.0 mg test item/L. Test solutions were exchanged every 24 hours. A water control was tested in parallel.

Observations were made on mortality and sub-lethal effects. Dead fish were removed upon observation. Sub-lethal effects were recorded and reported qualitatively. Fish were not fed during the course of the test. Fish tanks were aerated continuously. The pH and dissolved oxygen were measured in 24-hour intervals and were recorded continuously.

3. Preparation of test solutions

Stock solutions and test solutions were prepared daily. Stock solution was prepared by weighting the test item into a graduated flask. After filling the flask to the benchmark using test medium, the flask was softly agitated. The test item was evidently fully dissolved in test medium. Precipitations and/or undissolved particles were not noticed. Individual stock solutions per treatment group were prepared. These were mixed with untreated test medium in the fish tanks.

4. Analytical Methods

Test concentrations were verified by chemical analysis. Standard analytical methods were used to determine concentrations of Potassium phosphite (measured as Phosphonic acid equivalent) and Zoxamide in test solutions at test start, at test solution renewals and at test end as ‘fresh’ and ‘spent’ samples.

5. Statistics

Pre-testing included testing of monotonicity (qualitative trend analysis by contrasts ($p > 0.05$) and appropriate extra-binomial variance was found (Tarone’s test, $p \leq 0.01$)). For the time point 96 hours, a linear trend was found and the Cochran-Armitage test was used. 96 h-LC_x values were calculated using the Weibull modelling approach (maximum likelihood regression).

II. RESULTS AND DISCUSSION

A. Validity criteria

For a test to be valid, the mortality in the controls should not exceed 10 % at the end of the exposure (or one fish if less than ten are used) and was 0 % in this study. Further, the dissolved oxygen concentration should be ≥ 60 % of the air saturation value in all test vessels throughout the exposure and was > 60 % (i.e. > 81.5 %) throughout the study. Analytical measurement to confirm the test concentrations is compulsory and is provided below. Thus, the study is valid.

B. Analytical Results

Recoveries of both Potassium phosphite and Zoxamide were within 83 to 115 % in fresh and spent test solutions. Toxicity results were based on nominal concentrations.

Table 10.2.1-1: Summary of analytical results for Potassium phosphite and Zoxamide

nominal test item [mg/L]	**Nominal [mg/L]**	**Analysed [mg/L]**	**Recovery [% of nominal]**	**Analysed [mg/L]**	**Recovery [% of nominal]**
Potassium phosphite					
		0 h fresh		24 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.8683	0.8981	103.43	0.8184	94.25
5.00	1.7365	1.7649	101.63	1.6258	93.63
10.0	3.4730	3.5547	102.35	3.2514	93.62
20.0	6.9460	7.3977	106.50	6.8949	99.26
40.0	13.8920	12.3548	88.93	11.5420	83.08
		24 h fresh		48 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.8683	0.7823	90.10	0.9699	111.70
5.00	1.7365	1.5889	91.50	1.7556	101.10
10.0	3.4730	3.0574	88.03	3.5717	102.84
20.0	6.9460	6.7136	96.65	7.5871	109.23
40.0	13.8920	11.7686	84.72	11.6131	83.60
		48 h fresh		72 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.8683	0.8840	101.81	0.9228	106.28
5.00	1.7365	1.7114	98.56	1.9433	111.91
10.0	3.4730	3.4427	99.13	3.9626	114.10
20.0	6.9460	7.0704	101.79	7.8681	113.27
40.0	13.8920	11.6984	84.21	11.5691	83.28
		72 h fresh		96 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.8683	0.9380	108.04	0.7220	83.16
5.00	1.7365	1.9001	109.42	1.4409	82.98
10.0	3.4730	3.7428	107.77	2.8859	83.10
20.0	6.9460	8.0169	115.42	6.1200	88.11
40.0	13.8920	13.4446	96.78	14.1680	101.99
Zoxamide					
		0 h fresh		24 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.1063	0.1066	100.31	0.1053	99.12
5.00	0.2125	0.2101	98.86	0.2051	96.50
10.0	0.4250	0.4315	101.54	0.4067	95.69
20.0	0.8500	0.8443	99.33	0.8265	97.24
40.0	1.7000	1.8133	106.67	1.6988	99.93

nominal test item [mg/L]	Nominal [mg/L]	Analysed [mg/L]	Recovery [% of nominal]	Analysed [mg/L]	Recovery [% of nominal]
		24 h fresh		48 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.1063	0.1035	97.39	0.1022	96.17
5.00	0.2125	0.2046	96.30	0.2022	95.17
10.0	0.4250	0.4187	98.51	0.3820	89.87
20.0	0.8500	0.8552	100.61	0.8349	98.22
40.0	1.7000	1.7270	101.59	1.6880	99.29
		48 h fresh		72 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.1063	0.1042	98.10	0.0996	93.78
5.00	0.2125	0.2042	96.08	0.1950	91.76
10.0	0.4250	0.4128	97.13	0.3887	91.45
20.0	0.8500	0.8383	98.62	0.8223	96.74
40.0	1.7000	1.7371	102.18	1.6725	98.38
		72 h fresh		96 h spent	
0.00	0.0000	0.0000	-	0.0000	-
2.50	0.1063	0.0997	93.79	0.0995	93.60
5.00	0.2125	0.1958	92.12	0.1980	93.18
10.0	0.4250	0.3910	92.01	0.3944	92.80
20.0	0.8500	0.8220	96.71	0.8166	96.07
40.0	1.7000	1.6983	99.90	1.6581	97.54

C. Water quality

During the course of the test, the lowest measured oxygen content was 8.48 mg/L O_2 (corresponding to 81.5 % of the maximum air saturation; with 100 % being 10.4 mg/L O_2 at 13.5 °C). The pH was within a range of 7.14 to 7.94. The water temperature was recorded continuously (13.3 – 14.0 °C). Thus, physical and chemical parameters were all within acceptable limits.

D. Biological test results

The biological results are summarized in Table 10.2.1-2 (mortality) and 10.2.1-3 (sub-lethal effects). In the control and all test medium concentrations up to and including 10 mg GWN-10616/L all fish survived until the end of the test. At 20 and 40 mg test item/L mortalities were recorded from 48 hours onwards.

Table 10.2.1-2: Effects on mortality during the course of the test

time after application	**GWN-10616 (mg test item/L)**					
	control	**2.50**	**5.00**	**10.0**	**20.0**	**40.0**
	Mortality (%)					
3h	0.0	0.0	0.0	0.0	0.0	0.0
6h	0.0	0.0	0.0	0.0	0.0	0.0
24 h	0.0	0.0	0.0	0.0	0.0	0.0
30 h	0.0	0.0	0.0	0.0	0.0	0.0
48 h	0.0	0.0	0.0	0.0	14.3	14.3
54h	0.0	0.0	0.0	0.0	14.3	14.3
72 h	0.0	0.0	0.0	0.0	14.3	14.3
78 h	0.0	0.0	0.0	0.0	14.3	28.6
96 h	0.0	0.0	0.0	0.0	28.6*	57.1 *

*significantly different to the control (Step-down Cochran-Armitage Test, $p < 0.05$, one-sided greater)

Table 10.2.1-3: Sub-lethal effects during the course of the test

time after exposure start	GWN-10616 (mg test item/L)						observation
	control	2.50	5.00	10.0	20.0	40.0	
	number of fish affected/survivors						
3 h/6 h	0	0	0	0	0	0	abnormal horizontal orientation
	0	0	0	0	0	0	hypoactivity
24 h/30 h	0	0	0	0	0	0	abnormal horizontal orientation
	0	0	0	0	7/7	7/7	hypoactivity
48 h/54 h/ 72 h	0	0	0	0	0	0	abnormal horizontal orientation
	0	0	0	0	6/6	6/6	hypoactivity
78 h	0	0	0	0	0	0	abnormal horizontal orientation
	0	0	0	0	6/6	5/5	hypoactivity
96 h	0	0	0	1/7	0	0	abnormal horizontal orientation
	0	0	0	0	5/5	4/4	hypoactivity

No sub-lethal effects were recorded in the control and at measured concentrations up to and including 5 mg test item/L. At 10 mg test item/L abnormal horizontal orientation was observed in 1 fish at 96 h. At 20 and 40 mg test item/L hypoactivity was observed in all surviving fish from 24 h onwards.

Based on the test results, the 96-h LC_{50} for Rainbow trout (*Oncorhynchus mykiss*) exposed to GWN-10616 was calculated to be 34.8 mg test item/L (95 % confidence limits of 21.9 – 86.1 mg test item/L). The NOEC was determined to be 10 mg test item.

III. CONCLUSION

The acute effects of GWN-10616 to Rainbow trout (*Oncorhynchus mykiss*) were determined in a semi-static 96-hour test at nominal concentrations of 2.50, 5.00, 10.0, 20.0, 40.0 mg test item/L.
Based on the test results, the 96-h LC_{50} for Rainbow trout (*Oncorhynchus mykiss*) exposed to GWN-10616 was calculated to be 34.8 mg test item/L (95 % confidence limits of 21.9 – 86.1 mg test item/L). The NOEC was determined to be 10 mg test item.

Comments of zRMS:	The study was conducted to OECD guideline 202 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.2.1/02
Report: ACUTE IMMOBILISATION LIMIT TEST ON *DAPHNIA MAGNA* WITH GWN-10616 UNDER SEMI-STATIC CONDITIONS, Corboli, M., 2021, report No. BT208/21, Doc. No. 822-001
Guideline(s): OECD No. 202 (2004)
Deviations: None
GLP: Yes
Acceptability: Yes

Executive Summary

The acute immobilisation test was performed under semi-static conditions to assess the effects of the test item GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium

phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, on *Daphnia magna* after 48 hours of exposure. Young daphnids, less than 24 hours old, were exposed to a nominal concentration of 100 mg test item/L and an untreated control group. The test was performed in a limit test design in order to demonstrate that there is no significant toxic effect on the organisms at the tested concentration.

The analytical determination of the active substances content showed a mean recovery of 98.55% in fresh samples and 93.46% in aged samples for Zoxamide and 99.77% in fresh samples and 101.47% in aged samples for Potassium phosphite measured as Phosphonic acid equivalent. Accordingly, the assessment of the effects was based on the nominal concentrations of the test item and the active substances.

After 48 h, no effects on the exposed organisms were observed in the treatment group exposed to 100 mg test item/L.

The 48 h-EC_{50} was estimated to be > 100 mg test item/L, equivalent to 34.73 mg Potassium phosphite/L (measured as Phosphonic acid equivalent) and 4.25 mg Zoxamide/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GWN-10616
Active substance:	Potassium phosphite and Zoxamide
Description:	Liquid, beige (suspension concentrate)
Lot/Batch #:	P2102669001
Content of Active substance:	Potassium phosphite 507 g/L measured as Phosphonic acid equivalent (34.73 % w/w); Zoxamide 62 g/L (4.25 % w/w)

2. Vehicle and control:

Control:	Elendt M4 medium
Solvent:	none

3. Test animals:

Species:	*Daphnia magna* Straus
Age:	first instar, < 24 hours old
Source:	The animals are reared in the laboratory from a healthy culture maintained in conditions similar to those to be used in the test. The animals were maintained in reconstituted water and fed with an algae suspension (*Raphidocelis subcapitata*) three times a week.
Diet:	Daphnids were not fed throughout the exposure period
Test unit:	Glass vessel, 100 mL volume, filled with 50 mL medium

4. Environmental conditions/water quality:

Temperature:	19.7 – 20.4 °C
Dissolved oxygen:	Between 8.03 – 8.30 mg O_2/L throughout the study
Total hardness (as $CaCO_3$)	240 mg/L at the start of the test
pH:	7.05 – 8.08
Photoperiod:	16 h light : 8 h dark, 715 - 881 Lux
Test conditions:	Semi-static

B. STUDY DESIGN AND METHODS

1. Experimental phase: 24.082021 – 01.09.2021

2. Test design and test procedure
To determine the effects of GWN-10616 (containing Potassium phosphite 507 g/L; Zoxamide 62 g/L) to *Daphnia magna*, a 48-h semi-static exposure test at a nominal limit test concentration of 100 mg test item/L was performed in accordance with the guideline. A water control was tested in parallel.

The test consisted of four replicate test vessels for the treatment level and the control group. Each replicate contained 5 daphnids, corresponding to 20 organisms for treatment level and the control. The number of dead *Daphnia magna* in each replicate test vessel was recorded at 24 and 48 hours of exposure. Daphnids were considered to be immobile if they were unable to swim within approximately 15 seconds following gentle agitation of the test vessel.

Temperature, pH-value and oxygen concentration were measured at the start of the test and after 48 hours.

3. Preparation of test solutions
Stock solution (Ss) and test solution were prepared in M4 medium. At the start of the test and at the renewal after 24 hours, a solution of 100 mg test item/L was prepared by weighing 566.72 mg of the test item in a 500 mL graduated flask, to obtain SsA at the concentration of 1133.44 mg/L.; 44.11 mL of this solution were diluted in a 500 mL graduated flask, to obtain the test solution at 100 mg/L.

4. Analytical methods
The concentration of the active substances Potassium phosphite measured as Phosphonic acid equivalent and Zoxamide was determined by UHPLC-MS/MS analyses, in samples of all freshly prepared and aged test solutions.

5. Statistics
Two-sample comparisons between sample and control were made with Fisher's Exact Binominal Test. The statistical analysis was performed using software ToxRat Professional 3.3.0.

II. RESULTS AND DISCUSSION

A. Analytical Results
The results of the chemical analysis for Zoxamide and Potassium phosphite measured in samples of the test media are presented in Table 10.2.1-4.
The analytical determination of the active substance Zoxamide showed recovery values in the range of 96.71 - 103.27% at test start and 90.21 – 93.84% at test end, with mean recovery of 95.66% in fresh samples and 81.07% in aged samples. The analytical determination of the active substance Potassium phosphite (measured as Phosphonic acid equivalent) showed recovery values in the range of 97.09 - 113.23% at test start and 94.05 - 108.45% at test end, with mean recovery of 103.82% in fresh samples and 100.18% in aged samples.
Accordingly, the data evaluation was performed using the nominal concentrations of the test item.

Table 10.2.1-4: Measured concentrations of Zoxamide and Potassium phosphite (measured as Phosphonic acid) in the exposure solutions during the 48 h exposure of *Daphnia magna*

	Concentration nominal [mg a.s./L]	Concentration measured [mg a.s./L]	% of nominal	Mean recovery [%]*
Control	0	< LOD	n.a.	n.a.
Zoxamide	4.25	4.39 (0 h)	103.27	96.01
		4.11 (24 h fresh)	96.71	
		3.99 (24 h aged)	93.84	
		3.83 (48 h aged)	90.21	
Potassium phosphite (measured as Phosphonic acid)	34.75	34.82 (0 h)	100.27	100.62
		34.48 (24 h fresh)	99.27	
		36.21 (24 h aged)	104.25	
		34.28 (48 h aged)	98.70	

Data evaluation was performed using the nominal concentrations of the test item and geometric mean measured concentrations of Zoxamide and nominal concentrations of Phosphonic acid.
LOD (limit of detection) Zoxamide = 0.0721 µg a.s./L
LOD Phosphonic acid = 0.5937 µg a.s./L
n.a.: not applicable

All measured chemical and physical parameters (dissolved oxygen concentration, pH, and temperature) in the definitive test were within expected ranges.

B. Validity criteria

In order to be valid the immobilisation in the control must not exceed 10 % and was 0 % in the study. Further, the dissolved oxygen concentration must be ≥ 3 mg O_2/L at the end of the test in control and test vessels and was in the range of 8.03 to 8.30 mg O_2/L throughout the study. Thus, the study is valid in accordance with OECD 202.

C. Biological results

After 24 and 48 hours of exposure no immobilisation was observed in the control and the test item concentration of 100 mg test item/L (see Table 10.2.1-5).

Table 10.2.1-5: Immobilisation of *Daphnia magna* exposed to GWN-10616 for 48 h in a semi-static test

Nominal concentration [mg test item/L]	Number of immobilised daphnids	
	24 h	48 h
0 (control)	0	0
100	0	0

Based on nominal concentrations the 48-hour EC_{50} value (immobilisation) for *Daphnia magna* was estimated to be > 100 mg test item/L, equivalent to 4.25 mg Zoxamide/L and 34.73 mg Phosphonic acid/L, respectively. The NOEC was 100 mg test item/L.

III. CONCLUSIONS

The acute toxicity of GWN-10616 to *Daphnia magna* was determined in an unaerated, semi-static, 48-hour limit test according to OECD Guideline 202 (2004). The EC_{50} (48 h) for immobilisation was estimated to be > 100 mg test item/L.

Comments of zRMS:	The study was conducted to OECD guideline 201 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.2.1/03
Report: TOXICITY EVALUATION OF TEST ITEM GWN-10616 ON GREEN ALGA *RAPHIDOCELIS SUBCAPITATA* (FORMERLY KNOWN AS *PSEUDOKIRCHNERIELLA SUBCAPITATA*) IN A GROWTH INHIBITION TEST, Mantilacci, S., 2021, report No. BT207/21, Doc. No. 823-001
Guideline(s): OECD No. 201 (2011)
Deviations: None
GLP: Yes
Acceptability: Yes

Executive Summary

The toxicity of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, on the growth of the freshwater green algal species *Raphidocelis subcapitata* was investigated in a 72-hour static test with nominal concentrations of 12.35, 37.04, 111.11, 333.33 and 1000 µg test item/L, and a control. The study was conducted in accordance with OECD Guideline 201 (2011).

The analytical determination showed mean recoveries of 95.66 % in fresh and 81.07 % in aged samples for the active substance Zoxamide and 103.82 % in fresh and 100.18 % in aged samples for the active substance Potassium phosphite (measured as Phosphonic acid equivalent), respectively. Accordingly, the assessment of the effects was based on nominal concentrations of the test item.

After 72 hours, the E_rC_{50} and the E_yC_{50} values for *Raphidocelis subcapitata* were determined to be 656.03 µg test item/L and 351.14 µg test item/L, respectively. The NOEC for growth rate and yield was determined to be 37.04 µg test item/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material: GWN-10616
Active substances: Potassium phosphite and Zoxamide
Description: Liquid, beige
Lot/Batch #: P2102669001
Content of a.s.: Potassium phosphite (measured as Phosphonic acid equivalent): 507 g/L, Zoxamide: 62 g/L

2. Vehicle and control:

Control: algal nutrient medium according to OECD 201
Solvent: none

3. Test system:

Species: *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata*)
Source: Test facility culture
Test unit: 250 mL glass flasks with air-permeable cellulose caps
Volume: 100 mL

Initial inoculation: 10000 cells/mL

4. Environmental conditions/water quality:

Temperature:	24.3 – 24.8 °C
pH:	7.31 – 7.83
Photoperiod:	continuous uniform illumination
Light intensity:	Cool white fluorescence, 6641 – 7172 lux

B. STUDY DESIGN AND METHODS

1. Experimental phase: 23.08.2021 – 01.09.2021

2. Preparation of test solutions
All stock and test solutions were prepared in EPA (AAP) medium. At the start of the test, 108.74 test item were weighed in a 250 mL graduated flask and filled up to volume with EPA medium, to obtain a stock solution A (SsA) at the concentration of 434.96 mg/L. 50.0 mL of SsA were diluted with EPA medium, in a 1000 mL graduated flask, to obtain the SsB at 21.75 mg/L. The test solutions (C1 - C5) were prepared by dilution of the SsB in EPA medium.

3. Test design and test procedure
The toxicity of GWN-10616, a suspension concentrate formulation (containing active substances Potassium phosphite and Zoxamide) upon the growth of the freshwater green algae *Raphidocelis subcapitata* at nominal test concentrations of 12.35, 37.04, 111.11, 333.33 and 1000 µg test item/L and a control was observed over a period of 72 hours in a static test system. The test was performed in accordance with OECD Guideline 201 (2011). The test design included three replicates per test concentration and six replicates for the control.

Each flask was inoculated with an aliquot of algal culture (the inoculum culture was in the exponential growth phase) in order to have an initial density of 10^4 cells/mL in each culture.

Temperature and pH of control and test media was recorded at the start and end of the test. The temperature in the incubator and light intensity was continuously monitored during the study. Cell densities/biomass were counted at 0, 24, 48 and 72 hours during the definitive test. The biomass was determined by electronic particle counter (each sample was evaluated with three counts and the cell density was expressed as mean of the three obtained values). Microscopic observations were performed in order to assess any abnormal appearance of the algal cells that may have been caused by exposure to the test item.

4. Analytical methods
Test concentrations were verified by liquid chromatography technique (UHPLC-MS/MS). Samples were taken from control and test media at the start of the test and at 72 hours.

5. Statistics
The growth data evaluated during 72 hours of exposure at different concentrations of the test item were statistically evaluated using the software ToxRat Professional 3.3.0.

The determination of the ECx values together with the confidence limits was performed by Probit analysis using the linear maximum likelihood regression. For evaluation of LOEC/NOEC values for yield and growth rate, data was normally distributed (Shapiro-Wilk's Test) and variance homogeneity not given (Levene's Test), thus a Multiple sequentially-rejective Welch-t-test after Bonferroni-Holm ($\alpha = 0.05$, one-sided smaller) was applied.

II. RESULTS AND DISCUSSION

A. Analytical Results

The analytical determination of the active substance Zoxamide showed recovery values in the range of 92.76 – 100.75 % at test start and 76.16 – 86.50 % at test end, with mean recovery of 95.66 % in fresh samples and 81.07 % in aged samples.

The analytical determination of the active substance Potassium phosphite (measured as Phosphonic acid equivalent) showed recovery values in the range of 97.09 – 113.23 % at test start and 94.05 – 108.45 % at test end, with mean recovery of 103.82 % in fresh samples and 100.18 % in aged samples. The results are summarized in Table 10.2.1-6.

Accordingly, the assessment of the effects was based on nominal concentrations of the test item and active substances Zoxamide and Phosphonic acid.

Table 10.2.1-6: Analytical results for Zoxamide and Potassium phosphite (measured as Phosphonic acid) obtained in the 72 hours static toxicity test with *Raphidocelis subcapitata*

Nominal concentration [mg test item/L]	Measured concentrations [mg a.s./L]							
	0 hours				72 hours			
	Zoxamide	Recovery [%]	Phosphonic acid	Recovery [%]	Zoxamide	Recovery [%]	Phosphonic acid	Recovery [%]
Control	< LOD	-	< LOD	-	< LOD	-	< LOD	-
12.35	0.484	93.09	4.858	113.23	0.396	76.16	4.652	108.45
37.04	1.477	94.11	13.684	106.40	1.320	84.11	13.024	101.28
111.11	4.755	100.75	38.902	100.81	3.801	80.53	37.802	97.96
333.33	13.143	92.76	117.594	101.58	11.062	78.07	114.778	99.15
1000	41.482	97.61	337.171	97.09	36.761	68.50	326.650	94.05

LOD (limit of detection) Zoxamide = 0.0721 µg a.s./L
LOD Phosphonic acid = 0.5937 µg a.s./L
- not determined

All chemical and physical parameters (pH, and temperature) in the definitive test were within expected ranges.

B. Validity criteria

According to OECD 201 (2011) in order for the study to be valid, the cell concentration in the control cultures should have increased by a factor of at least 16 within three days (observed = 208, mean specific growth rate = 1.779 day^{-1}). The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures should not exceed 35 % (observed = 17.92 %). The coefficient of variation of average specific growth rates during the whole test period in the replicates of the control group should not exceed 7 % (observed = 1.74 %). Thus, the validity criteria were met.

C. Biological test results

The effect of GWN-10616 on the cell density of *Raphidocelis subcapitata* was determined at 24, 48 and 72 hours. Initial cell density was 1 x 10^4 cells/mL at test start. Mean cell densities are outlined in Table 10.2.1-7.

Table 10.2.1-7: Mean cell densities of *Raphidocelis subcapitata* exposed to GWN-10616 over 72 hours

Nominal concentrations [µg test item/L]	Mean cell density [10^4 cells/mL]		
	24 hours	48 hours	72 hours
0	4.80	40.90	208.41
12.35	4.72	38.90	206.70
37.04	4.64	37.84	204.51
111.11	4.58	31.89	173.49
333.33	3.18	23.35	124.13
1000	2.66	2.88	3.07

For the calculation of effect concentrations, the treatments were compared with the untreated control. Growth of *Raphidocelis subcapitata* was inhibited in a concentration-dependent manner, showing an increasing inhibition with increasing test concentration when compared to the untreated control.

For growth, a significant inhibitory effect was determined starting at 111.11 µg test item/L and above (Table 10.2.1-8).

Table 10.2.1-8: Effects of GWN-10616 on *Raphidocelis subcapitata* after exposure for 72 hours

Nominal concentrations [µg test item/L]	Inhibition after 72 hours			
	Yield	% inhibition	Growth rate[1]	% inhibition
0	207.41	-	1.779	-
12.35	205.70	0.82	1.777	0.11
37.04	203.51	1.88	1.773	0.34
111.11	172.49	16.84*	1.719	3.37*
333.33	123.13	40.63*	1.607	9.67*
1000	2.07	99.00*	0.372	79.09*

[1]: Growth rate 0-72 hours
* : statistically significant different from the control (Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm, $\alpha = 0.05$, one-sided smaller).

Based on these results, the E_yC_{50} for 72 hours was calculated to be 351.14 µg test item/L (95 % confidence limits: 297.06 – 415.71 µg test item/L). For growth rate, the E_rC_{50} for 0 – 72 hours was calculated to be 656.03 µg test item/L (95 % confidence limits: 630.68 – 681.32 µg test item/L). The NOEC for yield and growth rate was determined to be 37.04 µg test item/L.
All test and control cultures were inspected microscopically. No abnormalities were detected in any of the cultures examined.

Table 10.2.1-9: Effect concentrations of GWN-10616 on *Raphidocelis subcapitata* after exposure for 72 hours

Endpoint (72 h)	Concentration [µg test item/L] *	Endpoint (72 h)	Concentration [µg test item/L] *
E_yC_{10}	128.15 (78.98 – 168.66)	E_rC_{10}	336.45 (311.53 – 359.93)
E_yC_{20}	181.13 (127.99 – 223.53)	E_rC_{20}	423.13 (398.03 – 446.77)
E_yC_{50}	351.14 (297.06 – 415.71)	E_rC_{50}	656.03 (630.68 – 681.32)
LOE_yC	111.11	LOE_rC	111.11
NOE_yC	37.04	NOE_rC	37.04

* Values in parentheses refer to 95 % confidence limits.

III. CONCLUSION

A growth inhibition test was performed to assess the effects of GWN-10616 (containing active substances Potassium phosphite and Zoxamide) to green algae (*Raphidocelis subcapitata*).

Based on these results, the E_yC_{50} for 72 hours was calculated to be 351.14 µg test item/L (95 % confidence limits: 297.06 – 415.71 µg test item/L). For growth rate, the E_rC_{50} for 0 – 72 hours was calculated to be 656.03 µg test item/L (95 % confidence limits: 630.68 – 681.32 µg test item/L). The NOEC for yield and growth rate was determined to be 37.04 µg test item/L.

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, the test is considered valid and acceptable. Given that measured concentrations were within the 80% to 120% of nominal acceptance range, the study results were based on nominal test item concentrations.

Agreed endpoints:

Based on nominal concentrations:

The 96-hour LC50 (static test conditions) for *Oncorhynchus mykiss* ≥100 mg a.s./L (nom)

The NOEC (static test conditions) for *Oncorhynchus mykiss* = 100 mg a.s./L (nom)

Reference:	KCA 8.2.1
Report:	XXXX, 2020: RH-163353: Fish, acute toxicity test – Amended final report 1 XXXX, Report No. 3202385, GLP, Not published
Guideline(s):	OECD 203 (adopted 18 June 2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-163353 (HHGCP001-00-2)
Purity	98.97 % (w/w) (re-certified under GLP: 99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate))
Species	*Oncorhynchus mykiss* (rainbow trout)
Age:	young fish of the same age (4.4-6.0 cm length)
Number of animals:	7 (per treatment group and control)
Weight:	0.62-1.90 g
Source:	Hebden, Skipton, North Yorkshire, United Kingdom
Acclimation period:	at least 9 days prior to testing
Feeding:	The fish were fed with a proprietary fish food, which was added to the holding tank in quantities dictated by the size of the fish. On a fortnightly basis during the holding period, a sub sample of 10 fish was weighed and a mean wet weight calculated. The wet weight was used to determine the quantity of food required for the number of fish remaining in the holding tank. The amount of food required per holding tank was weighed and stored in labelled feed containers placed in the fish holding room. The feed was distributed to the holding tank over two feeding intervals during the day. Uneaten food and debris were siphoned or cleaned from the tanks as required.
Housing:	20 L glass aquaria, each fitted with an appropriate lid, containing 12 L of media, placed in a temperature-controlled room under artificial light and under continuous water renewal (flow-through) conditions
Environmental conditions	
Temperature:	11.5 – 12.9 °C
Photoperiod:	16 hours light, 8 hours dark
Test medium:	mains water, filtered (particulate filter and activated carbon filter) and UV sterilised
pH:	6.77 – 7.48
Dissolved oxygen:	93.6 – 101.4 % air saturation
Application rate(s)	Range- finding test: Nominally 10 and 100 mg/L (static conditions) Definitive test: Nominally 100 mg/L (static conditions)
Post exposure observation period	96 hours
Remarks	None

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-163353 to *Oncorhynchus mykiss* was tested at an application rate of nominally 100 mg/L (limit test) over 96-hours in a static test design. An untreated group served as control.

Concentrations of RH-163353 were determined by treating samples with acetonitrile containing 0.5% formic acid, then diluting further with acetonitrile/treated mains water (1:4 v:v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated

according to SANCO 3029/99 rev. 4 (SMV 3202385-01V and updated by SMV 3202385-02V to include storage stability data). The limit of quantification (LOQ) was 0.001 mg/L.

RH-163353 is a racemate. The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure, SMV 3202586-01V). A combination of the analytical procedures SMV 3202385-01V and SMV 3202586-01V was used to assess the enantiomeric ratio of the test substance in the test medium and calibration standard solution.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.77-7.48, temperature 12.7-13.3°C, dissolved oxygen 93.6 – 101.4 % air saturation). The water hardness was 77 mg $CaCO_3$ /L, the chlorine content amounted to 0.01 mg Cl_2/L.

Analysis of the freshly prepared media at 0 hours and the corresponding old media at 96 hours showed measured concentrations of 108 and 102 mg/L, respectively. Given that measured concentrations were within the 80% to 120% of nominal acceptance range, the study results were based on nominal test item concentrations.

Table 10.2.1-10: Test item concentrations [mg/L]

	Measured concentration (mg/L)		% nominal	
Nominal test item concentration (mg/L)	**0 hours (new media)**	**96 hours (old media)**	**0 hours (new media)**	**96 hours (old media)**
Control	< LOQ	0.0037*	--	--
100	108	102	108	102

LOQ = 0.001 mg/L

* Result was in excess of the LOQ probably due to an unintended carryover of residues. Re-analysis of the sample showed a measured concentration of 0.0039 mg/L therefore the 'back-up' samples were analysed. The results from the 'back-up- samples showed measured concentrations of 0.0047 and 0.0038 mg/L.

RH-163353 is a racemate. The ratio of its two enantiomers has been checked in a 96-hour test sample and in a freshly prepared calibration standard solution. As a result, the isomer ratio of the 96-hour 100 mg/L sample was within ±1.5% of the calibration standard and the ratio from the certificate of analysis for the test item batch. Thus, the enantiomeric ratio is deemed to be stable in the fish water during the course of the study.

No mortality of fish occurred during the course of the study. For completeness, the results of the range finder and of the definitive test are given.

Table 10.2.1-11: Cumulative mortality of fish

Nominal test item concentration (mg/L)	No. of fish exposed	Cumulative Mortality				
		ca.2 hours	24 hours	48 hours	72 hours	96 hours
Range-finding test						
Control	3	0	0	0	0	0
10	3	0	0	0	0	0
100	3	0	0	0	0	0
Definitive test						
Control	7	0	0	0	0	Control
100	7	0	0	0	0	100

The fish were showing no sublethal effects.
Based on nominal concentrations, the 96-hour LC_{50} value was determined to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 100 mg/L.
Given that no effects were observed throughout the test, the results were determined empirically.
The validity criteria for control mortality (≤ 10 %) and Dissolved oxygen (≥ 60 % air saturation) were met. Therefore, the test was considered valid.

Table 10.2.1-12: Acute toxicity of RH-163353 on fish – study endpoints

Parameter	Test item concentration (mg/L)			
	24 hours	48 hours	72 hours	96 hours
LC_{50}	>100	>100	>100	>100
NOEC	100	100	100	100

Conclusion

The acute toxicity of the zoxamide metabolite RH-163353 to *Oncorhynchus mykiss* was tested at an application rate of nominally 100 mg/L (limit test) over 96-hours in a static test design.
Based on nominal concentrations, the 96-hour LC50 value was determined to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 100 mg/L.

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on nominal concentrations:

The 96-hour LC50 (static test conditions) for *Oncorhynchus mykiss* >100 mg a.s./L (nom)

The NOEC (static test conditions) for *Oncorhynchus mykiss* = 100 mg a.s./L (nom)

Reference:	KCA 8.2.1
Report:	XXXX, 2020: RH-141455: Fish, acute toxicity test XXXX, Report No. 3202716, GLP, Not published
Guideline(s):	OECD 203 (adopted 18 June 2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication	No

(if vertebrate study)

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (HHGCP017-00-1)
Purity	99.6 % (w/w)
Species	*Oncorhynchus mykiss* (rainbow trout)
Age:	young fish of the same age (3.8-4.3 cm length)
Number of animals:	7 (per treatment group and control)
Weight:	0.40 - 56 g
Source:	Northern Trout, Hebden, Skipton, United Kingdom
Acclimation period:	at least 9 days prior to testing
Feeding:	The fish were fed with a proprietary fish food, which was added to the holding tank in quantities dictated by the size of the fish. On a fortnightly basis during the holding period, a sub sample of 10 fish was weighed and a mean wet weight calculated. The wet weight was used to determine the quantity of food required for the number of fish remaining in the holding tank. The amount of food required per holding tank was weighed and stored in labelled feed containers placed in the fish holding room. The feed was distributed to the holding tank over two feeding intervals during the day. Uneaten food and debris was siphoned or cleaned from the tanks as required.
Housing:	15 L glass aquaria, each fitted with an appropriate lid, containing 10 L of media, placed in a temperature-controlled room under artificial light and under continuous water renewal (flow-through) conditions
Environmental conditions	
Temperature:	12.1– 12.4°C
Photoperiod:	16 hours light, 8 hours dark
Test medium:	mains water, filtered (particulate filter and activated carbon filter) and UV sterilised
pH:	6.15 – 7.77
Dissolved oxygen:	94.2 – 104.7 % air saturation
Application rate(s)	Nominally 4.1, 9.1, 20, 45 and 100 mg/L (static conditions)
Post exposure observation period	96 hours
Remarks	A first definitve test (limit test) was performed, but terminated due mortalities observed at the 100 mg/L test concentration. Therefore, the test was repeated using a range of concentrations. In addition, fresh test item was used for the final test.

The acute toxicity of the zoxamide metabolite RH-141455 to *Oncorhynchus mykiss* was tested at application rates of nominally 4.1, 9.1, 20, 45 and 100 mg/L over 96-hours in a static test design. An untreated group served as control.
Concentrations of RH-141455 were determined by treating samples with acetonitrile containing 0.2% formic acid, then diluting further with acetonitrile/treated mains water (1:1 v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system. The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202716-01V, updated by SMV 3202716-02V to include extended storage stability data). The limit of quantification (LOQ) was 0.1 mg/L.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.15-7.77, temperature 12.1– 12.4°C, dissolved oxygen 94.2 – 104.7 % air saturation). The water hardness was 87 mg $CaCO_3$/L, the chlorine content amounted to 0.01 mg Cl_2/L.
Analysis of 0-hour samples showed measured concentrations ranging from 96-113 % of nominal, the 96-hour samples showed measured concentrations ranging from 120-126% of nominal. Analysis of the 0-hour and 96-hour 'back-up' samples showed measured concentrations ranging from 112-125 % of nominal and 112-120 % of nominal, respectively. The results confirmed that the test substance was stable in the test media over the period of the test. Although some measured concentrations were slightly above 120 % of nominal, the study results were based on nominal test item concentrations.

Table 10.2.1-13: Test item concentrations [mg/L]
Values given for initial A sample/back-up B/back-up C samples

	Measured concentration (mg/L)		% nominal	
Nominal test item concentration (mg/L)	**0 hours (new media)**	**96 hours (old media)**	**0 hours (new media)**	**96 hours (old media)**
Control	all < LOQ	all < LOQ	--	--
4.1	4.62/4.93/5.13	4.98/4.74/4.79	113/120/125	121/116/117
9.1	9.61/10.7/11.3	11.1/10.5/10.6	106/118/124	122/115/116
20	19.5/23.2/23.8	25.1/23.8/24.0	98/116/119	126/119/120
45	43.2/54.1/51.9	54.1/52.1/50.5	96/120/115	120/116/112
100	101/112/117	125/117/120	101/112/117	125/117/120

LOQ = 0.1 mg/L

No mortality of fish occurred during the course of the study.

Table 10.2.1-14: Cumulative mortality of fish

Nominal test item concentration (mg/L)	No. of fish exposed	Cumulative Mortality			
		24 hours	**48 hours**	**72 hours**	**96 hours**
Control	7	0	0	0	0
4.1	7	0	0	0	2*
9.1	7	0	0	0	0
20	7	0	0	0	0
45	7	0	0	0	0
100	7	0	0	0	0

*Mortalities not considered to be test item related, but due to aggressive fish

The fish were showing mild sublethal effects in the higher test rates (swimming normally but abnormal bottom distribution/behaviour, lethargy, coughing), 1-2 fish also at the two lowest test concentrations (abnormal bottom distribution/behaviour, lethargic with dark pigmentation, coughing).
Based on nominal concentrations, the 96-hour LC_{50} value was considered to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) was considered to be 100 mg/L.
Given that no effects were observed throughout the test, the results were determined empirically.
The validity criteria for control mortality (≤ 10 %) and dissolved oxygen (≥ 60 % air saturation) were both satisfied. Therefore, the test is valid.

Table 10.2.1-15: Acute toxicity of RH-141455 on fish – study endpoints

Parameter	Test item concentration (mg/L)			
	24 hours	**48 hours**	**72 hours**	**96 hours**
LC_{50}	>100	>100	>100	>100
NOEC	100	100	100	100

Conclusion

The acute toxicity of the zoxamide metabolite RH-141455 to *Oncorhynchus mykiss* was tested at application rates of nominally 4.1, 9.1, 20, 45 and 100 mg/L over 96-hours in a static test design.
Based on nominal concentrations, the 96-hour LC_{50} value was determined to be >100 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 100 mg/L.

EFSA (2017) requested in its Peer Review Conclusion: *"Further data or refinement (exposure and/or effects) for the acute risk assessment of fish for the metabolites RH-127450, RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 4 and 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on time-weighted mean measured substance concentrations:

The 96-hour LC50 (semi-static test conditions) for *Oncorhynchus mykiss* = 4.17 mg a.s./L (mm)

The NOEC (semi-static test conditions) for *Oncorhynchus mykiss* = 1.96 mg a.s./L (mm)

Reference:	KCA 8.2.1
Report:	XXXX 2020: RH-127450: Fish, acute toxicity test XXXX, Report No. 3202373, GLP, Not published
Guideline(s):	OECD 203 (adopted 18 June 2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-127450 (116286)
Purity	94.29 % (w/w); enantiomeric ratio 49.57 : 50.43
Species	*Oncorhynchus mykiss* (rainbow trout)
Age:	young fish of the same age (3.9-4.9 cm length)
Number of animals:	7 (per treatment group and control)
Weight:	0.49 – 0.84 g
Source:	Northern Trout, Hebden, Skipton, United Kingdom
Acclimation period:	at least 9 days prior to testing
Feeding:	The fish were fed with a proprietary fish food, which was added to the holding tank in quantities dictated by the size of the fish. On a fortnightly basis during the holding period, a sub sample of 10 fish was weighed and a mean wet weight calculated. The wet weight was used to determine the quantity of food required for the number of fish remaining in the holding tank. The amount of food required per holding tank was weighed and stored in labelled feed containers placed in the fish holding room. The feed was distributed to the holding tank over two feeding intervals during the day. Uneaten food and debris was siphoned or cleaned from the tanks as required.
Housing:	10 L glass aquaria, each fitted with an appropriate lid, containing 7 L of media, placed in a temperature-controlled room under artificial light and with daily renewal of the test media
Environmental conditions	
Temperature:	12.0 – 13.8°C
Photoperiod:	16 hours light, 8 hours dark
Test medium:	mains water, filtered (particulate filter and activated carbon filter) and UV sterilised
pH:	7.34 – 7.69
Dissolved oxygen:	95.6 -104.5 % air saturation
Application rate(s)	Range-finding test: Nominally 1, 10 and 100 % saturated solution (static conditions) Definitive test: Nominally 10, 18, 32, 56 and 100 % saturated solution (semi-static conditions)
Post exposure observation period	96 hours

Remarks	None

Based on the results of a range-finder, the acute toxicity of the zoxamide metabolite RH-127450 to *Oncorhynchus mykiss* was tested at application rates of nominally 10, 18, 32, 56 and 100 % saturated solution over 96-hours in a semi-static test design. An untreated group served as control.
Concentrations of RH-127450 were determined by mixing aqueous samples with acetonitrile containing 0.5% formic acid, then diluting further with treated mains water/acetonitrile (4:1 v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples are analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system or a liquid chromatography triple quadrupole mass spectrometry (LC-TQMS) system. The method has been validated according to SANCO/3029/99 rev. 4 (SMV 3202373-01V, updated by SMV 3202373-02V to include storage stability and method validation data of LC-TQMS). The limit of quantification (LOQ) was 0.001 mg/L.

RH-127450 is a racemate. The enantiomeric ratio of the RH-127450 isomers has been determined in 96-hour test samples with method SMV 3202373-01V.E. The test solutions were extracted with acetonitrile, then diluted with acetonitrile/HPLC grade water (1:4 v/v) to bring the response within the calibration range. Samples were thereafter analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.34-7.69, temperature 12.0– 13.8°C, Dissolved oxygen 95.6 – 104.5% air saturation). The water hardness was 65-96 mg $CaCO_3$/L, the chlorine content amounted to 0.0-0.08 mg Cl_2/L.

Samples of test media were analysed at 0 (fresh media), 24 (old media), 72 (fresh media) and 96 hours (old media). The results obtained are presented below.

Table 10.2.1-16: Test item concentrations [mg/L]

Nominal test item concentration (% saturated solution)	Measured concentration (mg/L)				Time-weighted mean measured concentration (mg/L)
	0 hours (fresh media)	24 hours (old media)	72 hours (fresh media)*	96 hours (old media)	
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	0.699	0.663	0.601	0.576	0.635
18	1.30	1.02	1.09	0.947	1.09
32	2.20	1.80	1.94	1.90	1.96
56	3.69	3.11	3.59	3.59	3.49
100	6.20	5.57	5.89	5.65	5.82

LOQ = 0.001 mg/L
* Initial batch failed analytical acceptance criteria therefore one set of the 'back-up' samples was analysed alongside the 96-hour samples

The test item concentrations decreased slightly over each 24-hour dosing period. As such, it was considered appropriate to base the results on the time-weighted mean measured test concentrations. These were calculated to be 0.635, 1.09, 1.96, 3.49 and 5.82 mg/L for the 10, 18, 32, 56 and 100% saturated solution test concentrations, respectively.

The measured ratio of the two enantiomers of RH-127450 in the 96-hour test samples was within ±1.1% of the ratio in the CoA of the test item and has therefore not changed during the lifetime of the study.
The fish showed a test item related mortality at 56 and 100 % saturated RH-127450 solution.

Table 10.2.1-17: Cumulative mortality of fish

Nominal test item concentration (% saturated solution)	No. of fish exposed	Cumulative Mortality			
		24 hours	48 hours	72 hours	96 hours
Control	7	0	0	0	0
10	7	0	0	0	0
18	7	0	0	0	0
32	7	0	0	0	0
56	7	0	1	1	1
100	7	3	6	6	7

The fish were showing mild to severe toxic effects at 56 and 100% saturated RH-127450 solution, starting with effects like hyperventilation, pigment changes, different swimming positions, showing thereafter abnormal swimming or lying on the bottom of the tank, resulting in dead fish mainly at the top concentration.
Based on time-weighted mean measured substance concentration, the 96-hour LC_{50} value was determined to be 4.17 mg/L. The corresponding No Observed Effect Concentration (NOEC) was determined to be 1.96 mg/L.
The validity criteria for control mortality (≤ 10 %) and dissolved oxygen (≥ 60 % air saturation) were both satisfied. Therefore, the test is valid.

Table 10.2.1-18: Acute toxicity of RH-127450 on fish – study endpoints

Parameter	Test item concentration (mg/L) Bracketed values present the 95% confidence limits			
	24 hours	48 hours	72 hours	96 hours
LC_{50}	>5.82	4.51 (3.94 – 5.15)	4.51 (3.94 – 5.15)	4.17 (3.61 – 4.82)
NOEC	3.49	1.96	1.96	1.96

Conclusion

The acute toxicity of the zoxamide metabolite RH-127450 to *Oncorhynchus mykiss* was tested at application rates of nominally 10, 18, 32, 56 and 100 % saturated solution (measured time-weighted mean test item concentrations of 0.635, 1.09, 1.96, 3.49 and 5.82 mg/L) over 96-hours in a semi-static test design.
Based on time-weighted mean measured substance concentrations, the 96-hour LC_{50} value was determined to be 4.17 mg/L. The corresponding No Observed Effect Concentration (NOEC) is 1.96 mg/L.

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 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on nominal concentrations:

The 48-hour EC50 (static test conditions) for *Daphnia magna* > 100 mg a.s./L (nom)

The NOEC (static test conditions) for *Daphnia magna* = 100 mg a.s./L (nom)

Reference:	KCA 8.2.4.1
Report	Jarrom, R., 2020: RH-163353: Acute toxicity to *Daphnia magna* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202386, GLP, Not published
Guideline(s):	OECD 202 (adopted April 13 2004)
Deviations:	A temperature deviation was noted during the range finding and definitive test because the continuous temperature vessel variation was >2°C (range finding test = 2.3°C; definitive test = 2.1°C). This slight deviation (protocol specification = within 2°C) was not considered to have had an impact on the integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Batch No.)	RH-163353 (HHGCP01-00-2)
Purity	99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)
Species	*Daphnia magna*
Age:	juveniles, < 24 hours old
Number of animals:	5 / 4 replicates (20)
Weight:	not relevant
Source:	MicroBioTests Inc., Belgium
Acclimation period:	not relevant
Feeding:	*Daphnia magna* were not fed during the test.
Test vessels:	100 mL glass beakers, filled with 50 mL test or control medium, in which the test organisms were suspended.
Environmental conditions	
Temperature:	18.6 – 20.7°C (water)
Photoperiod:	16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle
Test medium:	Elendt M4

pH:	7.26 – 7.81
Dissolved oxygen:	103.9 – 106.2% air saturation
Application rate(s)	Range-finding test: Nominally 0.10, 1.0, 10 and 100 mg/L (static conditions) Definitive test: Nominally 100 mg/L (static conditions)
Post exposure observation period	48 hours
Remarks	None

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-163353 to groups of 5 first instar *Daphnia magna* (4 replicates per test item and control) was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design. After 24 and 48 hours, the *Daphnia magna* in each test vessel were observed for evidence of immobility. Untreated groups of the freshwater planktonic crustacean (in test medium without test item) served as control.
Concentrations of RH-163353 were determined by mixing the aqueous samples with acetonitrile containing 0.5% formic acid, then diluting further with Elendt: acetonitrile (4:1 v/v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202386-01V and updated SMV 3202386-02V to include storage stability data). The limit of quantification (LOQ) was 0.001 mg/L.
RH-163353 is a racemate. The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure, SMV 3202586-01V). A combination of the analytical procedures SMV 3202385-01V and SMV 3202586-01V was used to assess the enantiomeric ratio of the test substance in the test medium and calibration standard solution.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.26-7.81, temperature 18.6-20.7°C, dissolved oxygen 103.9–106.2% air saturation).
Analysis of the freshly prepared media at 0 hours and the corresponding old media at 48 hours showed measured concentrations of 104 and 69.8 mg/L, respectively. Given that measured concentrations were within the 80% to 120% of nominal acceptance range, the study results are based on nominal test item concentrations.

Table 10.2.1-19: Test item concentrations

	Measured concentration (mg/L)		**% nominal**	
Nominal test item concentration (mg/L)	**0 hours (new media)**	**48 hours (old media)**	**0 hours (new media)**	**48 hours (old media)**
Control	< LOQ	< LOQ	--	--
100	104	96.8	104	93.1

LOQ = 0.1 mg/L

RH-163353 is a racemate. The ratio of its two enantiomers has been checked in a 96-hour test sample and in a freshly prepared calibration standard solution. As a result, the isomer ratio of the 96-hour 100 mg/L sample was within ±1.5% of the ratio from the certificate of analysis for the test item batch. Thus, the enantiomeric ratio is deemed to be stable in the *Daphnia* media during the course of the study.
There was no immobilisation of the daphnids in the control and 100 mg/L treatment during the test.

Table 10.2.1-20: Toxicity to *Daphnia magna*

Nominal test item concentration (mg/L)	No. of *Daphnia magna* exposed	Immobility at 24-hours (%)	Immobility at 48-hours (%)
Control	20	0	0
100	20	0	0

Based on nominal concentrations, the 48-hour EC_{50} value was determined to be > 100 mg/L. The corresponding no observed effect concentration (NOEC) is 100 mg/L.
Given that no effects were observed throughout the test, the results were determined empirically.
The validity criteria for control immobility (≤ 10 %) and dissolved oxygen (≥ 3 mg/L) were met. The test is therefore considered valid.

Table 10.2.1-21: Acute toxicity of RH-163353 on *Daphnia* – study endpoints

Parameter	Test item concentration (mg/L)	
	24 hours	48 hours
LC_{50}	>100	>100
NOEC	100	100

Conclusion

The acute toxicity of the zoxamide metabolite RH-163353 to *Daphnia magna* was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design.
Based on nominal concentrations, the 48-hour EC_{50} value was determined to be > 100 mg/L, respectively.
The corresponding no observed effect concentrations (NOEC) is 100 mg/L.
All validity criteria were met. Therefore, the test was considered valid.

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 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on nominal concentrations:

The 96-hour LC50 (static test conditions) for *Oncorhynchus mykiss* ≥100 mg a.s./L (nom)

The NOEC (static test conditions) for *Oncorhynchus mykiss* = 100 mg a.s./L (nom)

Reference: KCA 8.2.4.1

Report	Hugill, E., 2020: RH-141455: Acute toxicity to *Daphnia magna* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202380, GLP, Not published
Guideline(s):	OECD 202 (adopted April 13 2004)
Deviations:	There was no confirmation in the range finding data that the vitamin stock was added to the Elendt M4 media by error. This protocol deviation has no impact on the integrity of the study because the main test was performed without deviation and the immobilisation at all treatments was ≤ 10% - indicating that there was no impact on *Daphnia* if the vitamin stock was not added. A temperature deviation was noted during the test because the 100 mg/L treatment vessel temperature deviated by 2.2°C from the initial vessel temperature. This slight deviation (protocol specification = within 2°C) was not considered to have had an impact on the integrity of the study, as there was not test substance immobilisation during the test.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (A19X08291)
Purity	92.77 % (w/w)
Species	*Daphnia magna*
Age:	juveniles, < 24 hours old
Number of animals:	5 / 4 replicates (20)
Weight:	not relevant
Source:	MicroBioTests Inc., Belgium
Acclimation period:	not relevant
Feeding:	*Daphnia magna* were not fed during the test
Test vessels:	100 mL glass beakers, filled with 50 mL test or control medium, in which the test organisms were suspended.
Environmental conditions	
Temperature:	19.6 - 20.2°C (water)
Photoperiod:	16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle
Test medium:	Elendt M4 medium
pH:	6.00 – 7.80
Dissolved oxygen:	98.8 – 101.5% air saturation
Application rate(s)	Range-finding test: Nominally 0.10, 1.0, 10 and 100 mg/L (static conditions) Definitive test: Nominally 100 mg/L (static conditions)
Post exposure observation period	48 hours
Remarks	None

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-141455 to groups of 5 first instar *Daphnia magna* (4 replicates per test item and control) was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design. After 24 and 48 hours, the *Daphnia magna* in each test vessel were observed for evidence of immobility. Untreated groups of the freshwater planktonic crustacean (in test medium without test item) served as control.
Concentrations of RH-141455 were determined by mixing the aqueous samples with acetonitrile containing formic acid, then diluting further with Elendt: acetonitrile (4:1 v/v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202380-03V). The limit of quantification (LOQ) was 0.1 mg/L.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.00-7.80, temperature 19.6°-20.2°C, Dissolved oxygen 98.8-101.5% air saturation).
The analytical results confirm the correct test substance dosing, as the 100 mg/L 0-hour result was within 80 - 120% nominal range (i.e. 93.1%). The test item remained stable throughout the course of the study with a recovery of 110 % of the initially measured concentration. Given that measured concentrations were within the 80% to 120% of nominal acceptance range, the study results are based on nominal test item concentrations.

Table 10.2.1-22: Test item concentrations

	Measured concentration (mg/L)		% of initial measured	
Nominal test item concentration (mg/L)	**0 hours (new media)**	**48 hours (old media)**	**0 hours (new media)**	**48 hours (old media)**
Control	< LOQ	< LOQ	--	--
100	93.1	102	93.1	110

LOQ = 0.1 mg/L

There was no immobilisation of the daphnids in the control and 100 mg/L treatment during the test.

Table 10.2.1-23: Toxicity to *Daphnia magna*

Nominal test item concentration (mg/L)	No. of *Daphnia magna* exposed	Immobility at 24-hours (%)	Immobility at 48-hours (%)
Control	20	0	0
100	20	0	0

Based on nominal concentrations, the 48-hour EC_{50} value was determined to be > 100 mg/L. The corresponding no observed effect concentration (NOEC) is 100 mg/L.
Given that no effects were observed throughout the test, the results were determined empirically.
The validity criteria for control immobility (≤ 10 %) and Dissolved oxygen (≥ 3 mg/L) were met. The test is therefore considered valid.

Table 10.2.1-24: Acute toxicity of RH-141455 on *Daphnia* – study endpoints

Parameter	Test item concentration (mg/L)	
	24 hours	**48 hours**
LC_{50}	>100	>100
NOEC	100	100

Conclusion

The acute toxicity of the zoxamide metabolite RH-141455 to *Daphnia magna* was tested at an application rate of nominally 100 mg/L (limit test) over 48-hours in a static test design.
Based on nominal concentrations, the 48-hour EC_{50} value was determined to be > 100 mg/L, respectively.
The corresponding no observed effect concentrations (NOEC) is 100 mg/L.
All validity criteria were met. Therefore, the test was considered valid.

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 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on mean measured concentrations:

The 96-hour LC50 for *Americamysis bahia* (static test conditions) = 2.93 mg a.s./L (mm)

The NOEC for *Americamysis bahia* (static test conditions) = 1.86 mg a.s./L (mm)

Reference:	KCA 8.2.4.2
Report	Hugill, E., 2020: RH-127450: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202374, GLP, Not published
Guideline(s):	OCSPP 850.1035 (2016)
Deviations:	During the range finding test, the salinity of the control and highest concentration (100% saturated solution) exceed the ± 1 ppt protocol requirement, as these were measured to be 22 and 18 ppt, respectively at the start of the test. This was not identified at the time in error, however, has no impact as the protocol requirement was tighter than the guideline (± 2 ppt). Additional Dissolved oxygen concentrations were taken from the treatment vessels where 100% mortality was noted at the Study Director's direction. These were taken to confirm that the high mortality was not related to low oxygen levels, as mysids are very sensitive to oxygen levels. However, this protocol deviation has no impact on the integrity of the study. The protocol only required statistical analysis to be conducted on the 48 and 96-hour results, but should have included the conduct of statistical analysis on the 24 and 72-hour data, as this is a requirement of the OCSPP guideline.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-127450 (HHGCP-002-00-1)
Purity	99.22 % (w/w) (re-certiffied under GLP: 99.51 % (w/w))
Species	*Americamysis bahia*
Age:	juveniles, < 24 hours old
Number of animals:	5 / 4 replicates (20)
Weight:	not relevant
Source:	in-house breeding of the test facility
Acclimation period:	not relevant
Feeding:	24-hour old *Artemia sp. Nauplii*
Test vessels:	600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended
Environmental conditions	
Temperature:	25.1-25.6°C (water)
Photoperiod:	16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle
Test medium:	artificial (brackish) seawater The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰).
pH:	7.79 – 8.16
Dissolved oxygen:	81.9 – 102.7% air saturation
Salinity:	20 – 21 (‰/ppt)
Application rate(s)	nominally 6.25, 12.5, 25, 50 and 100% saturated solution (static conditions)
Post exposure observation period	96 hours
Remarks	None

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-127450 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100% saturated solution over 96 hours in a static test design. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.
Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.
Concentrations of RH-127450 were determined by treating brackish water samples with acetonitrile containing formic acid, then diluting further with brackish water: acetonitrile (4:1, v:v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202374-01V (later updated SMV 3202374-02V to add the stability information). The limit of quantification (LOQ) was 0.01 mg/L.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.79-8.16, temperature 25.1°-25.6°C, Dissolved oxygen 81.9 – 102.7% air saturation).

The analytical results demonstrated that the final definitive test was dosed at the following concentrations: 0.462, 0.859, 1.77, 3.82 and 7.46 mg/L for the 6.25, 12.5, 25, 50 and 100% saturated solution concentrations, respectively. The test item remained stable throughout the course of the study with a recovery of 99.1 - 114% of the initial measured concentrations. As the test solutions were prepared by saturated solution, the study results are based on arithmetic mean measured concentrations (0.460, 0.910, 1.86, 3.99 and 7.71 mg/L for the 6.25, 12.5, 25, 50 and 100% saturated solution concentrations, respectively).
The control and test item solutions were observed to be colourless throughout the during the duration of the test.

Table 10.2.1-25: Test item concentrations

Nominal concentration (% saturated solution)	Sample analysed	Mean measured concentration (mg/L)		Arithmetic mean measured concentration (mg/L)
		0 hours (new media)	96 hours (old media)	
Control	A	< LOD	< LOQ	< LOQ
6.25	A	0.462	0.458	0.460
12.5	A	0.713	0.954	0.910
	B1*	0.855	0.932	
	B2*	1.010	0.994	
	Mean	0.859	0.960	
25	A	1.77	1.94	1.86
50	A	3.82	4.15	3.99
100	A	7.46	7.96	7.71

LOQ = 0.01 mg/L, LOD = 0.0001 mg/L
* The 0 and 96-hour "B" samples were analysed to confirm the test concentrations at this treatment, as the initial "A" sample results for 0 and 96-hour were quite different (0-hour results were *ca* 25% lower than the 96-hour results). As all the three results were relatively close, the mean measured concentration has been calculated using all three results at each time point.

The toxicity results are summarised in the following table.

Table 10.2.1-26: Percentage mortality

Nominal concentration (% saturated solution)	Mean measured concentration (mg/L)	Percent mortality (%)			
		24-hours	48-hours	72-hours	96-hours
Control	Control	5	5	5	5
6.25	0.460	5	5	5	5
12.5	0.910	10	10	15	15
25	1.86	0	10	10	10
50	3.99	35[1]	75[3]	75	75
100	7.71	40[2]	100	100	100

[1] Two surviving mysids at this treatment appeared confused and were swimming very fast in circles
[2] Twelve surviving mysids at this treatment were observed to be lethargic
[3] Two surviving mysids at this treatment were observed to be lethargic

Statistical analysis was performed using the CETIS program v 1.8.6.8. The 24-hour LC_{50} values were calculated using a Linear Interpolation (ICPIN). The 48, 72 and 96-hour LC_{50} values were calculated using untrimmed Spearman Kärber. Where possible the 95% confidence limits were calculated. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was calculated using the Cochran-Armitage Trend Step-Down test. Where applicable, outliers according to Grubbs were considered.

Table 10.2.1-27: Acute toxicity of RH-127450 on *Americamysis bahia* – study endpoints

Parameter	Mean measured concentration (mg/L) Bracketed values present the 95% confidence limits			
	24-hour	**48-hour**	**72-hour**	**96-hour**
LC_{50}	>7.71 (NC)	3.05 (2.56 – 3.62)	2.93 (2.43 – 3.53)	2.93 (2.43 – 3.53)
NOEC	1.86	1.86	1.86	1.86

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid.

Conclusion

The acute toxicity of the zoxamide metabolite RH-127450 to *Americamysis bahia* was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100% saturated solution over 96 hours in a static test design.
The test substance remained stable throughout the course of the study. However, since the test solutions were prepared by saturated solution, the study endpoints are based on arithmetic mean measured concentrations.
Based on mean measured concentrations, the 96-hour LC_{50} value was determined at 2.93 mg/L, the 96-hour NOEC at 1.86 mg/L.
All validity criteria were met. Therefore, the test was considered valid.

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 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on mean measured concentrations:

The 96-hour LC50 for *Americamysis bahia* (static test conditions) = 23.2 mg a.s./L (mm)

The NOEC for *Americamysis bahia* (static test conditions) = 5.67 mg a.s./L (mm)

Reference:	KCA 8.2.4.2
Report	Hugill, E., 2020: RH-24549: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202394, GLP, Not published
Guideline(s):	OCSPP 850.1035: Mysid Acute Toxicity Test
Deviations:	The test media was prepared using reverse osmosis water rather than deionised water as per the definitive protocol. This protocol deviation has no impact on the integrity of the study because reverse osmosis water is acceptable according to

OCSPP 850.1035.
The salinity in the highest concentration dropped to 18 ppt (protocol requirement = 20±1 ppt during the test). This protocol deviation has no impact on the integrity of the study because salinity at 20±2 ppt is acceptable according to OCSPP 850.1035.
The protocol required statistical analysis to be conducted on the 48 and 96-hour results. However, it should have included additional statistical analysis on the 24 and 72-hour data. This protocol deviation has no impact on the integrity of the study since the necessary calculations have been performed.
A protocol deviation occurred at the highest concentration, because at 0-hours the pH in one test vessel was below pH 7.5 (allowed range: pH 7.5-8.5). This pH deviation was considered to have not an impact on the integrity of the study, because the 50% lethal effects concentration was between 25-50 mg/L where the pH measurements were within the acceptable range.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): No

Materials and methods

Test material (Lot/Batch No.)	RH-24549 (FCC25806)
Purity	99.59 % (w/w)
Species	*Americamysis bahia*
Age:	Juveniles, < 24 hours old
Number of animals:	5 / 4 replicates (20)
Weight:	not relevant
Source:	in-house breeding of the test facility
Acclimation period:	not relevant
Feeding:	24-hour old *Artemia sp. Nauplii*
Test vessels:	600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended
Environmental conditions	
Temperature:	24.6-25.6°C (water)
Photoperiod:	16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle
Test medium:	artificial (brackish) seawater The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰).
pH:	7.25 – 8.09
Dissolved oxygen:	70.5 – 99.0% air saturation
Salinity:	18 – 21 (‰/ppt)
Application rate(s)	nominally 6.25, 12.5, 25, 50 and 100 mg/L (static conditions)
Post exposure observation period	96 hours
Remarks	None

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-24549 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.
Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.

Concentrations of RH-24549 were determined by treating brackish water samples with acetonitrile containing formic acid, then diluting further with brackish water: acetonitrile (4:1, v/v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) or triple quad mass spectrometry (LC-TQMS/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202394-02V, later updated to SMV 3202394-05V to include stability data) The limit of quantification (LOQ) was 0.1 mg/L.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.25 to 8.09, temperature 24.6 - 25.6°C, Dissolved oxygen 70.5 to 199.0% air saturation).
The analytical results demonstrated that the test substance was dosed correctly because with exception to the 25 mg/L treatment (77.2% of nominal) the initial measured concentrations (0-hour) were within the 80 – 120% nominal range (86.7 - 94.8%). The test item remained stable throughout the course of the study with a recovery of 91.0 - 109% of initial measured concentrations. As the 25 mg/L treatment was not within the 80-120% nominal range, the study results are based on arithmetic mean measured concentrations.

Table 10.2.1-28: Test item concentrations

Nominal test item concentration (mg/L)	Measured concentration (mg/L)			Recovery	
	0 hours (new media)	96 hours (old media)	Arithmetic Mean	% of nominal concentration	% of initial measured concentration
Control	<LOQ	<LOQ	<LOQ	--	--
6.25	5.42	5.92	5.67	86.7	109
12.5	11.1	11.4	11.3	88.8	103
25	19.3	21.1	20.2	77.2	109
50	47.4	44.8	46.1	94.8	94.5
100	92.8	84.4	88.6	92.8	91.0

LOQ = 0.1 mg/L

The toxicity results are summarised in the following table.

Table 10.2.1-29: Percentage mortality

Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Percent mortality (%)			
		24-hours	48-hours	72-hours	96-hours
Control	Control	0	0	0	0
6.25	5.67	0	0	0	0
12.5	11.3	0	5	5	10
25	20.2	0	10	35	35
50	46.1	20	40	95	100

100	88.6	100	100	100	100

Statistical analysis was performed using the CETIS program v 1.8.6.8. The 24, 48, 72 and 96-hour LC_{50} values were calculated using untrimmed Spearman Kärber. Where possible the 95% confidence limits were calculated. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was calculated using the Cochran-Armitage Trend Step-Down test.

Table 10.2.1-30: Acute toxicity of RH-24549 on *Americamysis bahia* – study endpoints

Parameter	Mean measured concentration (mg/L) Bracketed values present the 95% confidence limits			
	24-hour	**48-hour**	**72-hour**	**96-hour**
LC_{50}	55.1 (48.3 – 62.9)	42.9 (35.3 – 52.3)	24.0 (20.1 – 28.6)	23.2 (19.3 – 28.0)
NOEC	20.2	11.3	11.3	5.67

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid.

Conclusion

The acute toxicity of the zoxamide metabolite RH-24549 to *Americamysis bahia* was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L over 96 hours in a static test design.
The test substance remained stable throughout the course of the study. However, since one application rate was not within the 80-120% nominal range, the study results are based on arithmetic mean measured concentrations.
Based on mean measured concentrations, the 96-hour LC_{50} value was determined at 23.2 mg/L, the corresponding 96-hour NOEC values at 5.67.
All validity criteria were met. Therefore, the test was considered valid.

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 (relevant for all representative uses evaluated; submission date pro-posed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on mean measured concentrations:

The 96-hour LC50 for *Americamysis bahia* (static test conditions) = 6.95 mg a.s./L (mm)

The NOEC for *Americamysis bahia* (static test conditions) = 3.59 mg a.s./L (mm)

Reference: KCA 8.2.4.2

Report Hugill, E., 2020: RH-139432: Mysid acute toxicity test

Gowan Crop Protection Ltd., UK
Smithers ERS Ltd., UK, Report No. 3202398, GLP, Not published

Guideline(s): OCSPP 850.1035 (2016)

Deviations: The test media was prepared using reverse osmosis water rather than deionised water as per the definitive protocol. This protocol deviation has no impact on the integrity of the study because reverse osmosis water is acceptable according to OCSPP 850.1035.
The study protocol requested statistical analysis only for the 48 and 96-hour results. However, according to OCSPP the protocol should also have been mentioned statistical evaluation of the 24 and 72-hour data, what has finally been performed and reported.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test material (Lot/Batch No.)	RH-139432 (HHGCP005-00-1)
Purity	99.01 % (w/w) (re-certified under GLP: 99.35 % (w/w))
Species	*Americamysis bahia*
Age:	juveniles, < 24 hours old
Number of animals:	5 / 4 replicates (20)
Weight:	not relevant
Source:	in-house breeding of the test facility
Acclimation period:	not relevant
Feeding:	24-hour old *Artemia sp. Nauplii*
Test vessels:	600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended
Environmental conditions	
Temperature:	24.9-25.5°C
Photoperiod:	16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle
Test medium:	artificial (brackish) seawater The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰).
pH:	7.95 – 8.19
Dissolved oxygen:	87.2 – 102.5% air saturation
Salinity:	19 – 21 (‰/ppt)
Application rate(s)	nominally 1.56, 3.125, 6.25, 12.5, 25 and 50% saturated solution (static conditions)
Post exposure observation period	96 hours
Remarks	The mortality results for the initial definitive test suggested that the 96 hour LC_{50} value would be between the 6.25 and 12.5% saturated

	solution and that the NOEC below 6.25 % saturated solution. The mortality results of an initial definitive test (with several protocol deviations) suggested that the 96-hour LC_{50} value would be between the 6.25 and 12.5% saturated solution and the NOEC below 6.25 % saturated solution (the lowest concentration tested). Therefore, a second and final definitive test has been performed at refined application rates.

Based on the results of the range-finding and initial definitive test, the acute toxicity of the zoxamide metabolite RH-139432 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested at final nominal concentrations of 1.56, 3.125, 6.25, 12.5, 25 and 50% saturated solution over 96 hours in a static test design. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.
Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.
Concentrations of RH-139432 were determined by treating brackish water samples with acetonitrile containing 0.5% formic acid, then diluting further with brackish water: acetonitrile (4:1, v:v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) or triple quadrupole mass spectrometry (LC-TQMS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202398-02V). The limit of quantification (LOQ) was 0.1 mg/L.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.95-8.19, temperature 24.6-25.5°C, dissolved oxygen 87.2-102.5% air saturation).
The analytical results demonstrated that the final definitive test was dosed at the following concentrations: 0.909, 1.78, 3.63, 7.24, 14.4 and 29.8 mg/L for the 1.56, 3.13, 6.25, 12.5, 25 and 50% saturated solution concentrations, respectively. The test item remained stable throughout the course of the study with a recovery of 95 - 101% of the initial measured concentrations. As the test solutions were prepared by saturated solution, the study results have been based on arithmetic mean measured concentrations.
The control and test item solutions were observed to be colourless throughout the during the duration of the test.

Table 10.2.1-31: Test item concentrations

Nominal concentration (% saturated solution)	Mean measured concentration (mg/L)		Arithmetic mean measured concentration (mg/L)	% of initial measured concentration
	0 hours (new media)	96 hours (old media)		
Control	< LOD	< LOQ	< LOQ	--
1.56	0.909	0.862	0.886	94.8
3.125	1.78	1.690	1.74	94.9
6.25	3.63	3.55	3.59	97.8
12.5	7.24	7.16	7.20	98.9
25	14.4	14.5	14.5	101
50	29.8	28.7	29.3	96.3

LOQ = 0.1 mg/L

The toxicity results are summarised in the following tables. For completeness, the results of the first and of the final definitive test are given.

Table 10.2.1-32: Percentage mortality of the initial definitive test

Nominal concentration (% saturated solution)	Percent mortality (%)			
	24-hours	48-hours	72-hours	96-hours
Control	0	5	5	5
6.25	10	30	35	35
12.5	20	50	55	55
25	20	75	95	100
50	85	100	100	100
100	100	100	100	100

Table 10.2.1-33: Percentage mortality of the final definitive test

Nominal concentration (% saturated solution)	Mean measured concentration (mg/L)	Number of *Americamysis bahia* exposed	Percent mortality (%)			
			24-hours	48-hours	72-hours	96-hours
Control	Control	20	10	10	10	10
1.56	0.886	20	0	0	0	0
3.125	1.74	20	0	0	0	0
6.25	3.59	20	5	5	5	5
12.5	7.20	20	5	45	45	55
25	14.5	20	30	80	100	100
50	29.3	20	100	100	100	100

Statistical analysis was performed using the CETIS program v 1.8.6.8. The 24, 48, 72 and 96-hour LC_{50} values were calculated using untrimmed Spearman Kärber. Where possible the 95% confidence limits were calculated. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was calculated using the Cochran-Armitage Trend Step-Down test.

Table 10.2.1-34: Acute toxicity of RH-139432 on *Americamysis bahia* – study endpoints

Parameter	Mean measured concentration (mg/L) Bracketed values present the 95% confidence limits			
	24-hour	48-hour	72-hour	96-hour
LC_{50}	16.6 (14.2 – 19.3)	8.64 (7.04 – 10.6)	7.47 (6.37 – 8.77)	6.95 (5.92 – 8.17)
NOEC	7.20	3.59	3.59	3.59

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid.

Conclusion

The acute toxicity of the zoxamide metabolite RH-139432 to *Americamysis bahia* was tested at nominal concentrations of 1.56, 3.125, 6.25, 12.5, 25 and 50% saturated solution over 96 hours in a static test design.

The test substance remained stable throughout the course of the study. However, since the test solutions were prepared by saturated solution, the study endpoints are based on arithmetic mean measured concentrations.

Based on mean measured concentrations, the 96-hour LC_{50} value was determined at 6.95 mg/L, the 96-hour NOEC at 3.59 mg/L.
All validity criteria were met. Therefore, the test was considered valid.

EFSA Peer Review Conclusion (2017) requested "*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 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

based on nominal concentrations:

The 96-hour LC50 for *Americamysis bahia* (static test conditions) ≥100 mg a.s./L (nom)

The 96-hour NOEC for *Americamysis bahia* (static test conditions) = 100 mg a.s./L (nom)

Reference:	KCA 8.2.4.2
Report	Jarrom, R., 2020: RH-163353: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202387, GLP, Not published
Guideline(s):	OCSPP 850.1035 (2016)
Deviations:	The measured pH of the 100 mg/L solution at test initiation was 7.37 (0.13 outside of the specified range of 7.5 – 8.5), pH adjustment was not made. This protocol deviation has no impact on the integrity of the study as no mortality was seen in the 100 mg/L replicates, demonstrating no negative effects on the test organisms.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-163353 (HHGCP001-00-2)
Purity	99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)
Species	*Americamysis bahia*
Age:	juveniles, < 24 hours old
Number of animals:	5 / 4 replicates (20)
Weight:	not relevant

Source:	in-house breeding of the test facility
Acclimation period:	not relevant
Feeding:	24-hour old *Artemia sp. Nauplii*
Test vessels:	600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended
Environmental conditions	
Temperature:	24.3-25.8°C (water)
Photoperiod:	16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle
Test medium:	artificial (brackish) seawater The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰).
pH:	7.37 – 7.97
Dissolved oxygen:	85.4 – 101% air saturation
Salinity:	20 – 21 (‰/ppt)
Application rate(s)	nominally 6.25, 12.5, 25, 50 and 100 mg/L (static test conditions)
Post exposure observation period	96 hours
Remarks	None

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-163353 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L. A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.
Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.
Concentrations of RH-163353 were determined by treating brackish water samples with acetonitrile containing 0.5% formic acid, then diluting further with brackish water: acetonitrile (4:1, v:v) containing 0.1% formic acid to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202387-01V, later updated SMV 3202387-02V to add stability information). The limit of quantification (LOQ) was 0.1 mg/L.
RH-163353 is a racemate. The 50:50 ratio of its enantiomers in the test media has been analytically verified using method SMV 3202387-01V.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.37-7.97, temperature 24.3°-25.8°C, Dissolved oxygen 85.4 to 101% air saturation).
The analytical results demonstrated that the test substance was dosed correctly because the initial measured concentrations (0-hour) were within the 80 – 120% nominal range (i.e. 93.5 - 100%). The test item remained stable throughout the course of the study with a recovery of 95.5 – 99.1% of initial measured concentrations. Since the analysed test item concentrations in the new and old media samples stayed within their nominal ranges (80 - 120%) throughout the test, the study results are based on nominal concentrations.
RH-163353 is a racemate. The 50:50 ratio of its enantiomers in the test media has been analytically verified in old media samples and compared to the validated enantiomeric ratio provided in the certificate of analysis for the test substance. As a result, the measured ratio of the 96-hours samples were within 2% (i.e. ≤1.13%) of the validated enantiomeric ratio and so was not deemed to have changed during the course of the study.

Table 10.2.1-35: Test item concentrations

Nominal test item concentration (mg/L)	Measured concentration (mg/L)		Recovery	
	0 hours (new media)	96 hours (old media)	% of nominal concentration	% of initial measured concentration
Control	<LOQ	<LOQ	--	--
6.25	5.84	5.79	93.5	99.1
12.5	12.5	12.0	100	95.5
25	24.3	23.9	97.0	98.6
50	47.8	46.2	95.5	96.8
100	97.7	96.2	97.7	98.5

LOQ = 0.1 mg/L

The toxicity results are summarised in the following table.

Table 10.2.1-36: Percentage mortality

Nominal concentration (mg/L)	Percent mortality (%)			
	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
6.25	0	0	0	0
12.5	0	0	0	0
25	5	5	5	5
50	0	0	0	0
100	0	0	0	0

As there was no mortality up to and including the test item application rate of 100 mg/L the LC_{50} is reported as being greater than the highest test concentration. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was determined empirically.

Table 10.2.1-37: Acute toxicity of RH-163353 on *Americamysis bahia* – study endpoints

Parameter	Mean measured concentration (mg/L)			
	24-hour	48-hour	72-hour	96-hour
LC_{50}	>100	>100	>100	>100
NOEC	100	100	100	100

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid.

Conclusion

The acute toxicity of the zoxamide metabolite RH-163353 to *Americamysis bahia* was tested at nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg/L over 96 hours in a static test design.
Since the analysed test item concentrations in the new and old media samples stayed within their nominal ranges (80 - 120%) throughout the test, the study results were based on nominal concentrations. The 96-hour LC_{50} value was determined at >100 mg/L, the corresponding 96-hour NOEC values at 100 mg/L.
RH-163353 is a racemate. Its 50:50 ratio during the course of the study in the test media has been analytically verified.

All validity criteria were met. Therefore, the test was considered valid.

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 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on nominal concentrations:

The 96-hour LC50 for *Americamysis bahia* determined (semi-static test conditions) ≥100 mg a.s./L (nom)

The 96-hour NOEC for *Americamysis bahia* (semi-static test conditions) = 100 mg a.s./L (nom)

Reference:	KCA 8.2.4.2
Report	Hugill, E., 2020: RH-141455: Mysid acute toxicity test Gowan Crop Protection Ltd., UK Smithers ERS Limited, UK, Report No. 3202381, GLP, Not published
Guideline(s):	OSPP 850.1035: Mysid Acute Toxicity Test
Deviations:	Only some minor deviations during the range-finder, which are considered to be not relevant for the integrity of the (main) study results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (A19X08291)
Purity	92.77 % (w/w)
Species:	*Americamysis bahia*
Age:	juveniles, < 24 hours old
Number of animals:	5 / 4 replicates (20)
Weight:	not relevant
Source:	in-house breeding of the test facility
Acclimation period:	not relevant
Feeding:	24-hour old *Artemia sp. Nauplii*

Test vessels:	600 mL glass beakers, filled with 400 mL test or control medium, in which the test organisms were suspended
Environmental conditions	
Temperature:	24.1–25.2°C
Photoperiod:	16-hour light, 8-hour dark, with a 30-minute dawn/dusk cycle
Test medium:	artificial (brackish) seawater The media was prepared by dissolving artificial marine salts (e.g. Tropic Marin®) in reverse osmosis water to provide a salinity of 20 ± 1 ppt (‰).
pH:	6.53–7.97
Dissolved oxygen:	87.4–102.5 air saturation
Salinity:	20-22 ‰/ppt
Application rate(s)	Range-finding test: Nominally 0.10, 1.0, 10 and 100 mg/L (static conditions) Definitive test: Nominally 100 mg/L (limit test; semi-static conditions)
Post exposure observation period	96 hours
Remarks	Since the test substance was found to have dropped the pH to below pH 7.5 at the 100 mg/L application rate, the range-finding test has been performed with and without pH adjustment at the 100 mg/L application rate. However, pH adjustment was finally regarded to be not necessary, and therefore the main test was performed without pH adjustment. An initial definitive/limit test had to be stopped after 24-hours due to high control mortality (>10%).

Based on the results of a range finder, the acute toxicity of the zoxamide metabolite RH-141455 to groups of 5 juvenile *Americamysis bahia,* less than 24 hours old (4 replicates per test item and control) was tested under semi-static conditions (one renewal at 48-hours) at a nominal concentration of 100 mg/L (limit test). A control group was also included. After 24, 48, 72 and 96-hours the marine crustacean *Americamysis bahia* was observed for survival.

Analysis of the test media samples was conducted at 0 and 48-hours from freshly prepared media at each treatment and at 48 and 96 hours from pooled old media test vessels at each treatment.
Concentrations of RH-141455 were determined by treating brackish water samples with Milli-Q water/acetonitrile (8:2, v:v) containing 0.1% formic acid, then diluting with brackish water/Milli-Q water/acetonitrile (1:8:2, v:v) containing 0.1% formic acid) as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202381-01V, later updated to SMV 3202381-03V to add stability data and update the gradient program). The limit of quantification (LOQ) was 0.25 mg/L.

The results were statistically evaluated using the CETIS program v 1.8.6.8. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was estimated using the Equal Variance t Two-Sample test. Outliers were excluded using Grubbs test – in case applicable.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 6.53-7.46, temperature 24.1–25.2°C, Dissolved oxygen 84.6 to 102.3% air saturation).

The analytical results demonstrated that the definitive/limit test was dosed correctly, as the measured concentrations for the fresh 0 and 48-hour samples were 100 and 111% of nominal, respectively. The test

item remained stable over the 48-hour renewal period because the measured concentrations in the old media samples at 48 and 96-hour were 102 and 94.6% of the initial measured concentrations, respectively. As the measured concentrations for the new and old media maintained within the nominal range (80 - 120%) throughout the test, the study results were based on nominal test item concentrations.

Table 10.2.1-38: Test item concentrations

Nominal test item conc. (mg/L)	Measured concentration (mg/L)					Recovery				
						% of nominal		% of initial measured		Mean measured
	0 h (new media)	48 h (old media)	48 h (new media)	96 h (old media)	Mean	0 h (new media)	48 h (new media)	48 h (old media)	48 h (old media)	% of nominal
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	--	--	--	--	--
100	100	102	111	105	105	100	111	102	94.6	105

LOQ = 0.25 mg/L

The toxicity results are summarised in the following table.

Table 10.2.1-39: Percentage mortality

Nominal concentration (mg/L)	Percent mortality (%)			
	24-hours	48-hours	72-hours	96-hours
Control	5	10	10	10
100	5	10	15	15

The mortality at the 100 mg/L treatment was shown to be statistically not significant. Therefore, the LC_{50} is reported as being greater than the highest test concentration. The 24, 48, 72 and 96-hour no observed effect concentration (NOEC) was determined empirically.

Table 10.2.1-40: Acute toxicity of RH-141455 on Americamysis bahia – study endpoints

Parameter	Nominal concentration (mg/L)			
	24-hour	48-hour	72-hour	96-hour
LC_{50}	>100	>100	>100	>100
NOEC	100	100	100	100

The validity criteria for control mortality (≤10%) and Dissolved oxygen (60 – 105% air saturation value) were both met. The test is therefore considered valid.

Conclusion

Based on the results of a range-finder, the acute toxicity of the zoxamide metabolite RH-141455 to *Americamysis bahia* was tested at a nominal concentration of 100 mg/L (limit test) over 96 hours under semi-static conditions (one renewal at 48-hours).
Since the analysed test item concentrations in the new and old media samples stayed within their nominal ranges (80 - 120%) throughout the test, the study results were based on nominal concentrations.
The 96-hour LC_{50} value was determined at >100 mg/L, the corresponding 96-hour NOEC values at 100 mg/L.
All validity criteria were met. Therefore, the test was considered valid.

EFSA (2017) requested in its Peer Review Conclusion: "*Further algae studies following the latest OECD 201 guideline are needed or further detailed information on all validity criteria requested by the latest OECD 201 guideline from the studies provided in the RAR for zoxamide, RH-127450 and RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

With regard to the EFSA request on alga studies with the parent compound zoxamide: The alga studies with zoxamide which are available in the RAR for zoxamide (2017) have been re-evaluated at a later stage during AIR based on additionally provided information. The results of the re-evaluation were included in the RAR, the alga studies were regarded valid. This was confirmed by Latvia as RMS for zoxamide. As such, the endpoints from the alga studies with the active substance zoxamide available in the RAR (2017) are valid and applicable for the aquatic risk assessment.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on geometric mean measured concentrations:

The 72-hour EyC50 for *Raphidocelis subcapitata* (static test conditions) = 5.98 mg a.s./L (mm)

The 72-hour ErC50 for *Raphidocelis subcapitata* (static test conditions) ≥6.60 mg a.s./L (mm)

The 72-hour NOEC for *Raphidocelis subcapitata* (for yield and average specific growth rate, static test conditions) = 2.77 mg a.s./L (mm)

Reference:	KCA 8.2.6
Report	Hugill, E., 2020: RH-127450: Inhibition of growth to the alga *Raphidocelis subcapitata* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202375, GLP, Not published
Guideline(s):	OECD 201 (adopted 23 March 2006, corrected 28 July 2011)
Deviations:	An initial definitive test was conducted at nominal concentrations of 1, 3.2, 10, 32 and 100% saturated solution. However, it was necessary to repeat the main test due to an equipment failure (lux meter) and since, by error, 24- and 48-hour samples were not taken for analysis. The appearance of the test item in the test media was only observed at the start and at the end of the test. This deviation has no impact on the integrity of the study as the non-inoculated media observation at 72-hours showed the test substance to be in solution.
GLP:	Yes
Acceptability:	Yes
Duplication	No

(if vertebrate study)

Materials and methods

Test material (Lot/Batch No.)	RH-127450 (HHGCP-002-00-1)
Purity	99.22 % (w/w) (re-certiffied under GLP: 99.51 % (w/w))
Species	Alga *Raphidocelis subcapitata* (strain 278/4; formerly known as *Pseudokirchneriella subcapitata)*
Age:	not applicable
Weight:	not applicable
Source:	Culture Collection of Algae and Protozoa (CCAP), SAMS Research Service Ltd., Oban, UK
Acclimation period:	72 hours
Test vessel:	250 mL Erlenmeyer (conical) glass flasks, filled with 100 mL of test or control medium, capped with foam bungs, continuously agitated at ~100 rpm
Environmental conditions	
Test medium:	OECD alga nutrient medium (according to Annex 3 of the OECD TG 201)
Temperature:	22.2 – 23.1°C
pH:	7.95 – 8.21
Light:	Continuous light at 4.66 – 5.53 kLux
Application rate(s)	1, 3.2, 10, 32 and 100 % saturated solution under static test conditions
Post exposure observation period	72 hours
Remarks	None

Based on the results of a range-finder, three replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-127450 at nominal concentrations of 1, 3.2, 10, 32 and 100 % saturated solution under static conditions over 72 h. A control treatment (6 replicate cultures) was also included in the test. A toxic reference test with potassium dichromate was separately performed in January 2019.
Each test vessel was inoculated with 1×10^4 algae cells/mL. Algae cell counts were conducted at 24-hour intervals. The test item concentrations were analytically verified at 0, 24, 48 and 72-hours.
Concentrations of RH-127450 were determined by treating OECD medium samples with acetonitrile containing formic acid, then diluting further with OECD medium: acetonitrile (4:1, v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (SMV 3202375-01V, later updated to SMV 3202337-02V to add stability data). The limit of quantification (LOQ) was 0.01 mg/L.
The results were statistically evaluated using the CETIS program v 1.8.6.8. The no observed effect concentrations for yield and growth rate at all timepoints were determined using a Dunnett multiple comparison test because the data showed a non-monotonic but parametric trend. Linear interpolation analysis was performed in order to estimate EC_{10}, EC_{20} and EC_{50} values for the 72-hour final yield and the 0-24, 0-48, 0-72 hours average specific growth rate (μ) time intervals. Where possible, 95% confidence limits were calculated for the EC_{10}, EC_{20} and EC_{50} values. Outliers were assessed using Grubbs extreme value – in case applicable.

Results and discussion

Environmental parameters remained within acceptable limits throughout the duration of the study (pH 7.95–8.21, temperature 22.2 – 23.1°C, light intensity range of 4.66 – 5.53 kLux).
The non-inoculated test media remained colourless throughout the course of the study, the inoculated changed to pale green besides the solution of the highest test item concentration, which was also colourless at the end of the incubation period.
No morphological abnormalities were observed in any of the tested concentrations.
The measured concentrations at the start of the test were 0.0826, 0.269, 0.816, 2.67 and 8.19 mg/L, respectively for the 1, 3.2, 10, 32 and 100% saturated solution concentrations. The test item remained relatively stable over the test period, as with exception of the highest concentration (100% saturated solution) all measured test substance concentrations between 24 and 72-hours were within the 80-120% nominal range (101 – 119% of initial measured). As the test concentrations were saturated solutions and the measured concentrations were not maintained at the 100% saturated solution concentration, the study results were based on geometric mean measured concentrations.

Table 10.2.1-41: Test item concentrations

Nominal test item conc. (% saturated solution)	Measured concentration (mg/L)	Recovery (% of initial measured) inoculated			Recovery (% of initial measured) non inoculated	Geometric mean measured concentration (mg/L)
	at 0 hours	at 24 hours	at 48 hours	at 72 hours	at 72 hours	0-72 hours
Control	< LOQ	--	-	-	-	< LOQ
1	0.0826	103	105	101	105	0.0842
3.2	0.269	119	108	104	110	0.289
10	0.816	108	103	105	116	0.849
32	2.67	107	104	105	112	2.77
100	8.19	79.8	69.8	75.6	86.9	6.60

LOQ = 0.01 mg/L

When compared with the control, substantial growth inhibition (>50% based on yield) was only observed at the highest test concentration.

Table 10.2.1-42: Effects of RH-127450 on *Raphidocelis subcapitata* growth rate and yield 72 hours after exposure

Geometric mean measured concentration (mg/L)	% inhibition of growth rate relative to control	% inhibition of yield relative to control
Control	--	--
0.0842	-0.767	-2.419
0.289	-4.850	-16.360
0.849	-1.806	-5.667
2.77	0.916	2.764
6.60	24.375	53.020

Negative values show an increase relative to control

Table 10.2.1-43: 72-hour yield (E_yC_x) and growth rate (E_rC_x) toxicity and no observed effect concentration (NOEC)

Parameter	Toxicity values based on geometric mean measured concentrations (mg/L) Bracketed values present the 95% confidence limits			
	Yield	Average Specific Growth Rate		
	0-72 hour	0-24 hour	0-48 hour	0-72 hour
EC_{10}	2.86 (0.436-3.52)	3.23 (0.570-3.68)	3.40 (1.19-4.13)	3.70 (2.98-4.53)
EC_{20}	3.47 (2.79-4.24)	4.00 (2.43-4.50)	4.69 (3.03-6.32)	5.38 (3.88-7.84)
EC_{50}	5.98 (4.37-NC)	>6.60 (NC)	>6.60 (NC)	>6.60 (NC)
NOEC	2.77	2.77	2.77	2.77

NC = not calculated

All validity criteria were met:

Parameter	Criterion	Observed value
Control cell density increase	Increase by a factor of at least 16	17
Coefficient of variation of average specific growth rates at 72h	≤ 7%	1.38
Mean coefficient of variation for specific growth rates (Individual replicates – 0-24, 24-48, 48-72 hours)	≤ 35%	24.1

Conclusion

Based on the results of a range-finder, three replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-127450 at nominal concentrations of 1, 3.2, 10, 32 and 100 % saturated solution under static conditions over 72 h.
The study results were based on geometric mean measured concentrations.
The EC_{50} for 72-hour yield and average specific growth rate were 5.98 and >6.60 mg/L, respectively. The NOEC for 72-hour yield and average specific growth rate were both 2.77 mg/L.
All validity criteria were met. Therefore, the test was considered valid.

EFSA (2017) requested in its Peer Review Conclusion: "*Further algae studies following the latest OECD 201 guideline are needed or further detailed information on all validity criteria requested by the latest OECD 201 guideline from the studies provided in the RAR for zoxamide, RH-127450 and RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).*" This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

With regard to the EFSA request on alga studies with the parent compound zoxamide: The alga studies with zoxamide which are available in the RAR for zoxamide (2017) have been re-evaluated at a later stage during AIR based on additionally provided information. The results of the re-evaluation were included in the RAR, the alga studies were regarded valid. This was confirmed by Latvia as RMS for zoxamide. As such, the endpoints from the alga studies with the active substance zoxamide available in the RAR (2017) are valid and applicable for the aquatic risk assessment.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_

like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

Based on nominal concentrations:

The 72-hour EyC50 and ErC50 for *Raphidocelis subcapitata* (static test condi-tions) ≥100 mg a.s./L (nom)

The 72-hour NOEC (yield and growth rate) for *Raphidocelis subcapitata* (static test conditions) = 100 mg a.s./L (nom)

Reference:	KCA 8.2.6
Report	Jarrom, R., 2020: RH-163353: Inhibition of growth to the alga *Raphidocelis subcapitata* Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202388, GLP, Not published
Guideline(s):	OECD 201 (adopted 23 March 2006, corrected 28 July 2011)
Deviations:	Additional light measurements were taken at 24 and 48-hours to ensure the light remained within the correct ranges. The maximum temperature recorded during the test was 24.6°C and therefore, this exceeded the protocol range (21 - 24°C). This temperature deviation occurred within the first 45-hour of the test. As the test temperatures were measured at approximately 24-hour intervals it is not possible to confirm the exact length of the out of specification period, however, the temperature was out of specification (actual temperature = 24.4°C) when recorded at ca 21-hours but was back in specification (actual temperature = 23.4°C) when recorded at 45-hours. As the temperature remained within 2°C, the algae growth was good and achieved all the control validity criteria, this protocol deviation was not considered to have an impact on the integrity of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-163353 (HHGCP001-00-2)
Purity	99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)
Species	Alga *Raphidocelis subcapitata* (strain 278/4; formerly known as *Pseudokirchneriella subcapitata)*
Age:	not applicable
Weight:	not applicable
Source:	Culture Collection of Algae and Protozoa (CCAP), SAMS Research Service Ltd., Oban, UK
Acclimation period:	72 hours
Test vessel:	250 mL Erlenmeyer (conical) glass flasks, filled with 100 mL of test

	or control medium, capped with foam bungs, continuously agitated at ~100 rpm
Environmental conditions	
Test medium:	OECD alga nutrient medium (according to Annex 3 of the OECD TG 201)
Temperature:	22.7 – 24.6 °C
pH:	7.94 – 9.79
Light:	Continuous light at 4.61 – 8.72 kLux
Application rate(s)	Range- finding test: 0.10, 1.0, 10 and 100 mg/L under static test conditions Definitive test: 100 mg/L under static test conditions (limit test)
Post exposure observation period	72 hours
Remarks	None

Based on the results of a range-finder, six replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-163353 at a nominal concentration of 100 g/L (limit test) under static conditions over 72 h. A control treatment (6 replicate cultures) was also included in the test. A toxic reference test with potassium dichromate was separately performed in December 2019.
Each test vessel was inoculated with 1×10^4 algae cells/mL. Algae cell counts were conducted at 24-hour intervals. The test item concentrations were analytically verified at 0 and 72-hours. The 0-hour samples were taken from bulk preparations prior to the addition of algae. The 72-hour inoculated samples were taken from pooled inoculated vessels at each treatment.
Concentrations of RH-163353 were determined by treating OECD medium samples with acetonitrile containing 0.5% formic acid, then diluting further with OECD medium: acetonitrile (4:1, v/v) containing 0.1% formic acid as required to bring the response within the calibration range. Samples were analysed by liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method has been validated according to SANCO 3029/99 rev. 4 (Smithers ERS study number 3202388, established analytical procedure SMV 3202388-01V). The limit of quantification (LOQ) was 0.1 mg/L.
RH-163353 is a racemate. The ratio of the two enantiomers was determined in each of the samples and compared with the validated certificate of analysis enantiomeric ratio. A combination of the analytical procedures SMV 3202388-01V and SMV 3202586-01V were used to assess the enantiomeric ratio.
No statistical analysis of the results was performed, as there was no growth inhibition noted compared to the control up to and including the highest test item concentration tested. The reported values have been derived empirically.

Results and discussion

Environmental parameters (pH and temperature) remained within acceptable limits throughout the study. The pH values ranged from 7.94 to 9.79, room temperature ranged from 22.7 to 24.6°C and the light intensity ranged from 4.61 to 8.73 kLux during of the study.
The non-inoculated test media remained colourless throughout the course of the study, the inoculated changed to pale green for both the control and the test item concentration.
No morphological abnormalities were observed.

The analytical results confirmed test substance dosing, as the 0-hour results was within the 80 - 120% nominal range (92.5%). The test item was stable over the test period with a mean measured test substance concentration at the end of the study within the 80 - 120% nominal range (i.e. 105%). Moreover, all the measured test substance concentrations were within the 80 - 120% nominal range. The study results were therefore based on nominal test item concentrations.
The ratio of the two enantiomers of RH-163353 in the 72-hours 100 mg/L sample was within 1.5% (0.3%) of the enantiomeric ratio provided for the test substance in the certificate of analysis.

Table 10.2.1-44: Test item concentrations

Nominal test item concentration (mg/L)	Measured concentration (mg/L)		Recovery (% of initial measured)
	at 0 hours	at 72 hours	at 72 hours
Control	< LOQ	< LOQ	-
100	92.5	96.7	105

LOQ = 0.1 mg/L

When compared with the control, there was no growth inhibition observed at the 100 mg/L treatment. The 72-hour growth rate (ErC_{50}) and yield (EyC_{50}) as well as the corresponding NOEC values were derived empirically.

Table 10.2.1-45: Effects of RH-163353 on *Raphidocelis subcapitata* growth rate and yield 72 hours after exposure

Nominal test item concentration (mg/L)	% inhibition of growth rate relative to control	% inhibition of yield relative to control
Control	--	--
100	-2.596	-13.655

Negative values show an increase relative to control

Table 10.2.1-46: 72-hour yield (E_yC_x) and growth rate (E_rC_x) toxicity and no observed effect concentration (NOEC)

Parameter		Toxicity values based on nominal test item concentrations (mg/L)
		72 hours
Yield	E_yC_{50}	>100
	NOEC	100
Average specific growth rate	E_rC_{50}	>100
	NOEC	100

All validity criteria were met:

Parameter	Criterion	Observed value
Control cell density increase	Increase by a factor of at least 16	114
Coefficient of variation of average specific growth rates at 72h	≤ 7 %	2.09
Mean coefficient of variation for specific growth rates (Individual replicates – 0-24, 24-48, 48-72 hours)	≤ 35 %	8.76

Conclusion

Based on the results of a range-finder, six replicate cultures of the green alga *Raphidocelis subcapitata* (strain 278/4; alga formerly known as *Pseudokirchneriella subcapitata*) in an exponential growth phase were exposed to RH-163353 at a nominal concentration of 100 g/L (limit test) under static conditions over 72 h.

The 72-hour E_yC_{50} and E_rC_{50} values based on nominal concentrations were both >100 mg/L. The 72-hour NOEC values for yield and growth rate were both estimated to be 100 mg/L.

All validity criteria were met; therefore, the test was considered valid.

The endpoints from a *Lemna* study with zoxamide (XXXX, 1998b) were re-evaluated during AIR. The 7-days EC_{50} endpoint (here: 7-days IC_{50} value) of 18 µg a.s./L was regarded as not valid any more due to lack of information and changed to the NOEC of 9.0 µg a.s./L for growth rate. Therefore, a new *Lemna* study with zoxamide technical has been performed and has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

The biomass 7-days ErC50 for *Lemna gibba* = 0.0237 mg a.s./L (nom) (semi-static test conditions)

The biomass 7-days ErC10 for *Lemna gibba* = 0.00709 mg a.s./L (nom) (semi-static test conditions)

The biomass 7-days EyC50 for *Lemna gibba* = 0.0122 mg a.s./L (nom) (semi-static test conditions)

The biomass 7-days EyC10 for *Lemna gibba* = 0.00283 mg a.s./L (nom) (semi-static test conditions)

The biomass and frond number 7-days NOEC for *Lemna gibba* (both yield and growth rate) = 0.00301 mg a.s./L (nom) (semi-static test conditions)

Reference:	KCA 8.2.7
Report	Juckeland, D., 2020: Effects of Zoxamide technical on *Lemna gibba* in a growth inhibition test under semi-static test conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 18 48 ALE 0005, GLP, Not published
Guideline(s):	OECD 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Zoxamide technical (2016051601)
Purity	97.62 % (w/w) ratio of R- and S-isomer of zoxamide: 50.65 : 49.35 (racemate)
Species	Duckweed - *Lemna gibba* L.
Age:	initial colonies consisted of 2 - 4 fronds having a similar size; free from other organisms (algae and protozoa)

Weight:	not applicable
Source:	Purchased from "Institut für allgemeine Botanik" at the University of Jena, Germany, in June 2007 and since then cultivated in the test facility
Acclimation period:	7 days
Test vessel:	glass beaker with a glass lid (volume: 150 mL), filled with 100 mL of test or control medium; 3 fronts/colony or 9 fronts/vessel inserted at study initiation
Environmental conditions	
Test medium:	20X AAP growth medium (according to OECD 221 guideline)
Temperature:	22.6 – 23.2°C (recorded in the water bath)
pH:	7.39 - 7.95
Light:	continuous illumination (on average 111 µE $*m^{-2}*s^{-1}$)
Application rate(s)	0.33, 1.00, 3.01, 9.01, 27.03, 81.05, 243.02 µg/L test item (nominal) under semi-static conditions
Vehicle:	Dimethylformamide (DMF)
Post exposure observation period	7 days
Remarks	None

Three replicate cultures of *Lemna gibba* L. were exposed under semi-static conditions to zoxamide technical (batch no.: 2016051601, analysed purity of 97.62 ± 2.02% w/w or 967.2 ± 20.2 g/kg) at nominal concentrations of 0.33, 1.00, 3.01, 9.01, 27.03, 81.05, 243.02 µg test item/L over a period of 7 days. Dimethylformamide saved as solvent for the active substance. An untreated control and a solvent control were included in the study, with 6 replicate each. A toxic reference test with 3,5-dichlorophenol confirmed the sensitivity of the test system.
Each test vessel was inoculated with 3 fronts/colony or 9 fronts/vessel. During the course of the study frond numbers per vessel and any changes in plant development were observed on days 0, 3, 5 and 7, the dry weight of plants per test vessel was determined on days 0 and 7 of the study.
Specimens were stabilised using methanol (1:1, v/v) and stored deep frozen (≤ -18 °C) for a maximum of 27 days until analysis.
The test item concentrations were analytically verified at test start, at each renewal, and at the end of the test in fresh and spent solutions. The test item concentrations were measured by reversed phase high performance liquid chromatography (RP-HPLC) and mass spectrometric determination with a method validated according to SANCO 3029/99 rev. 4 at an LOQ of 0.168 µg/L zoxamide.
After homogenisation of the specimens, the aquatic medium was diluted 1: 1 (v/v) with methanol and analysed.
The two untreated controls (with and without solvent (vehicle)) were compared by STUDENT's t-test. As a result, there were no statistically significant differences between control and solvent control. Therefore, the solvent control was used for comparison with the treated samples. Regression analysis was performed using individual replicate responses, not treatment group means. From average specific growth rates and yield, recorded in a series of test solutions, effect concentrations of ErC_{10}, ErC_{20} and ErC_{50}, (average specific growth rate) and EyC_{10}, EyC_{20} and EyC_{50} (yield) was determined using concentrations-response modelling (non-linear regression, 3 or 4 parameters Normal CDF = cumulative distribution function). To determine a LOEC and to derive a NOEC for effects on growth rate, it was necessary to compare treatment means using analysis of variance (ANOVA) techniques. Shapiro-Wilk´s Test on Normal Distribution was performed. The mean for each concentration was compared with solvent control means using an appropriate multiple comparison test method. Williams's t-test was used if variance-homogeneity requirements are fulfilled. The Multiple Sequentially rejective Median (2x2-table) test for non-homogeneous variances with Bonferroni-Holm-adjustment was performed. As a test for homogeneity of variances, Levene's test was done. All statistical analysis was performed with ToxRat Professional

version 3.2.1 (02.11.2015).

Results and discussion

Environmental parameters (pH and temperature) remained within acceptable limits throughout the study. The cultures showed a pH of 7.39-7.95 during the course of the study and were exposed at 22.6 – 23.2°C under continuous illumination (111 µE *m-2*s-1).

The measured concentrations of zoxamide remained within a range of 81 – 117% of nominal in the freshly prepared test solutions at test start and at each renewal in the freshly prepared test solutions. The zoxamide concentrations in the spent test solutions were determined at 72 – 131% of nominal at each renewal and at the end of the study (day 7). The calculated study endpoints were based on nominal and geometric mean measured test item concentrations.

Table 10.2.1-47: Test item concentrations

Nominal test item concentration (mg/L)		**Concentration** µg/L test item								
		Control	**Solvent control**	**0.33**	**1.00**	**3.01**	**9.01**	**27.0**	**81.1**	**243.0**
day 0 (fresh)	measured	<LOQ	<LOQ	0.270	1.015	3.177	8.925	26.85	74.24	191.7
	% of nominal a.s.	**-**	**-**	**83**	**104**	**108**	**101**	**102**	**94**	**81**
day 3 (spent)	Measured	<LOQ	<LOQ	0.297	0.893	2.940	8.195	25.84	73.65	187.8
	% of nominal a.s.	**-**	**-**	**91**	**91**	**100**	**93**	**98**	**93**	**79**
day 3 (fresh)	Measured	<LOQ	<LOQ	0.312	1.017	3.423	9.348	27.79	87.09	205.9
	% of nominal a.s.	**-**	**-**	**95**	**104**	**116**	**106**	**105**	**110**	**87**
day 5 (spent)	Measured	<LOQ	<LOQ	0.429	1.050	3.243	10.57	25.20	80.15	170.6
	% of nominal a.s.	**-**	**-**	**131**	**107**	**110**	**120**	**95**	**101**	**72**
day 5 (fresh)	Measured	<LOQ	<LOQ	0.296	1.071	3.182	8.934	28.2	82.11	266.6
	% of nominal a.s.	**-**	**-**	**91**	**109**	**108**	**102**	**107**	**104**	**112**
day 7 (spent)	Measured	<LOQ	<LOQ	0.253	0.896	2.485	8.358	24.07	72.42	224.9
	% of nominal a.s.	**-**	**-**	**77**	**92**	**85**	**95**	**91**	**92**	**95**
geometric mean measured a.s. concentration over 7 days		-	-	0.30	0.98	3.06	8.95	26.29	77.49	203.37
% of nominal a.s.		**-**	**-**	**90**	**98**	**102**	**99**	**97**	**96**	**84**

<LOQ: not detected or detected at concentration below LOQ = Limit of quantification (0.055 µg/L zoxamide)

The two untreated controls (with and without solvent (vehicle)) were compared by Student's t-test. As a result, there were no statistically significant differences between control and solvent control. Therefore, the solvent control was used for comparison with the treated samples.

Table 10.2.1-48: Effects of Zoxamide technical on growth rate and yield for Lemna gibba

Treatment group µg/L test item nominal	**Final frond number** replicate mean day 7	**Biomass (dry weight)** replicate mean day 7 (mg)	**% Inhibition**			
			Average specific growth rate (% I_r)		**yield (% I_y)**	
			frond number	biomass	frond number	biomass
Control	70.3	11.3	-	-	-	-
Solvent control	72.0	11.2	-	-	-	-
0.33	74.3	12.2	-1.5*	-3.1*	-3.7*	-9.0*
1.00	73.3	11.1	-0.9*	0.3	-2.1*	1.0
3.01	71.7	10.4	0.3	3.3	0.5	8.4
9.01	58.7	7.6	9.9 +	15.7 +	21.2 +	34.7 +
27.03	23.7	5.4	53.6 +	28.7 +	76.7 +	55.9 +
81.05	19.7	2.6	62.4 +	57.7 +	83.1 +	83.3 +
243.02	19.0	2.5	64.2 +	58.9 +	84.1 +	83.9 +

* negative values show an increase compared to the solvent control
+ significantly different to the solvent control (Williams t-test; $p \leq 0.05$, one-sided)

No statistically significant effect on the average specific growth rate and yield of *Lemna* based on frond number and biomass was observed at the nominal concentrations ≤ 3.01µg/L test item, whereas statistically significant effects ($p \leq 0.05$) were calculated for nominal concentrations ≥ 9.01 µg/L test item. As a result, the NOEC for average specific growth rate and yield for frond number and biomass was determined to be 3.01 µg/L test item (equivalent to 3.06 µg/L test item/L, mean measured) and the LOEC was determined to be 9.01 µg/L test item (equivalent to 8.95 µg/L test item/L, mean measured), based on nominal concentrations.

The frond number EC-values (0-7 d) were 7.59, 9.70 and 15.5 µg/L test item for growth rate (E_rC_{10}, E_rC_{20} and E_rC_{50}) and 4.52, 7.01 and 16.2 µg/L test item for yield (E_yC_{10}, E_yC_{20} and E_yC_{50}), based on nominal concentrations, respectively.
The frond number EC-values (0-7 d) were 7.57, 9.62 and 15.2 µg/L test item for growth rate (E_rC_{10}, E_rC_{20} and E_rC_{50}) and 4.40, 6.86 and 16.0 µg/L test item for yield (E_yC_{10}, E_yC_{20} and E_yC_{50}), based on mean measured a.s. concentrations, respectively.
The biomass EC-values (0-7 d) were 7.09, 10.7 and 23.7 µg/L test item for growth rate (E_rC_{10}, E_rC_{20} and E_rC_{50}) and 2.83, 4.67 and 12.2 µg/L test item for yield (E_yC_{10}, E_yC_{20} and E_yC_{50}), based on nominal concentrations, respectively.
The biomass EC-values (0-7 d) were 6.93, 10.5 and 23.1 µg/L test item for growth rate (E_rC_{10}, E_rC_{20} and E_rC_{50}) and 2.79, 4.60 and 12.0 µg/L test item for yield (E_yC_{10}, E_yC_{20} and E_yC_{50}), based on mean measured a.s. concentrations, respectively.

Table 10.2.1-49: 7-days LOEC, NOEC and effect concentrations EC_x of zoxamide technical for growth rate and yield based on frond number and biomass for *Lemna gibba*

Effect concentration	Zoxamide, µg/L			
	average specific growth rate inhibition		yield inhibition	
	Frond number	Biomass	Frond number	Biomass
NOEC				
test item, nominal	**3.01**	**3.01**	**3.01**	**3.01**
Zoxamide, mean measured	3.06	3.06	3.06	3.06
LOEC				
test item, nominal	**9.01**	**9.01**	**9.01**	**9.01**
Zoxamide, mean measured	8.95	8.95	8.95	8.95
EC_{10}	**E_rC_{10}**	**E_rC_{10}**	**E_yC_{10}**	**E_yC_{10}**
(95th confidence interval)				
test item, nominal	**7.59**	**7.09**	**4.52**	**2.83**
	(6.33 – 9.11)	(4.13 – 12.2)	(2.64 – 7.75)	(2.31 – 3.48)
Zoxamide, mean measured	7.57	6.93	4.40	2.79
	(6.33 – 9.05)	(4.01 – 12.0)	(2.57 – 7.52)	(2.59 – 3.00)
EC_{20}	**E_rC_{20}**	**E_rC_{20}**	**E_yC_{20}**	**E_yC_{20}**
(95th confidence interval)				
test item, nominal	**9.70**	**10.7**	**7.01**	**4.67**
	(8.19 – 11.5)	(6.42 – 18.0)	(4.19 – 11.7)	(3.87 – 5.67)
Zoxamide, mean measured	9.62	10.5	6.86	4.60
	(8.15 – 11.4)	(6.22 – 17.7)	(4.11 – 11.4)	(4.30 – 4.93)
EC_{50}	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
(95th confidence interval)				
test item, nominal	**15.5**	**23.7**	**16.3**	**12.2**
	(12.7 – 18.9)	(12.5 – 44.1)	(8.66 – 30.2)	(9.80 – 15.3)
Zoxamide, mean measured	15.2	23.1	16.0	12.0
	(12.6 – 18.6)	(12.0 – 43.6)	(8.58 – 29.7)	(11.0 – 13.0)

No chlorotic effects were observed in the control and solvent group during the test. Some chlorotic effects of zoxamide technical were observed at nominal test item concentrations ≥ 27.03 µg/L on day 5 and 7.

According to the guideline, the doubling time of the frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a 7-fold increase in biomass in 7 days and an average specific growth rate of 0.275 d^{-1}. The measured doubling time of the frond numbers in the control was on average 2.36 days (1.9 days for dry weight), corresponding to a 7.8-fold increase in frond number over the 7-day study period (mean of 9 to 70.3 fronds in the control vessels) and a 13.0-fold increase in dry weight (0.867 mg to 11.3 mg dry weight). The average specific growth rate in the control was 0.294 d^{-1} for frond number and 0.367 d^{-1} for dry weight. Therefore, all validity criteria were met in this study.
The E_rC_{50} (growth rate based on frond number) value for the reference item (toxic standard) 3,5 - dichlorophenol was 3.04 mg/L. This value is included in the range 2.2 - 3.8 mg/L 3,5 - dichlorophenol as stated in Guideline ISO 20079, demonstrating that the test system was sensitive.

Conclusion

Three replicate cultures of *Lemna gibba* L. were exposed under semi-static conditions to zoxamide technical at nominal concentrations of 0.33, 1.00, 3.01, 9.01, 27.03, 81.05, 243.02 µg test item/L over a period of 7 days. Dimethylformamide saved as solvent for the active substance.
Since the 0-7 days measured test item concentrations amounted to 84-102 % of nominal and thus were within the required 80-120% range, the study results were based on nominal test item concentrations.
The 7-days E_rC_{50} and E_yC_{50} values based on nominal test item concentrations were 23.7 and 12.2 µg/L.
The 7-days E_rC_{10} and E_yC_{10} values based on nominal test item concentrations were 7.09 and 2.83 µg/L test item. The corresponding 7-days NOEC values were 3.01 µg/L test item for both yield and growth rate.
All validity criteria were met, the study is valid.

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

An additional fish ELS study with sheepshead minnow (XXXX, 1998) is available from the authorisation of zoxamide and its products in the US. The study has been evaluated by US EPA, but not yet by European authorities. It is therefore provided with this submission to complete the picture on chronic toxicity of zoxamide to fish and to be used in the aquatic risk assessment (i.e. for a species sensitivity distribution).

The study has been performed at Wildlife International in the US. To support the report of XXXX (1998), the legal successor of Wildlife International in the US has been asked to re-evaluate the findings with regard to current guidelines based on the report data and the raw data still available in the laboratory. Please refer to the “Final Report Addendum for RH-117,281 Technical: An Early Life-stage Toxicity Test with The Sheepshead Minnow (*Cyprinodon variegatus*)” (Milligan et al., 2020), which is presented in the following together with the summary of the original report findings.

This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the study valid and acceptable.

Agreed endpoints:

The results of the study (XXXX, 1998) were based on mean measured test concentrations:

The 34d NOEC (flow-through test) for *Cyprinodon variegatus* = 0.04 mg a.s./L (mm)

The 34d LOEC (flow-through test) for *Cyprinodon variegatus* = 0.078 mg a.s./L (mm)

The results of the Milligan et al., 2020 study (LC/ECx values estimated based on mean measured concentrations and data from the original study):

The EC10 for *Cyprinodon variegatus* = 0.093 mg a.s./L (fish wet weight)

The EC10 value for the fish dry weight could be estimated but was determined to be equivocal due to a slightly wide 95% confidence interval. Other ECx values could not be reliably estimated.

Reference:	KCA 8.2.2.1
Report:	XXXX, 2020: Final report addendum for RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*) XXXX, Report No. 129A-143A, GLP, Not published
Guideline(s):	OCSPP 850.1400 (2016) SANCO 3029/99 rev. 4 (2000)
Deviations:	No
Acceptability:	Yes
and	
Reference:	KCA 8.2.2.1
Report:	XXXX, 1998: RH-117,281 technical: An early life-stage toxicity test with the sheepshead minnow (*Cyprinodon variegatus*) XXXX, Report No. 97RC-0078 XXXX, Report No. 129A-143A, GLP, Not published
Guideline(s):	850.1500 (1996)
Deviations:	Two sets of Day 0 analytical samples were collected. The first set of samples contained an interference in the chromatography. An additional set of samples was collected and analysed using a direct injection method which removed the interference. The protocol states that method validation which bracelets the concentrations of the study will be conducted and approved by Dr. Sandra Ferris prior to initiation of the definitive. The method was actually validated after test initiation since the first set of samples contained an interference in the chromatography. A new analytical method was developed concurrently with the Day 0 samples. However, formal validation of the method occurred after test initiation. The deviations did not adversely affect the results and the integrity of the study.
GLP:	Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test material (Lot/Batch No.)	RH-117,281 Technical (Lot No. DSR-9510; TD No.95-161)
Active substance content or purity	92.3 % (w/w)
Test organism	sheepshead minnow *(Cyprinodon variegatus)*
Age:	eggs < 24 hours old
Number of test organisms:	80 embryos
Weight:	Biomass loading (the total wet weight of the fish in the negative control replicate A test chamber per liter of test water) at the end of the test was calculated to be 0.034 g of fish per liter of test water that passed through the test chamber during a 24-hour period. Instantaneous loading was 0.22 g of fish per liter of test water in the test chamber at any given time.
Source:	Wildlife International Ltd. cultures Easton, Maryland 21601
Acclimation period:	not relevant
Diet:	Live brine shrimp nauplii *(Artemia sp.)*
Feeding:	Newly-hatched larvae were fed live brine shrimp nauplii *(Artemia sp.)* 3 times per day during the first 7 days post-hatch. On Days 8 through 26 post-hatch, all fish were fed live brine shrimp nauplii 3 times daily on weekdays and at least 2 times daily on weekends. On Day 27 and 28 post-hatch, fish were not fed to allow for clearance of the digestive tracts before weight measurements were made.
Housing:	9-L glass aquaria filled with approximately 7 L of test solution
Environmental conditions	
Temperature:	25 ± 2 °C
Photoperiod:	16 hours light (640 lux), 8 hours dark, with 30 min transition period
Test medium:	filtered salt water (sand filtered 25 µm), particle filter (0.2 µm), UV sterilised
pH:	8.0 – 8.3
Dissolved oxygen:	≥ 64 % of saturation (4.7 mg/L)
Salinity:	20 %
Application rate(s)	0.019, 0.040, 0.078, 0.15 and 0.25 mg a.s./L (flow-through conditions)
Negative control:	filtered salt water solvent control (acetone; 0.10 mL/L)
Post exposure observation period	34 days
Remarks	None

The objective of this study was to determine the effects of a test substance, RH-117,281 (zoxamide) technical (92.3 % w/w pure) on the sheepshead minnow, *Cyprinodon variegatus,* during early life-stage development. Groups of 80 sheepshead minnow embryos were exposed to a geometric series of five test concentrations, a solvent (acetone) control and a negative (filtered saltwater) control for 34 days.
A primary stock solution of RH-117,281 was prepared by dissolving the test substance in acetone at a concentration of 3.00 mg a.s./mL. The primary stock was sonicated and inverted to aid in solubilisation of the test substance. Aliquots were proportionally diluted with acetone to prepare four additional stocks at concentrations of 1.50, 0.750, 0.375 and 0.188 mg a.s./mL. The five working stocks were injected into the diluter mixing chambers (at a rate of 0.0125 mL/minute) where they were mixed with dilution water (at a rate of 125 mL minute) to achieve the desired test concentrations. Acetone was injected into the mixing chamber for the solvent control. Nominal test concentrations used in the study were 0.019, 0.038, 0.075, 0.15 and 0.30 mg a.s./L. The concentration of acetone in the solvent control and all treatment groups was 0.10 mL/L in order to maximise the solubility of RH-117,281 technical in saltwater. All test solutions appeared clear and colorless.
A continuous-flow diluter was used to deliver each concentration of the test substance, a solvent control and a negative (dilution water) control. Syringe pumps (Harvard Apparatus) were used to deliver the five test substance stock solutions and the solvent for the solvent control into mixing chambers assigned to each treatment and the solvent control. The stock solutions were diluted with filtered saltwater in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters. Rotameters were calibrated prior to test initiation and at approximately weekly intervals thereafter. The flow of test water from each mixing chamber was split and allowed to flow into four replicate test chambers. The proportion of the test water that was split into each replicate was checked prior to the test, and at weekly intervals thereafter to ensure that flow rates varied by no more than ±10% of the mean for the four replicates.
The diluter was adjusted so that each test chamber received approximately 6.4 volume additions of test water every 24 hours. The test substance delivery pumps were calibrated before the test. The general operation of the diluter was checked visually at least two times per day during the test and once at the end of the test. The delivery of test substance to test chambers began approximately 53 hours prior to test initiation in order to establish equilibrium of the concentrations of the test substance.
The test chambers were 9-L glass aquaria filled with approximately 7 L of test solution. The depth of the test water in a representative test chamber was approximately 16 cm, they were impartially positioned in a temperature-controlled environmental chamber.
The embryo incubation cups were suspended in the water column of each test chamber and attached to a rocker arm. The reciprocating motion of the rocker arm (approximately 2 rpm) facilitated circulation of test water around the embryos during incubation. The incubation cups were constructed from glass cylinders approximately 50 mm in diameter with 425 J.im Nytex screen mesh attached to the bottom with silicone sealant.
The water used for testing was natural seawater collected at Indian River Inlet, Delaware, and diluted to a salinity of approximately 20% with Wildlife International Ltd. well water. The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank and aerated with spray nozzles. The water again was filtered (0.2 µm) to remove microorganisms and particles. Prior to use, a UV sterilizer was provided as an additional method of water treatment.
Lighting used to illuminate test chambers during culturing and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (Colortone® 50). A photoperiod of 16 hours of light and 8 hours of dark was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity at test initiation was approximately 640 lux over a negative control replicate at the surface of the water. Light intensity was measured using a SPER Scientific Ltd. light meter. Temperature was measured in each test chamber at the beginning and end of the test and at weekly intervals during the test using a liquid-in-glass thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ERIC recorder. The target test temperature during the study was 25±2°C. The pH of the water was measured in alternate replicates at the beginning and end of the test and at weekly intervals during the test. Dissolved oxygen content was measured daily in alternate replicates of each treatment and control group during the first 7 days of the test, at weekly intervals during the test, and at

test termination. Salinity was measured in alternating replicates of the negative control at the beginning of the test, once a week during the test, and at test termination. Measurements of pH were made using a Fisher Accumet Model 915 pH meter and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Salinity was measured using an Aquafauna Bio-Marine, Inc. refractometer.

Sheepshead minnow embryos used in this test were less than 24 hours old and were obtained from a culture maintained by Wildlife International Ltd., Easton, Maryland. Newly-hatched larvae were fed live brine shrimp nauplii (*Artemia sp.*) 3 times per day during the first 7 days post-hatch. On days 8 through 26 post-hatch, all fish were fed live brine shrimp nauplii 3 times daily on weekdays and at least 2 times daily on weekends. On day 27 and 28 post-hatch, fish were not fed to allow for clearance of the digestive tracts before weight measurements were made. Brine shrimp nauplii were obtained by hatching cysts purchased from Bonneville Artemia International Inc., Salt Lake City, Utah. Rations were adjusted each week to account for losses due to mortality and growth of the fish.

Biomass loading (the total wet weight of the fish in the negative control replicate test chamber per liter of test water) at the end of the test was calculated to be 0.034 g fish per liter of test water that passed through the test chamber during a 24-hour period. Instantaneous loading was 0.22 g fish per liter of test water in the test chamber at any given time.

Concentrations of RH-117,281 technical in the test solutions were measured at pretest, days 0, 7, 14,21, 28 and at the end of the test by HPLC-UV with a method validated by the laboratory. Water samples were collected during the test and analyzed for RH-117,281 by HPLC. Water samples were collected from one replicate test chamber of each treatment and control group prior to the test (pre-test) to evaluate diluter performance. Water samples were also collected from one alternating replicate test chamber of each treatment and control group on Days 0, 7, 14, 21, 28 and at test termination. An additional set of samples was collected on Day 0 due to an analytical interference. All water samples were collected from mid-depth of each test chamber. The analytical method and the results of the measurements were verified in an addendum to the report (Milligan et al., 2020).

Observations of mortality were made twice during the first 24 hours of the embryo exposure period and daily until hatch. After hatching, larvae were observed daily to evaluate the number of mortalities. The number of individuals exhibiting clinical signs of toxicity or abnormal behavior during the 28-day post-hatch exposure period were also evaluated. From these observations, hatching success, time to hatch, and post-hatch growth and survival were evaluated. Hatching success was calculated as the percentage of viable embryos that hatched successfully. Post-hatch survival was calculated as the number of larvae surviving to test termination divided by the number of embryos which hatched successfully. Post-hatch growth of the sheepshead minnows was evaluated at the conclusion of the 28-day post-hatch exposure period. Total lengths for each surviving fish were determined with the SIGMA SCANTM scientific measurement system and wet and dry weights were measured using an analytical balance

In the original study, hatching success, post-hatch survival, wet weight, dry weight, and total length of the juvenile fish were test endpoints that were analyzed statistically. Negative and solvent control groups were compared using either Student's t-test or 2X2 contingency tables. When no differences were detected between the two control groups ($p \leq 0.05$), those data were pooled and used to assess treatment-level effects. Survival data were analysed using 2X2 contingency tables and the chi-square test to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the pooled control group. Growth data were evaluated for normality using the Shapiro-Wilks' test and for homogeneity of variance using Bartlett's test. For data with unequal replicate sample sizes which passed both homogeneity of variance and normality tests, the Bonferroni t-test was used to evaluate differences between treatment and control means. The results of the statistical analyses were used to aid in the determination of the NOEC and the LOEC. All statistical tests were performed on a personal computer using SPSSIPC Version 2.0 (5) or TOXSTAT® 3.4 (6) statistical software.

In the addendum to the report (Milligan et al, 2020), the test endpoints were analysed again statistically. The statistical analyses used to estimate the LC/EC_x values were based on the procedures provided in current study guidelines and the OECD (2006). It was performed in the light of approaches in the statistical analysis of ecotoxicity data guidance. It was checked if LC/ECx values could be estimated (e.g., LC_{10}, LC_{20} and LC_{50} for survival (hatching success and post-hatch larval survival); EC_{10}, EC_{20} and EC_{50} for growth). These values were determined from a regression model, and calculations were based on the following conditions: the test concentrations must bracket the LC/ECx so that the LC/ECx comes from

interpolation rather than extrapolation; the LC/ECx was estimated so that (i) the 95% confidence interval reported for LC/ECx does not contain zero and is not overly wide, (ii) the 95% confidence interval for the predicted mean at LC/ECx does not contain the control mean, and (iii) there is no significant lack-of-fit of regression model to the data. The LCx values for hatching success were estimated with the Log-Gompertz model, while the LCx values for post-hatch survival were estimated with the linear interpolation method, using CETIS software (version 1.9.3.0) if possible. The ECx values for the growth (total length, wet weight and dry weight) data were estimated with the Bruce-Versteeg method in CETIS software, when possible. When reliable LCx or ECx values together with their 95% confidence intervals could not be determined, an explanation was provided – in line with data requirements of Commission Regulation (EU) No. 283/2013 and EFSA Technical Report (2019) on general recurring issues in ecotoxicology.

Results and discussion

All water quality measurements remained within acceptable limits throughout the test. Measurements of salinity in the negative control test chambers were consistently 20 parts per thousand. Measurements of pH ranged from 8.0 to 8.3 and showed no obvious difference between control and treatment groups. Dissolved oxygen concentrations remained ≥60 percent of saturation and all temperature measurements were within the desired range of 25 ± 2°C.

Nominal concentrations selected for use in this study were 0.019, 0.038, 0.075, 0.15 and 0.30 mg a.s./L. Mean measured concentrations were 0.019, 0.040, 0.078, 0.15 and 0.25 mg a.s./L. These mean values represent 100, 105, 104, 100 and 83%, respectively, of the nominal test concentrations. No precipitates were observed in the test solutions.

Table 10.2.2-1: Measured test item concentrations

Nominal test concentration mg a.s./L)	Measured concentration (mg a.s./L)						Mean	% of nominal
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 34		
Negative control	< LOQ[1]	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	--	--
Solvent control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	--	--
0.019	0.0183	0.0218	0.0175	0.0200	0.0193	0.0200	0.019	100
0.038	0.0403	0.0405	0.0371	0.0434	0.0390	0.0417	0.040	105
0.075	0.0744	0.0727	0.0791	0.0783	0.0799	0.0816	0.078	104
0.15	0.147	0.126	0.141	0.155	0.153	0.155	0.15	100
0.30	0.242	0.210	0.216	0.209	0.297	0.302	0.25	83

[1] limit of quantification (LOQ) = 0.010 mg a.s./L

Hatching success and time to hatch

Sheepshead minnow embryos either hatched on day 5 or day 6. Hatching success in the negative and solvent control groups averaged 88 and 89%, respectively. There were no statistically significant ($p>0.05$) differences in hatching success between the negative and solvent control groups and the controls were pooled for comparisons among the treatment groups. No statistically significant ($p>0.05$) differences in hatching success existed between the pooled control group and any RH-117,281 treatment group. Daily observations of embryos and newly hatched larvae indicated that there were no apparent differences in time to hatch between the control groups and any of the treatments tested.

Table 10.2.2-2: Hatching success of sheepshead minnow embryos exposed to the test substance

Mean measured test concentration (mg a.s./L)	Repli-cate	No. of eggs exposed	Total number of hatched embryos							No. hatched	Replicate % hatching success[1]	Treatment % hatching success
			Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6			
Negative Control	A	20	0	0	0	0	0	10	18	18	90	88
	B	20	0	0	0	0	0	10	19	19	95	
	C	20	0	0	0	0	0	6	15	15	75	
	D	20	0	0	0	0	0	7	18	18	90	
Solvent Control	A	20	0	0	0	0	0	5	19	19	95	89
	B	20	0	0	0	0	0	6	18	18	90	
	C	20	0	0	0	0	0	6	18	18	90	
	D	20	0	0	0	0	0	5	16	16	80	
0.019	A	20	0	0	0	0	0	5	16	16	80	83
	B	20	0	0	0	0	0	5	17	17	85	
	C	20	0	0	0	0	0	5	18	18	90	
	D	20	0	0	0	0	0	6	15	15	75	
0.040	A	20	0	0	0	0	0	6	15	15	75	81
	B	20	0	0	0	0	0	8	16	16	80	
	C	20	0	0	0	0	0	7	16	16	80	
	D	20	0	0	0	0	0	6	18	18	90	
0.078	A	20	0	0	0	0	0	6	15	15	75	86
	B	20	0	0	0	0	0	6	16	16	80	
	C	20	0	0	0	0	0	5	18	18	90	
	D	20	0	0	0	0	0	10	20	20	100	
0.15	A	20	0	0	0	0	0	10	17	17	85	84
	B	20	0	0	0	0	0	8	16	16	80	
	C	20	0	0	0	0	0	10	18	18	90	
	D	20	0	0	0	0	0	12	16	16	80	
0.25	A	20	0	0	0	0	0	6	14	14	70	80
	B	20	0	0	0	0	0	5	14	14	70	
	C	20	0	0	0	0	0	4	17	17	85	
	D	20	0	0	0	0	0	4	19	19	95	

[2] Percent hatching success = number hatched / number exposed

Clinical observations and post-hatch survival

There were no effects on hatching success or post-hatch survival in any RH-117,281 treatment group and all surviving organisms appeared normal throughout the test.

All fish were observed to determine the number of mortalities. The numbers of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated. Surviving fish in the negative and solvent control groups appeared normal throughout the test. The survival percentages in the negative and solvent control groups during the post-hatch period were 97 and 96%, respectively. There were no statistically significant ($p > 0.05$) differences in post-hatch survival between the negative and solvent control groups and the controls were pooled to evaluate treatment-related effects. All surviving fish in the RH- 117,281 treatments appeared normal throughout the test and no statistically significant ($p > 0.05$), differences in post-hatch survival existed when compared to the pooled control group. During the test, 4 fish were unaccounted for and were assumed dead.

Table 10.2.2-3: Survival of larvae exposed to the test substance for 28 days post-hatch

Mean measured test concentration (mg a.i/L)	Replicate	Initial number of larvae	Approximate live counts[1] (days post-hatch)					Replicate percent survival[2]	Treatment percent survival
			0	7	14	21	28		
Negative control	A	18	18	16	16	16	16	89	97
	B	19	19	19	19	19	19	100	
	C	15	15	15	15	15	15	100	

Mean measured test concentration (m g a.i/L)	Replicate	Initial number of larvae	Approximate live counts[1] (days post-hatch)					Replicate percent survival[2]	Treatment percent survival
			0	7	14	21	28		
	D	18	18	18	18	18	18	100	
Solvent control	A	19	19	19	19	19	19	100	96
	B	18	18	17	17	17	17	94	
	C	18	18	17	17	17	16	89	
	D	16	16	16	16	16	16	100	
Pooled controls	-	141	141	137	137	137	136	-	96
0.019	A	16	16	15	15	15	15	94	97
	B	17	17	16	16	16	16	94	
	C	18	18	18	18	18	18	100	
	D	15	15	15	15	15	15	100	
0.040	A	15	15	15	15	15	15	100	98
	B	16	16	16	16	16	16	100	
	C	16	16	16	16	16	16	100	
	D	18	18	18	18	18	17	94	
0.078	A	15	15	15	15	15	14	93	96
	B	16	16	16	16	16	15	94	
	C	18	18	17	17	17	17	94	
	D	20	20	20	20	20	20	100	
0.15	A	17	17	17	17	17	17	100	99
	B	16	16	16	16	16	15	94	
	C	18	18	18	18	18	18	100	
	D	16	16	16	16	16	16	100	
0.25	A	14	14	14	14	14	14	100	94
	B	14	14	12	12	12	12	86	
	C	17	17	16	16	16	16	94	
	D	19	19	18	18	18	18	95	

[1] Live counts made on days 0 through 21 post-hatch were variable due to the difficulty in counting large numbers of living fish. The counts made on day 28 were exact counts of all fish surviving at the end of the test (i.e., the number of fish weighed and measured).

[2] Percent survival = $\frac{\text{Live counts on day 28 post-hatch}}{\text{Initial number of larvae}} \times 100$

Growth

Growth was evaluated at test termination by measuring the total length, wet weight and dry weight of each surviving fish. Total length, wet weight and dry weight measurements of the negative and solvent control fish were compared using Student's t-test. No statistically significant differences between the two control groups were found ($p>0.05$) for total length, wet weight and dry weight measurements. Therefore, those data were pooled for evaluation of treatment-related growth effects.

There were no apparent differences in fish growth between the pooled control group and the 0.019 and 0.040 mg a.s./L treatment groups. Any differences in mean total lengths, mean wet weights or mean dry weights were slight and not statistically different ($p > 0.05$) from the pooled control group. However, wet weight was reduced in a dose-dependent, statistically significant ($p > 0.05$), manner in the 0.078, 0.15 and 0.25 mg a.s./L treatment groups when compared to the pooled control group. Length and dry weight of sheepshead minnows in the 0.25 mg a.s./L treatment group were also reduced in comparison to the pooled controls ($p > 0.05$). Therefore, the LOEC for growth was 0.078 mg a.s.1L and the NOEC was 0.040 mg a.s./L.

Table 10.2.2-4: Total length, wet weight and dry weight of sheepshead minnow larvae at the end of the 28-day post-hatch observation period

Mean measured test concentration (mg a.s./L)	Total length (mm) Mean (± SD)	Wet weight (mg) Mean (± SD)	Dry weight (mg) Mean (± SD)
Negative control	18.3 ± 1.94	93.8 ± 27.4	20.8 ± 6.1
Solvent control	18.6 ± 1.79	91.9 ± 26.6	20.2 ± 6.0
Pooled controls	18.5 ± 0.38	93.0 ± 3.62	20.6 ± 1.4
0.019	18.4 ± 0.40	88.5 ± 5.76	20.5 ± 1.19
0.040	18.5 ± 0.38	88.8 ± 3.16	19.7 ± 0.97
0.078	17.9 ± 0.53	84.6 ± 8.11*	19.1 ± 1.94
0.15	18.0 ± 0.39	80.3 ± 5.85*	18.6 ± 1.21
0.25	17.5 ± 0.43*	76.5 ± 6.44*	17.3 ± 1.87*

* Indicates a significant difference from the pooled controls using the Bonferroni t-test ($p \leq 0.05$).

Growth was the most sensitive biological factor. The wet weight of sheepshead minnows in the 0.078, 0.15 and 0.25 mg a.s./L treatment groups were statistically reduced ($p \leq 0.05$) when compared to the pooled control group. Length and dry weight of sheepshead minnows in the 0.25 mg a.s./L treatment group were also statistically reduced *(*$p \leq 0.05$) when compared to the pooled control group.
For the study, the no-observed-effect-concentration (NOEC) was 0.040 mg a.s./L, while the lowest-observed-effect-concentration (LOEC) was 0.078 mg a.s./L. The maximum acceptable toxicant concentration (MATC) was 0.056 mg a.s./L (the geometric mean of the NOEC and LOEC). The results of the study were based on mean measured test concentrations.

Table 10.2.2-5: Study results with regard to total length, wet weight and dry weight of sheepshead minnow larvae at the end of the 28-day post-hatch observation period

NOEC	0.040 mg a.s./L
LOEC	0.078 mg a.s./L
Maximum acceptable toxicant concentration (MATC)	0.056 mg a.s./L

In the addendum to the report (Milligan et at., 2020), the EC_{10} value for fish wet weight was estimated to be 0.093 mg a.s./L, with a 95 % confidence interval of 0.054 to 0.14 mg a.s./L. The EC_{20} and EC_{50} values for wet weight were not reportable, since the ECx values were extrapolated above the highest mean measured test concentration and the 95 % confidence intervals were overly wide. The decreases in wet weight in the treatment groups were ≤17 % compared to the pooled control group. The lack of a strong concentration dependent response supports the inability to reliably estimate EC_{20} and EC_{50} values for fish wet weight.

Conclusion

The effects of RH-117,281 technical (zoxamide technical) on the early life stage development of sheepshead minnows *(Cyprinodon variegatus)* were assessed during a 34-day toxicity test. Up to a nominal concentration of 0.30 mg a.s./L (mean measured concentration of 0.25 mg a.s./L, or 83% of the nominal test concentration) were tested. Acetone (100 µL acetone/L) served as a solvent for zoxamide. As a result of the study, no effects from the untreated control and the solvent control were found. As treatment-related effects of zoxamide, statistically significant decreases were evident in total length and fish dry weight at a mean measured concentration of 0.25 mg a.s./L, and a statistically significant decrease in wet weight at mean measured concentrations of 0.078, 0.15 and 0.25 mg a.s./L ($p \leq 0.05$). The effect on wet weight was slight (<10%) in the 0.078 mg a.s./L treatment group, while the effects on all growth endpoints in the 0.25 mg a.s./L treatment group were more pronounced. Based on effects on wet weight, the overall LOEC for the study was identified as 0.078 mg a.s./L (or 78 µg a.s./L).
The NOEC for this study was 0.040 mg a.s./L. The LOEC, based on wet weight, was 0.078 mg a.i/L. The MATC was calculated to be 0.056 mg a.s./L.
The EC_{10} value for fish wet weight was estimated to be 0.093 mg a.s./L, with a 95 % confidence interval of 0.054 to 0.14 mg a.s./L. The EC_{20} and EC_{50} values for wet weight were not reportable, since the ECx

values were extrapolated above the highest mean measured test concentration and the 95 % confidence intervals were overly wide.
The study is regarded valid.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No new data is submitted with this application.

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

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was conducted to OECD 213 and 214 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.1/01
Report:	ACUTE ORAL AND ACUTE CONTACT TOXICITY EFFECTS OF GOW F716 (GWN-10616) TO ADULT WORKER HONEYBEES *APIS MELLIFERA* L., LABORATORY LIMIT TEST, Venturi, S., 2021, report No. BT135/17, Doc. No. 832-001
Guideline(s):	OECD No. 213 (1998), OECD No. 214 (1998)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Executive Summary

The potential acute oral and contact toxicity of test item GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphonate (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances on worker bees (*Apis mellifera*) was determined in the laboratory in a limit test. The 48 hour-LD_{50} for oral and contact toxicity was > 300 µg test item/bee. The NOEC was not statistically determined but is estimated to be ≥ 300 µg test item/bee.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GOW F716 (GWN-10616)
Active substances	Potassium phosphonate and Zoxamide
Description:	Brownish Liquid
Lot/Batch #:	L1704669001
Content of a.s.:	Potassium phosphonate (measured as Phosphonic acid equivalent): 518 g/L, Zoxamide: 64 g/L

2. Vehicle and control:

Control:	50 % (w/v) sucrose solution (oral test), deionised water containing wetting agent (Tween80) (contact test)
Positive control:	Dimethoate

3. Test animals:

Species:	Larval honey bee (*Apis mellifera* L.)
Source:	Healthy colonies maintained at test facility
Age:	Larvae
Test unit:	Disposable and well-ventilated cardboard cages (5.0 x 9.5 x 6.5 cm)
Food	50 % w/v aqueous sucrose solution *ad libitum*

4. Environmental conditions

Temperature:	25.67 – 26.0 °C
Humidity:	65 – 70 %
Photoperiod:	Darkness (except during observations)

B. STUDY DESIGN AND METHODS

1. Experimental phase: 26.06.2017 – 28.06.2017

2. Experimental treatments

The acute oral and contact toxicity of test item GWN-10616 (containing active substances Potassium phosphonate and Zoxamide) to honey bees (*Apis mellifera*) was tested in a laboratory study (limit test design) for 48 hours.

An oral limit test according to OECD Guideline 213 (1998) was performed at single dose of the test item (300 µg test item/bee) dispersed in a 50 % (w/v) aqueous sucrose solution and provided *ad libitum* to honeybees over a period of 4 h. A control group with untreated 50 % (w/v) aqueous sucrose solution and a reference item group exposed to 0.35 µg reference item/bee were tested in parallel. Five replicates with 10 honeybees each were tested for each treatment group. After treatment, the honeybees were fed *ad libitum* with untreated diet.

A limit contact test according to OECD Guideline 214 (1998) was performed where adult worker honeybees were topically exposed to a single dose of the test item (300 µg test item/bee) dissolved in deionised water, by direct application to the thorax (droplets). The wetting agent (Tween80) was used at a concentration of 0.5 % v/v in the preparation. A control group with water and wetting agent and a reference item group at the doses of 0.35 µg reference item/bee were tested in parallel. Five replicates with 10 honeybees each were tested for each treatment group.

3. Observations

Mortality and behavioural abnormalities in all treatments were observed 4, 24 and 48 hours after start of the test.

4. Statistics

NOED was evaluated with Fisher's Exact Binomial Test ($\alpha = 0.05$, one-sided greater). The software ToxRatPro 3.2.1 was used for the statistical analysis.

II. RESULTS AND DISCUSSION

A. Validity criteria

Control mortality in the oral and contact toxicity test was 0 % and 4 %, respectively, and thus not exceeding 10 %. The average, control-corrected mortality in the reference item groups, tested at the dose of 0.35 µg a.s./bee, was 100% at the end of the oral test and 81.25 % at the end of the contact test. Thus,

as all validity criteria were met, the acute oral and the contact toxicity study is regarded as valid.

B. Biological results

In all control groups, mortality ranged between 0 and 4 % after 48 hours. Following oral and contact treatment of 300 µg test item/bee, mortality after 48 hours was 8 % and 0 %, respectively. The 48-hour LD_{50} for both oral and contact toxicity was estimated to be > 300 µg test item/bee (equivalent to > 109.08 µg Phosphonic acid/bee and > 13.47 µg Zoxamide/bee). No sub-lethal effects were recorded in the treatment or control groups. Please refer to Table 10.3.1.1-1 for details.

Table 10.3.1.1-1: Mean mortality of honey bees in the acute oral and contact toxicity test with test item GWN-10616

Treatment Group	Nominal dose [µg test item/bee]	Mean mortality after 48 hours [%]	
		Oral test	Contact test
Control	-	0	4
GWN-10616	300	8	0
Reference item	0.35	100	81.25

III. CONCLUSIONS

The 48 hour-LD_{50} for oral and contact toxicity of the test item GWN-10616 (containing active substances Potassium phosphonate and Zoxamide) on honeybees determined in the laboratory was greater than 300 µg test item/bee.

Comments of zRMS:	The study was conducted to OECD 246 and 247 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable.

Reference: KCP 10.3.1.1/02
Report: ACUTE ORAL AND ACUTE CONTACT TOXICITY EFFECTS OF GWN-10616 TO ADULT WORKER BUMBLEBEES *BOMBUS TERRESTRIS* L., LABORATORY TEST, Venturi, S., 2021, report No. BT210/21, Doc. No. 832-004
Guideline(s): OECD No. 246 (2017), OECD No. 247 (2017)
Deviations: None
GLP: Yes
Acceptability: Yes

Executive Summary

The potential acute oral and contact toxicity of test item GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances on bumblebee (*Bombus terrestris*) was determined in the laboratory in a dose-response test design.

The 48 hour-LD_{50} for oral toxicity was > 3159.4 µg test item/bee (corresponding to 1097.1 µg Potassium phosphite/bee and 134.2 µg Zoxamide/bee, respectively). The NOED was determined to be 1861.2 µg test item/bee.

The 48 hour-LD_{50} for contact toxicity was > 2000 µg test item/bee (corresponding to 694.5.3 µg Potassium phosphite/bee and 87.9 µg Zoxamide/bee, respectively). The NOEC was not statistically determined but is estimated to be ≥ 2000 µg test item/bee.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GWN-10616
Active substances	Potassium phosphite and Zoxamide
Description:	Beige homogeneous and viscous liquid
Lot/Batch #:	P2102669001
Content of a.s.:	Potassium phosphite (measured as Phosphonic acid equivalent): 507 g/L, Zoxamide: 62 g/L

2. Vehicle and control:

Control:	50 % (w/v) sucrose solution (oral test), deionised water containing wetting agent (Triton X-100) (contact test)
Positive control:	Dimethoate

3. Test animals:

Species:	Adult worker bumblebee (*Bombus terrestris* L.)
Source:	Collected from adequately fed, healthy and apparently diseases-free, medium sized bumblebee colonies, having brood at all stages of development and a laying queen, containing 60 - 80 bumblebee workers (BioPlanet S.r.l. – Cesena, Italy).
Age:	Adult worker bumblebees
Test unit:	Nicot® cages were used as single-housing test cages. Each cage is a well-ventilated, semi-transparent, 7-cm long plastic cylinder with a diameter of approx. 2.5 cm: one half is grid-shaped with a hinged lid, the other half is opened with a removable, holed lid in which a syringe (e.g. 1 mL, 5 mL) can be easily inserted.
Food	50 % w/v aqueous sucrose solution *ad libitum*

4. Environmental conditions

Temperature:	24.2 – 24.9 °C
Humidity:	58.4 – 62.3 %
Photoperiod:	Darkness (except during observations)

B. STUDY DESIGN AND METHODS

1. Experimental phase: 19.10.2021 – 27.10.2021

2. Experimental treatments

The acute oral and contact toxicity of test item GWN-10616 (containing active substances Potassium phosphite and Zoxamide) to bumblebee (*Bombus terrestris*) was tested in a laboratory study (dose-response design) for 48 hours.

The acute oral toxicity test in the laboratory was performed as a dose-response test: the test item was diluted in water and then in 50% (w/v) aqueous sucrose solution in a series of five increasing doses (nominal: 250.0, 500.0, 1000.0, 2000.0 and 4000.0 µg test item/bee) and provided to adult worker bumblebees over a period of 4 h. A control group with untreated 50% (w/v) aqueous sucrose solution and a reference item group exposed to nominal dosage of 4.0 µg dimethoate/bee were tested in parallel. Forty bumblebees per group were tested: evaluations of the consumptions were done to identify non-feeder bumblebees in each group, where present. After treatment, the bumblebees were fed *ad libitum* with untreated diet.

The acute contact toxicity test in the laboratory was performed as a dose response test: adult worker

bumblebees were topically exposed to five increasing doses of test item (125.0, 250.0, 500.0, 1000.0 and 2000.0 µg test item/bee) dissolved in water, by direct application to the dorsal thorax (droplets). A control group treated with water and a reference item group exposed to nominal dosage of 10.0 µg dimethoate/bee were tested in parallel. The wetting agent (Triton X-100) was used at a concentration of 0.5 % v/v in the preparation of all the solutions. Thirty bumblebees per group were tested.

3. Observations

Mortality and behavioural abnormalities in all treatments were observed 4, 24 and 48 hours after start of the tests.

4. Statistics

The oral NOED was determined with Chi2 2x2 Table Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) and the contact NOED was determined with Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (one-sided greater, $\alpha = 0.05$). The software ToxRatPro ver. 3.3.0 was used for the statistical evaluation.

II. RESULTS AND DISCUSSION

A. Validity criteria

In accordance with OECD Test Guidelines 246 and 247, control mortality in the oral and contact toxicity test was 5.13 % and 0 %, respectively, and thus not exceeding 10 %. The average, control-corrected mortality of the reference item groups was 97.15% in the oral test and 100% in the contact test.

Thus, as all validity criteria were met, the acute oral and the contact toxicity study is regarded as valid.

B. Biological results

Oral toxicity: Based on the mean food intake per treatment group, the actual test item uptake of the test item group was calculated. The identified non-feeder bumblebees (1 in the control group, 2 in the 250 µg test item/bee group, 4 in the 500 µg test item/bee group, 4 in the 1000 µg test item/bee group, 5 in the 2000 µg test item/bee group, 12 in the 4000 µg test item/bee group and 3 in the reference test item group) were excluded from the calculations of the mean mortalities of each group as well as of the endpoints.

The results of the acute oral toxicity test show no adverse effects of the test item on mortality at the end of the test. No behavioural abnormalities were observed, except for a greater reduction in consumptions of the feeding solution at the highest test item dose, as well as a greater number of non-feeder bumblebees in that group. Based on the results, the 24-hour and 48-hour LD_{50} was estimated to be > 3159.4 µg test item/bee (corresponding to 1097.1 µg Potassium phosphite/bee and 134.2 µg Zoxamide/bee, respectively). The NOED was determined to be 1861.2 µg test item/bee after 24 and ≥ 3159.4 µg test item/bee after 48 hours. Please refer to Table 10.3.1.1-2 for details.

Table 10.3.1.1-2: Mean mortality of bumblebees in the acute oral toxicity test with test item GWN-10616

Dose [µg test item/bee]		Mortality after 24 h [%][2]	Mortality after 48 h [%][2]
nominal	Effective[1]		
0	0	2.56 (n/a)	5.13 (n/a)
125	241.71	2.63 (0.07)	5.26 (0.14)
250	472.31	5.56 (3.08)	5.56 (0.45)
500	969.09	2.78 (0.23)	2.78 (0)
1000	1861.19	8.57 (6.17)	8.57 (3.63)
2000	3159.43	21.43* (19.37)	21.43 (17.18)
4 (Reference)	3.82	97.30 (97.23)	97.30 (97.15)

M = mortality; CM = corrected mortality, using the Abbott's formula (1925) modified by Schneider-Orelli (1947): negative values were replaced with 0.00; n/a = not applicable.
[1]Calculated based on actual consumption.
[2]Control-corrected mortality in parenthesis.
*Significantly different from control (Chi2 2x2 Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$)

The results of the acute contact toxicity test show no adverse effect of the test item on mortality at the end of the test. No behavioural abnormalities were observed. Based on the results the 24-hour and the 48-hour LD_{50} were estimated to be > 2000.0 µg test item/bee. The NOED was determined to be 2000.0 µg test item/bee (corresponding to 694.5.3 µg Potassium phosphite/bee and 87.9 µg Zoxamide/bee, respectively) after 24 and 48 hours. Please refer to Table 10.3.1.1-3 for details.

Table 10.3.1.1-3: Mean mortality of bumblebees in the acute contact toxicity test with test item GWN-10616

Doses [µg test item/bee]	Mortality after 24 h [%]	Mortality after 48 h [%]
0	0	0
125	0	3.33
250	0	0
500	6.67	6.67
1000	3.33	3.33
2000	0	0
10 (Reference)	90	100

III. CONCLUSIONS

The toxicity effects of the test item GWN-10616 to adult worker bumblebees (*Bombus terrestris* L.) after oral or topical exposure, were assessed in a GLP compliant laboratory dose-response test with five doses, respectively. The 48 hour-LD_{50} for oral toxicity was > 3159.4 µg test item/bee (corresponding to 1097.1 µg Potassium phosphite/bee and 134.2 µg Zoxamide/bee, respectively). The NOED was determined to be 1861.2 µg test item/bee. The 48 hour-LD_{50} for contact toxicity was > 2000 µg test item/bee (corresponding to 694.5.3 µg Potassium phosphite/bee and 87.9 µg Zoxamide/bee, respectively). The NOEC was not statistically determined but is estimated to be ≥ 2000 µg test item/bee.

This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, RMS considers the test valid and acceptable.

Agreed endpoints:

Oral test:

The 48 hours LD50 >1802.4 µg consumed Zoxium 240 SC/bumblebee, corresponding to >391.1 µg consumed a.s./bumblebee

The 48 hours NOED ≥ 1802.4 µg consumed Zoxium 240 SC/bumblebee

Contact test:

The 48 hours LD50 >1843.2 µg Zoxium 240 SC/bumblebee, corresponding to >400.0 µg a.s./bumblebee.

The 48 hours NOED ≥ 1843.2 µg Zoxium 240 SC/bumblebee

Reference:	KCA 8.3.1.1
Report	Amsel, K., 2018: Acute toxicity of Zoxium 240 SC to the bumblebee *Bombus terrestris* L. under laboratory conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 17 48 BBA 0017, GLP, Not published
Guideline(s):	OECD 246 (2017) OECD 247 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (18011201-72-52)
Active substance content (measured)	240 g/L Zoxamide (nominal) 21.7 % w/w (analysed)
Species:	*Bombus terrestris* L. (Hymenoptera, Apidae)
Age:	adult bumblebee, workers
Number:	50 replicates with 1 bumblebee each for control(s) and treatment 30 for reference item
Weight:	Not applicable; very small and large bumblebees were excluded from the test by visual inspection
Source:	Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium; delivered from Katz Biotech AG
Acclimation period:	18 hours (and a starving period of 4 hours in the oral toxicity test)
Feeding:	50% (w/v) sucrose solution
Feeding:	During the test (after application) food was provided continuously using a syringe which was set up to the Nicot cage.
Housing**:**	Nicot cages (part of the Nicot queen bee rearing system) with a length of 7 cm and a diameter of 2 cm.
Environmental conditions	
Temperature:	23.4° 24.5 °C
Photoperiod:	constant darkness (diffuse artificial light only during handling and assessments)
Relative humidity:	50 - 64%
Application rate(s)	Oral test: Test item: 1844.0 µg product/bumlebee (400.1 µg a.s./bumblebee) (nominal); 1802.4 µg product/bumlebee (391.1 µg a.s./bumblebee) (mean consumed) Control: Sucrose solution Contact test: Test item: 1843.2 µg product/bumlebee (400.0 µg a.s /bumblebee) Control: Deionised water Vehicle control: 0.5% v/v TritonX
Positive control:	Dimethoate EC 400 (10.0 µg a.s./bee)

Post exposure observation period	48 hours
Remarks	None

The acute toxicity of Zoxium 240 SC (SC formulation containing nominally 240 g/L zoxamide) to the bumblebee *Bombus terrestris* L. has been determined in a laboratory test after oral and contact exposure for 48 hours.
Based on the results of range-finders, the final tests were done as limit tests with a topical application rate of 1843.2 µg Zoxium 240 SC/bumblebee (equivalent to 400.0 µg zoxamide) and a nominal oral application rate of 1844.0 µg Zoxium 240 SC/bumblebee (equivalent to 400.1 µg zoxamide) with a mean consumed oral application rate of 1802.4 µg Zoxium 240 SC/bumblebee (equivalent to 391.1 µg zoxamide). All zoxamide application rates were calculated based on analysed active substance concentrations in the product.
In the contact test, deionised water as well as deionised water with a wetting agent (0.5% (v/v) Triton X) - used as vehicle for the test and reference item - served as controls. In the oral test, the control group was treated with 50% (w/v) sucrose solution. In both tests, Dimethoate EC 400 (containing 429.0 g/L dimethoate analysed content) served as reference item.
In both studies, assessments of mortality and behavioural effects were done after 4, 24 and 48 hours. The statistical calculations were performed with the computer program ToxRat Professional 3.2.1 (2015).
The test item concentrations (ratio of R- and S-zoxamide and sum of isomers) were analytically verified with a method fully validated according to SANCO/3029/99. The determination was conducted by reversed phase – high performance liquid chromatography (RP-HPLC) with DAD-detection. The maximum storage time of deep-frozen stock solutions was < 30 days. Sample extracts were stored for < 24 hours in the refrigerator until analysis.

Results and discussion

Environmental parameters (temperature, light intensity and relative humidity) remained within acceptable limits throughout the study. The bees were exposed at 23.4°- 24.5 °C in the darkness with relative humidity of 50 - 64%.
The test item concentration in the oral and contact toxicity test solutions was analytically confirmed (100% and 98% of the nominal zoxamide concentration was measured in the oral and contact toxicity test, respectively), as well as the 1:1 ratio of the R- and S-isomers of zoxamide in the test item solution.
No active substance was detected in the control samples.

Table 10.3.1.1-4: Mortality and behaviour of bumblebees in the oral toxicity test

Treatment group (dosage unit)	**Dosage applied**	**After 4 hours**				**After 24 hours**				**After 48 hours**			
		Mean mortality (%)	**Behavioural abnormalities**			**Mean mortality (%)**	**Behavioural abnormalities**			**Mean mortality (%)**	**Behavioural abnormalities**		
		Total	**∑**	**A**	**M**	**Total**	**∑**	**A**	**M**	**Total**	**∑**	**A**	**M**
Control	Sucrose	**0.0**	0	0	0	**0.0**	0	0	0	**0.0**	0	0	0
Test item (µg product/ bumblebee)	1802.4	**0**	0	0	0	**0**	0	0	0	**0**	0	0	0
Reference item (µg a.s./ bumblebee)	1.43	**66.7**	10	10	0	**76.7**	7	7	0	**83.3**	5	5	0

Mortality results are averages based on 50/30 replicates consisting of 1 bumblebee each, calculations are performed with non-rounded values
A: affected; M: moribund

In the oral toxicity test, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. In the test item treatment, no mortality occurred after mean oral consumption of 1802.4 µg Zoxium 240 SC within 48 hours. Behavioural effects of bumblebees were not noted in the oral toxicity test.

In the contact toxicity test, no mortality occurred in the control groups treated with deionised water or Triton®X solution. In the test item treatment group, no mortality was seen 48 hours after thoracic application of 1843.2 µg Zoxium 240 SC/bumblebee. Behavioural effects of bumblebees were not noted in the contact toxicity test.

Table 10.3.1.1-5: Mortality and behaviour of bumblebees in the contact toxicity test

Treatment group (dosage unit)	Dosage applied	After 4 hours				After 24 hours				After 48 hours			
		Mean mortality (%)	Behavioural abnormalities			Mean mortality (%)	Behavioural abnormalities			Mean mortality (%)	Behavioural abnormalities		
		Total	∑	A	M	Total	∑	A	M	Total	∑	A	M
Control	Water	**0.0**	0	0	0	**0.0**	0	0	0	**0.0**	0	0	0
	0.5% TritonX	**0.0**	0	0	0	**0.0**	0	0	0	**0.0**	0	0	0
Test item (µg product/ bumblebee)	1843.2	**0**	0	0	0.0	**0**	0	0	0.0	**0**	0	0	0
Reference item (µg a.s./ bumblebee)	10.0	**0.0**	30	30	0	**93.3**	0	0	0	**100.0**	-	-	-

Mortality results are averages based on 50/30 replicates consisting of 1 bumblebee each; calculations performed with non-rounded values
A: affected; M: moribund

The resulting LD_{50} after 48 hours was estimated to be > 1802.4 µg consumed Zoxium 240 SC/bumblebee and the NOED at ≥ 1802.4 µg consumed Zoxium 240 SC/bumblebee after 48 hours. The resulting LD_{50} in the contact toxicity test was > 1843.2 µg Zoxium 240 SC/bumblebee with the NOED ≥ 1843.2 µg Zoxium 240 SC/bumblebee after 48 hours.

Table 10.3.1.1-6: LD_{50}-values of the contact and oral toxicity test

LD_{50}	Contact test		Oral test[1]	
	24 h	48 h	24 h	48 h
LD_{50} [µg product/bumblebee]	> 1843.2	> 1843.2	> 1802.4	> 1802.4
LD_{50} [µg a.s./bumblebee]	> 400.0	> 400.0	> 391.1	> 391.1

[1] Doses of the oral toxicity are referring to mean consumed dose

All validity criteria of the tests were met:
- The mean mortality in the reference item treatments was ≥ 50% in the contact and the oral test (i.e. 100.0 and 83.3%, respectively) after 48 hours.
- The mean control mortality was ≤ 10% (here: 0%) in the control groups.

Conclusion

The acute toxicity of Zoxium 240 SC (240 g/L Zoxamide SC formulation) was determined after contact and oral exposure to bumblebees. Based on the results of range-finders, the main tests were performed as limit tests. As a result, the LD_{50} in the contact test was obtained at > 1843.2 µg Zoxium 240 SC/bumblebee (equivalent to > 400.0 µg zoxamide) and the NOED at ≥ 1843.2 µg Zoxium 240 SC/bumblebee (equivalent to ≥ 400.0 µg zoxamide) after 48 hours. The LD50 in the oral test was > 1802.4 µg consumed Zoxium 240 SC/bumblebee (equivalent to > 391.1 µg zoxamide) and the NOED after 48 hours was ≥ 1802.4 µg consumed Zoxium 240 SC//bumblebee (equivalent to ≥ 391.1 µg zoxamide) after 48 hours. The test item concentrations were analytically confirmed with a method fully validated according to SANCO/3029/99, as well as the 1:1 ratio of the zoxamide isomers in the test item solutions. All zoxamide application rates were calculated based on analysed active substance concentrations in the product (i.e. 21.7% w/w).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

See KCP 10.3.1.1.1 above.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted to OECD 245 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.2/01
Report:	CHRONIC ORAL EFFECTS OF GOW F716 (GWN-10616) TO ADULT WORKER HONEYBEES *APIS MELLIFERA* L. 10-DAY FEEDING LABORATORY TEST, Colli, M., 2021, report No. BT147/17, Doc. No. 832-002
Guideline(s):	OECD No. 245 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Executive Summary

The chronic oral effects of the test item GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphonate (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances on adult worker honeybees *Apis mellifera* were tested in a laboratory study according to the OECD guideline 245 in a 10-day feeding test.

Apis mellifera (max. 2-day-old adults) were exposed to test item GWN-10616 at concentrations of 228.8, 457.5, 915.0, 1830.0 and 3660.0 mg test item/kg 50 % sucrose feeding solution (corresponding to calculated mean doses of 9.97, 19.72, 41.33, 71.3 and 111.64 µg test item/bee/day) during 10 days.

The 10-day NOAEDD and LDD_{50} values for survival were determined to be 71.303 and 137.08 µg test item/bee/day, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GOW F716 (GWN-10616)
Active substances	Potassium phosphonate and Zoxamide
Description:	Brownish liquid
Lot/Batch #:	L1704669001
Content of a.s.:	Potassium phosphonate measured as Phosphonic acid equivalent: 518 g/L, Zoxamide: 64 g/L

2. Vehicle and control:

Control:	Deionised water
Positive control:	Dimethoate

3. Test animals:

Species:	Honeybee (*Apis mellifera* L.)
Source:	Test facility cultures

Age:	Max. 2 days old adult worker bees
Number of organisms:	3 replicates with 10 bees per replicate
Feeding:	50% w/v aqueous sucrose solution *ad libitum*
Test vessel:	Disposable and well-ventilated cardboard cages (5.0 x 9.5 x 6.5 cm)

4. Environmental conditions

Temperature:	29.5 – 33.0°C (average = 32.4°C)
Humidity:	39.5 – 61.6% (average = 54.0%)
Photoperiod:	Darkness (except during observations and renewal of diets)

B. STUDY DESIGN AND METHODS

1. Experimental phase: 21.06.2017 – 01.07.2017

2. Experimental treatments

The 10-day chronic oral feeding test in the laboratory was performed as a dose-response test: the test item GWN-10616 (containing active substances Potassium phosphonate and Zoxamide) was dissolved in water and subsequently diluted, and each water solution was used to make up a treated 50 % (w/v) aqueous sucrose solution at five increasing concentrations (228.8, 457.5, 915.0, 1830.0 and 3660.0 mg test item/kg feeding solution). The treated feeding solutions were prepared freshly every day and administered to the bees for a period of 10 days (from Day 0 to Day 9 of the test). The reference item Dimethoate was tested at 1 mg a.s./kg diet. An untreated control was run in parallel with 50 % (w/v) aqueous sucrose solution.

3. Observations

Mortality and sub-lethal effects were recorded every 24 ± 2 h, from Day 1 to Day 10 of the test. The amount of feeding solution consumed was determined by weighing separate feeders at the start and at the end of each 24-h period of feeding.

4. Analytics

The content of the active substances in samples of test solutions was determined by LC/MS-MS. The highest and the lowest concentrations of the test item in stock and feeding solutions, prepared on Day 0, were analysed.

5. Statistics

NOEDD/NOEC evaluated with Step-Down Cochran-Armitage Test Procedure Test ($\alpha = 0.05$, one-sided greater). LDDx/LCx was evaluated with probit analysis using linear max. likelihood regression. The software ToxRatPro 3.3.0 was used for the statistical analysis.

II. RESULTS AND DISCUSSION

A. Validity criteria

The test is considered to be valid in accordance with the OECD guideline as the cumulative control mortality was less than 15 % (actual 3.3 %) and the mortality in the reference item group was greater than 50 % (actual 100 %).

B. Analytical results

The analytical results demonstrate that the Phosphonic acid and Zoxamide content in the stock solutions prepared on Day 0 at the highest and lowest concentrations was in the range of ± 20% of nominal concentrations. The results of the stock solution analysis are presented in Table 10.3.1.2-1.

Table 10.3.1.2-1: Mean recovery of active substances Zoxamide and Phosphonic acid in the lowest and highest stock and feeding solutions for the diet in the 10 d chronic oral feeding test with honeybee (*Apis mellifera*) exposed to GWN-10616

Day	Nominal concentration [mg a.s./L] Phosphonic acid	Mean Recovery [%]	Nominal concentration [µg a.s./L] Zoxamide	Mean Recovery [%]
0	331.967 (stock solution)	81.30	4.099 (stock solution)	89
	530.856 (stock solution)	88.12	6.555 (stock solution)	88
	83.26 (feeding solution)	93.72	10.273 (feeding solution)	87
	1327 (feeding solution)	99.43	164.334 (feeding solution)	91

C. Biological results

Based on feeding syringe weights recorded at the beginning and end of each exposure day, the mean amount of consumed diet was 30.5 – 45.17 mg/bee/day for the test item treatment groups, respectively (Table 10.3.1.2-2). Based on the overall mean consumption rates, the bees in the adjusted test item treatment groups consumed 9.97, 19.72, 41.33, 71.3 and 111.64 µg test item/bee/day, respectively, over the 10-day test period.

The results of the control group showed low mortality (3.3 %). Behavioural effects were not observed in the control. The results of the reference item group indicated that the test system was sensitive to harmful substances (mortality: 100 % on day 7). The test item GWN-10616 had significant lethal effects on adult honeybees after being administered for 10 consecutive days at the concentration of 3660 mg test item/kg diet, corresponding to the dose of 111.635 µg test item/bee/day.

Table 10.3.1.2-2: Mean food uptake and cumulative mortality in the 10 d chronic oral feeding test with honeybee (*Apis mellifera*) exposed to GWN-10616

Concentration [mg test item/kg feeding solution]	Mean uptake [1]		Cumulative mortality [%]
	Feeding solution [mg diet/bee/day]	Dose [µg test item/bee/day]	
Control	44.59	-	3.3
228.75	43.59	9.972	0
457.50	43.11	19.721	0
915.00	45.17	41.331	10
1830.00	38.96	71.303	3.3
3660.00	30.50	111.635	46.7*
1.00 (Reference)	14.10	0.014	100

[1] Adjusted for evaporation from the feeders;
* = statistically significant difference to control group (Step-down Cochran-Armitage Test Procedure (α = 0.05, one-sided greater).

The NOEC and the NOED (evaluated by the Step-down Cochran-Armitage test) were 1830 mg test item/kg diet and 71.303 µg test item/bee/day, respectively. In terms of dose (related to the mean food consumption and considering evaporation), the LDD_{50} was calculated as 137.08 µg test item/bee/day; in terms of concentration, the LC_{50} was calculated as 4657.15 mg test item/kg diet (LDD/LC were evaluated by Probit analysis).

III. CONCLUSIONS

The effects of GWN-10616 on adult worker honeybees (*Apis mellifera* L.) were assessed in a 10-day oral chronic test. The 10-day survival NOAED and LD_{50} values were determined to be 71.303 µg test item/bee/day and 137.08 µg test item/bee/day, respectively.

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 OECD 239 and according to the principles of GLP. No deviations to the guideline were noted. All validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.3/01
Report:	EFFECTS OF GOW F716 (GWN-10616) TO HONEYBEES (*APIS MELLIFERA* L.), IN A LARVAL TOXICITY TEST FOLLOWING REPEATED EXPOSURE, Colli, M., 2021, report No. BT133/17, Doc. No. 832-003
Guideline(s):	OECD No. 239 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Executive Summary

The potential effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances applied at 5 dose rates (25 – 400 g test item/larva) on the larval development and subsequent adult emergence of honeybees (*Apis mellifera* L.) were measured in the laboratory via oral exposure over a period of 22 days according to OECD GD No. 239 (2016). The results were compared to a water treated control and to a reference item. Assessments on mortality and any developmental/behavioral abnormality were performed from D4 to D8 and on D15 and on D22. The pupal mortality and the adults' emergence rate on D22 were also assessed.

Under worst case laboratory conditions, the test item GWN-10616 caused statistically significant mortality to larvae on D8 (developmental period) at the two highest tested doses. Therefore, the NOED for larvae on D8 was determined to be 100 µg test item/larva (4.49 µg Zoxamide/larva and 36.36 µg Phosphonic acid/larva) equivalent to a NOEC of 649.35 mg test item/kg diet (29.16 mg Zoxamide/kg diet and 236.10 mg Phosphonic acid/kg diet). Regarding the effects on adult emergence on D22, the test item GWN-10616 caused statistically significant reduction in emergence rate with respect to the control only at the highest tested dose. The NOED and the NOEC for adult emergence rate were determined to be 200 µg test item/larva (8.98 µg Zoxamide/larva and 72.72 µg Phosphonic acid/larva) and 1298.70 mg test item/kg diet (58.31 mg Zoxamide/kg diet and 472.21 mg Phosphonic acid/kg diet), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GOW F716 (GWN-10616)
Active substances	Potassium phosphite and Zoxamide
Description:	Brownish Liquid
Lot/Batch #:	L1704669001
Content of a.s.:	Potassium phosphite (measured as Phosphonic acid equivalent): 518 g/L, Zoxamide: 64 g/L

2. Vehicle and control:

Control:	Ultrapure water
Positive control:	Dimethoate

3. Test animals:

Species:	Larval honey bee (*Apis mellifera* L.)
Source:	Test facility colonies
Age:	First instar (< 24 hours old)
Number of organisms:	3 replicates and 12 larvae per replicate (36 larvae per group)
Feeding:	Aqueous sugar solution mixed with royal jelly, varying with developmental stage. Day 1 = Diet A (50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose). Day 3 = Diet B (50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose). Days 4 to 6 = Diet C (50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose).
Test vessel:	48-well plates

4. Environmental conditions

Temperature:	33.9 – 35.0°C (average = 34.5°C)
Humidity:	from D1 to D8 = 91.8 % (average measured) from D8 to D15 = 83.8 % (average measured) from D15 to D22 = 72.7 % (average measured) Short term deviations from the specified range occurring for ≤ 2 hours per day (e.g. during the assessments)
Photoperiod:	Darkness (except during observations and renewal of diets)

B. STUDY DESIGN AND METHODS

1. Experimental phase 12.07.2017 – 31.07.2017

2. Experimental treatments

The test was performed with 5 doses of test item GWN-10616 at nominal doses of 25, 50, 100, 200 and 400 µg test item/larva corresponding to 162.34, 324.68, 649.35, 1297.7 and 2597.4 mg test item/kg diet, respectively. Control and reference item group (7.39 µg a.s./larva) were concurrently run. Each group contained 3 replicates (containing 12 larvae, for a total of 36 larvae per test group).

In the laboratory, the larvae were reared in crystal polystyrene grafting cells. Each cell was placed into a well of a 48-well plate. The top of the grafting cell was maintained at the level of the plate. From D1 to D8, these plates were placed into a hermetic Plexiglas desiccator and kept at a relative humidity of 95 % ± 5 %. The desiccator was placed into an incubator with a forced air circulation system at 34 - 35°C in the dark. At D8, the dental rolls were removed and the relative humidity was reduced to 80 % ± 5 %. The desiccator was placed in a ventilated incubator at 34 – 35°C in the dark. From D15 to D22, the test system was maintained at 50 – 80 % relative humidity. Larvae were exposed to treated royal jelly diet on day 3, 4, 5 and 6.

At each treatment day, the test item was dissolved in ultrapure water to prepare the stock solution and dilutions of the stock solution. The solutions were then further used to prepare the treated diets using 35.0 µL of the relevant water solution per 1000 mg larval diet. The total amount of test item treated diet administered to the larvae from D3 to D6 was 140 µL equivalent to 154 mg. The treated diets (feeding solutions) were prepared daily and warmed in an incubator before use. The reference item stock solution was prepared in ultrapure water once for the experimental and stored at about 2°C. All larvae on one plate received the same treatment. On each feeding day, the position of the plates was changed within the desiccator to ensure eliminating potential spatial bias.

3. Observations

Assessments on mortality and any developmental/behavioral abnormality were performed from D4 to D8 and on D15 and on D22. The pupal mortality and the adults' emergence rate on D22 were also assessed.

4. Analytics

On day D3, day D4, day D5 and day D6, samples of the up water solutions (at lowest and highest concentrations, S1 and S5) were frozen at ≤ -20°C until analysed for the determination of the actual concentrations of the test chemical.

5. Statistics

The NOEC/NOED of the test item were evaluated using a Step-down Cochran Armitage test at day 8 and using a Chi2 2x2 table test with Bonferroni correction at day 22 (one-sided greater, $\alpha = 0.05$). The LD/LCx values (larval mortality) of the test item at day 8 were determined with probit analysis. The $ED/EC_{10/20}$ values (emergence) of the test item at day 22 were determined with Weibull analysis. ED/EC_{50} values at D22 were estimated to be higher than the highest dose tested due to the lack of effects ≥ 50 %. The software ToxRatPro 3.3.0 was used for the statistical analysis.

II. RESULTS AND DISCUSSION

A. Validity criteria

The test is considered to be valid in accordance with the OECD guideline as the cumulative control mortality was less than 15 % (actual 2.8 % for control) and the larval mortality in the reference item group was greater than 50 % (actual 100 %). The adult emergence rate on D22 in the control plates was greater 70 % (actual 86.11 %).

B. Analytical results

The analytical results demonstrate that the Phosphonic acid and Zoxamide content in the stock solutions prepared on D3, D4, D5 and D6 at the highest and lowest concentrations was in the range of ± 20% of nominal concentrations. The results of the stock solution analysis are presented in Table 10.3.1.3-1.

Table 10.3.1.3-1: Mean recovery in the lowest and highest stock solutions for the diet in the 22 d *in vitro* exposure of honeybee larvae (*Apis mellifera*) to GWN-10616

Day	Nominal concentration [mg a.s./L] Phosphonic acid	Mean Recovery [%]	Nominal concentration [mg a.s./L] Zoxamide	Mean Recovery [%]
3	1705	110.83	210.5	84.39
	27270	104.77	3368	83.84
4	1705	109.43	210.5	85.58
	27270	108.31	3368	92.25
5	1705	116.40	210.5	98.72
	27270	93.01	3368	100.42
6	1705	103.0	210.5	100.62
	27270	114.04	3368	99.79

C. Biological results

The qualitative observations carried out during the test (e.g. larval and pupal behaviour and morphological differences) did not show abnormalities in the survived treated bees.

The mean larval mortality (day 8) was 2.78, 5.56, 2.78, 11.11, 19.44 and 25.0 0 % in the control, 25, 50, 100, 200, and 400 µg test item/larva treatment groups, respectively. Step-down Cochran-Armitage test determined a significant reduction in larval survival among honey bees exposed to doses > 100 µg test item/larva compared to the control. Therefore, the 8-day NOED value for honey bees was determined to be 100 (equivalent to 4.49 µg Zoxamide/larva and 36.36 µg Phosphonic acid/larva) equivalent to a NOEC

of 649.35 mg test item/kg diet (29.16 mg Zoxamide/kg diet and 236.10 mg Phosphonic acid/kg diet). The 8-day LD_{50} value was estimated to be > 400 µg test item/larva.

The mean mortality (day 22) was 13.89, 13.89, 16.67, 16.67, 27.78, and 44.44 % in the control, 25, 50, 100, 200, and 400 µg test item/larva treatment groups, respectively. Step-down Cochran-Armitage test determined significant reduction in pupal survival among honey bees exposed to the highest test item dose of 400 µg test item/larva compared to the control. Adult emergence was also significantly reduced at this test dose. Therefore, the 22-day NOED and NOEC for adult emergence rate were determined to be 200 µg test item/larva (8.98 µg Zoxamide/larva and 72.72 µg Phosphonic acid/larva) and 1298.70 mg test item/kg diet (58.31 mg Zoxamide/kg diet and 472.21 mg Phosphonic acid/kg diet), respectively. The 22-day ED_{10}, ED_{20} and ED_{50} values for adult emergence were estimated to be 156.58, 254.34 and > 400 µg test item/larva, respectively.

The mortality rates for larvae and pupae as well as the adult emergence rate are summarised in Table 10.3.1.3-2.

Table 10.3.1.3-2: Mortality of honeybee (*Apis mellifera*) larvae, pupae and adults and adult emergence

Cumulative dose [µg test item/larva]	Concentration [mg test item/ kg diet]	Cumulative mortality [%]				Adult emergence on D22 [%]
		Day 8	from D8 to D15[1]	from D8 to D22[2]	D22	
Control	0	2.78	11.43	11.43	13.89	86.11
25	162.34	5.56	5.88	8.82	13.89	86.11
50	324.68	2.78	2.86	14.29	16.67	83.33
100	649.35	11.11	0	6.25	16.67	83.33
200	1298.7	19.44*	0	10.34	27.78	72.22
400	2597.4	25.0*	14.81	25.93	44.44*	55.56*
Reference	48.0	100	n.a.	n.a.	n.a.	n.a.

[1]calculated in percentage comparing the number of dead pupae from D8 to D15 to the number of alive pupae on D8
[2]calculated in percentage comparing the number of dead pupae from D8 to D22 to the number of alive pupae on D8
* Significantly reduced compared to the control, based on Step-down Cochran-Armitage test $\alpha = 0.05$, one-sided greater
n.a. = not applicable

Following exposure to dimethoate, 100 % mortality was observed among the larvae exposed to the reference substance, demonstrating that this result was consistent with the expectations within the study guideline.

III. CONCLUSIONS

The effects of the test item GWN-10616 on the larval development and subsequent adult emergence of honeybees (*Apis mellifera*), were tested in a laboratory study. Regarding the effects on larvae on Day 8 (developmental period), the test item GWN-10616 caused statistically significant mortality at the two highest tested doses. Therefore, the NOED for larvae on Day 8 was determined to be 100 µg test item/larva. Regarding the effects on adult emergence on Day 22, the test item GWN-10616 caused statistically significant reduction in emergence rate with respect to the control only at the highest tested dose. The 22-day ED_{10}, ED_{20} and ED_{50} values for adult emergence were estimated to be 156.58, 254.34 and > 400 µg test item/larva, respectively. The NOED for adult emergence rate was determined to be 200 µg test item/larva.

EFSA (2017) requested *"Further information to address the risk to bee larvae (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

RMS considers the study valid and acceptable.

Agreed endpoints:

LD50 for *Apis mellifera* L. > 110 µg a.s/larvae (larval and pupal survival, adult emergence and adult weight at emergence)

NOED for *Apis mellifera* L. = 110 µg a.s./larvae (larval and pupal survival, adult emergence)

NOED for *Apis mellifera* L. = 49 µg a.s./larvae (adult weight at emergence)

Reference:	KCA 8.3.1.3
Report:	Picard, Ch. R., 2018: Zoxamide: Honey bee (*Apis mellifera* L.) larval toxicity, repeated exposure Exigent LLC, A Gowan Group Company, USA Smithers Viscient, USA, Report No. 12791.6307, GLP, Not published
Guideline(s):	OECD 239 (2016)
Deviations:	The protocol states that the cell culture plates will be located within a larval box inside the incubator and that the larval box assists in maintaining a relative humidity ≥90%. On test day 7 (16 July 2018), the humidity in the larval plates was recorded at 84% during a 15-minute interval. The decrease in humidity coincided with documented routine maintenance of the surrogate larval box where the temperature was monitored. During this maintenance it was necessary to disconnect the HOBO data logger probe, which likely resulted in the low humidity reading. The test itself was conducted in an identical larval box that had not been disturbed and it can be surmised that the relative humidity was maintained ≥90% for the duration of the larval stage. In addition, the control groups met the referenced acceptability criteria, the decrease in humidity was short in duration and well within the tolerance limits for the test organism. Therefore, this deviation is considered to not impact the integrity of the study and its results.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Zoxamide tech. (2015081302)
Active substance content or purity	99.1 % (w/w)

<table>
<tr><td>Species</td><td>Apis mellifera</td></tr>
<tr><td>Age:</td><td>≤ 24 hours old larvae</td></tr>
<tr><td>Number of larvae:</td><td>36 larvae (3 replicates of 12 larvae each)</td></tr>
<tr><td>Source:</td><td>Wood´s Beekeeping Supply, Lincoln, Rhode Island</td></tr>
<tr><td>Acclimation:</td><td>2 days</td></tr>
<tr><td>Diet:</td><td>The deionised water used in the diet preparation was boiled and then cooled to room temperature before use. Three untreated diets were prepared prior to test initiation and were stored frozen until use. The diets were prepared as follows:
<table>
<tr><th>Component (g)</th><th>Diet A[a]</th><th>Diet B[b]</th><th>Diet C[c]</th></tr>
<tr><td>Deionised water</td><td>800</td><td>800</td><td>720</td></tr>
<tr><td>D-glucose[d]</td><td>95</td><td>120</td><td>220</td></tr>
<tr><td>D-fructose[d]</td><td>95</td><td>120</td><td>220</td></tr>
<tr><td>Yeast extract[de]</td><td>16</td><td>24</td><td>48</td></tr>
<tr><td>Royal jelly[f]</td><td>800</td><td>800</td><td>1200</td></tr>
</table>
[a] Diet fed on day 1
[b] Used for treated diet fed on exposure day 3
[c] Used for treated diet fed on exposure days 4, 5 and 6
[d] Supplier: Sigma Aldrich, Saint Louis, Missouri
e Yeast extract is made from Saccharomyces cerevisiae species of yeast
[f] Supplier: Stakich, Inc., Troy, Michigan</td></tr>
<tr><td>Test system</td><td><u>larval test vessels:</u> sterile, 48-well cell culture plates (1.6 mL/well; Corning) containing a plastic queen cup grafting cell (Mann Lake) in 32 wells during acclimation and in 18 wells during exposure; the perimeter wells within each plate not containing larvae partially filled with deionized water to assist in maintaining the relative humidity at >90%
<u>pupation plates:</u> sterile, 24-well cell culture plates (3.4 mL/well; Corning), each containing two layers of sterilized dust-free Kimwipes</td></tr>
<tr><td>Environmental conditions</td><td></td></tr>
<tr><td>Temperature:</td><td>Larval phase (days 1-8): 31° - 34°C
Pupal phase (days 9-22): 32° - 34°C</td></tr>
<tr><td>Photoperiod:</td><td>near darkness, laboratory lighting only for approximately 30 minutes each day during observations and renewal of the diet</td></tr>
<tr><td>Relative humidity:</td><td>Larval phase (days 1-8): 95% - 99% (once 84%, see study plan deviation)
Pupal phase (days 9-22): 68% - 78%</td></tr>
<tr><td>Application rate(s)</td><td>38, 78, 150, 300 and 610 µg a.s./g (nominal diet concentration)
36, 67, 150, 300 and 640 µg a.s./g (mean measured diet concentration)
5.9, 11, 24, 49, and 110 µg a.s./larva (calculated dose)</td></tr>
<tr><td>Negative control:</td><td>deionised water
solvent control (acetone)</td></tr>
<tr><td>Positive control:</td><td>Dimethoate</td></tr>
<tr><td>Test duration</td><td>22 days</td></tr>
<tr><td>Remarks</td><td>None</td></tr>
</table>

The objective of this study was to evaluate the effect of zoxamide technical to honey bee larvae and successive pupae survival, adult emergence, and adult weight in an artificial *in vitro* testing design. The

22-day exposure of larvae to zoxamide treated diet was initiated on day 3 and continued through day 6, resulting in both dermal and oral exposure until pupation (typically day 7 to 8).
First instar larvae were transferred to 48-well plates for a 2-day acclimation phase and then exposed to zoxamide during four days of the larval treatment phase (days 3, 4, 5, and 6) as part of the 22-day period including larval, pupal, and adult stages. The test was conducted in near total darkness. Organisms were Only exposed to laboratory lighting for approximately 30 minutes each day during observations and diet renewal. Larvae were kept in an incubator at 33 ± 2 °C with a relative humidity of ≥90%.
Representative samples of the royal jelly diet, comprised of water and royal jelly and additional ingredients, were analysed periodically to ensure that they are free of toxic concentrations of PCBs, toxic metals, common pesticides, and antibiotics.
Based on the results of a range-finder, nominal cumulative test item concentrations of 38, 78, 150, 300, and 610 µg a.s./g diet, equivalent to doses of 6.3, 13, 25, 50, and 100 µg a.s./larva, a negative control, and a solvent (acetone) control were selected for the definitive exposure.
For test item treatment, a 150 mg a.s./mL primary stock solution was prepared by dissolving 15.0985 g of zoxamide (14.9626 g pure active ingredient) in 100 mL of acetone as solvent. This stock solution was used to prepare the treated royal jelly diets. The solvent control diet contained an equivalent amount of untreated acetone (0.48%) as each treated diet. Untreated diet was used for the negative control. An 8-day reference item test was included in the test and was conducted concurrently with the definitive exposure using larvae obtained from the same hives as those used to initiate the definitive exposure. Dimethoate served as reference item since it is known to be toxic to the honey bee larvae.
On day 3, individual larvae in all plates were fed by adding 20 µL of the appropriate treated diet B to each cell; on days 4, 5, and 6, respectively, larvae in all plates were fed by adding 30, 40, and 50 µL of the appropriate treated diet C to each cell. Larvae that were observed to completely consume their diet on day 7 or 8 were transferred to the appropriate labelled pupation plates; larvae that did not consume the entire diet were considered dead and not transferred to pupation plates. The number of replicates with uneaten diet was recorded. The health of the larvae was observed and recorded daily. Mortality of a larva was defined by lack of movement.
Upon transfer to the pupal plates, the plates were maintained within an incubator at the same temperature with as before, but with a lower relative humidity of 50 to 85%.
After pupation (non-feeding, developmental stage), test organisms were allowed to complete development to adulthood.
Survival of pupae was initially checked on day 15 to avoid disturbing the fragile pre-pupal stage earlier. Larvae that failed to develop into pupae by day 15 were classified as dead. Starting on day 15, the number of emerged adults was recorded. At the time of emergence, each adult bee was removed from the well plate, frozen, and individually weighed. The test was terminated on day 22. At test termination, after health observations and remaining individual bee weights were recorded. Pupae that had not emerged by day 22 were considered dead.
The 8-day (larval) and 22-day (pupal, adult emergence, and adult weight) No-Observed-Effect Dose (NOED), the dose which demonstrated no statistically adverse effect on survival, emergence, or body weight when compared to the control and the Lowest-Observed-Effect Dose (LOED), which is the lowest dose that demonstrated a statistically significant effect on survival, emergence, or adult weight compared to the control, were determined. The ED_{10}, ED_{20}, and ED_{50} (for sublethal endpoints) and the LD_{10}, LD_{20}, and LD_{50} (for survival endpoints) were determined. These statistical endpoints were also determined based on measured diet concentrations (i.e., NOEC, LOEC, LC_X, and EC_X). Calculations were performed using CETIS Version 1.8, applying applicable statistical tests. The results of the negative control and solvent control data for each endpoint were compared using Fisher's Exact Test for percent survival/emergence and Equal Variance two sample t-Test for adult weight data. Since no significant difference was observed between the controls, the negative control was used to evaluate treatment performance, per current U.S. EPA guidance.
The concentration of test substance was measured in the stock solutions on day 3 and in the treated diets on days 3 through 6 using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). The method was fully validated according to SANCO/3029/99 rev. 4 at a LOQ of 0.500 µg/g.
The dose rates within this study were based on the cumulative dose over four days. The resulting mean measured diet concentrations were used to adjust the nominal cumulative dosages. The results of this

study were based on mean measured diet concentrations (µg a.s./g diet) and calculated dose (µg a.s./larva) that reflect the mean measured diet concentrations.

Results and discussion

Environmental parameters (relative humidity and temperature) remained within acceptable limits throughout the study. The humidity was in the range for the larval phase (days 1-8): 84% - 99% and for pupal phase (days 9-22): 68% - 78%values, temperature ranged from 31° - 34°C (larval phase (days 1-8)) and 32° - 34°C (pupal phase (days 9-22)). Based on historical data, these parameters were considered acceptable for the survival and growth of the test organisms.

Prior to conducting the definitive exposure, the solubility, homogeneity, and stability of zoxamide in royal jelly diet has been confirmed in a non-GLP diet trial. Based on the results of this trial, zoxamide was applied at nominal concentrations of 610 µg a.s./g (equivalent to a dose of 100 µg a.s./larva) in royal jelly diet. The storage stability of zoxamide in refrigerated royal jelly diet was confirmed for at least four days.

Analysis of the stock solutions resulted in measured concentrations ranging from 86 to 95% of nominal concentrations. These results confirmed the appropriate amount of zoxamide in the stock solution. Mean measured diet concentrations of zoxamide in royal jelly diets on day 3, 4, 5, and 6 for the nominal concentrations of 38, 78, 150, 300, and 610 µg a.s./g were 36, 67, 150, 300, and 640 µg a.s./g, respectively, and ranged from 86 to 110% of nominal concentrations. These means measured diet concentrations (µg a.s./g diet) were used to adjust the nominal cumulative dose (µg a.s./larva) for each treatment and were expressed as calculated doses of 5.9, 11, 24, 49, and 110 µg a.s./larva.

The mean larval survival (days 3 to 8) was 97, 97, 97, 92, 97, 97, and 94% in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.s./larva treatments, respectively.

Fisher's Exact Test indicated that the days 3 to 8 larval survival data for the negative control and solvent control data were not significantly different; therefore, treatment data were compared to the negative control data to define treatment effects.

Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant reduction in larval survival among honey bees exposed to any of the treatments compared to the negative control. Therefore, the 8-day larval survival NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.s./larva, respectively. Since no concentration resulted in ≥10, 20, or 50% mortality, the 8-day LD_X values of zoxamide to honey bee larvae were all empirically estimated to be >110 µg a.s./larva, the highest calculated dose tested.

The mean pupal survival (days 8 to 22) was 91, 97, 89, 91, 97, 94, and 94% in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.s./larva treatments, respectively. Fisher's Exact Test indicated that the pupal survival data for the negative control and solvent control data were not significantly different; therefore, treatment data were compared to the negative control data to define treatment effects.

Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant reduction in pupal survival among honey bees exposed to any of the treatments compared to the negative control. Therefore, the 22-day pupal survival NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.s./larva, respectively. Since no concentration tested resulted in ≥10, 20, or 50% mortality, the 22-day LD_X values of zoxamide to honey bee pupae were empirically estimated to be >110 µg a.s./larva, the highest calculated dose tested.

Table 10.3.1.3-3: Larval survival and mortality

Mean measured diet concentration (µg a.s./g diet)	Calculated dose (µg a.s./ larval)	% survival (number of surviving larvae)					Day 8		
		Day 3	Day 4	Day 5	Day 6	Day7	% survival (no. of surviving larvae)	% mortality (no. of dead larvae)	Corrected % mortality[ab]
Negative control	Negative control	100 (36)	100 (36)	100 (36)	100 (36)	100 (36)	97 (35)	3 (1)	NA

Solvent control	Solvent control	100 (36)	100 (36)	100 (36)	100 (36)	100 (36)	97 (35)	3 (1)	0
36	5.9	100 (36)	100 (36)	100 (36)	100 (36)	100 (36)	97 (35)	3 (1)	0
67	11	100 (36)	100 (36)	100 (36)	100 (36)	100 (36)	92 (33)	8 (3)	6
150	24	100 (36)	100 (36)	100 (36)	100 (36)	100 (36)	97 (35)	3 (1)	0
300	49	100 (36)	100 (36)	100 (36)	100 (36)	100 (36)	97 (35)	3 (1)	0
640	110	100 (36)	100 (36)	100 (36)	100 (36)	100 (36)	94 (34)	6 (2)	3

a per the study guideline, mortality values were also corrected and reported using Abbott´s formula (Abbott, 1925)
b Relative to the negative control.
NA = not applicable

At test termination, the mean adult percent emergence (days 3 to 22) in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.s./larva treatments was 89, 94, 86, 83, 94, 92, and 89%, respectively. Fisher's Exact Test indicated that the day 22 adult percent emergence data for the negative control and solvent control data were not significantly different; therefore, treatment data were compared to the negative control data to define treatment effects. Fisher's Exact Test with Bonferroni-Holm's Adjustment determined no significant reduction in adult percent emergence for honey bees exposed to any of the levels tested compared to the negative control. Therefore, the 22-day adult emergence NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.s./larva, respectively. Since no concentration tested resulted in ≥10, 20, or 50% reduction in emergence, the 22-day ED_X values of zoxamide to honey bee larvae through adulthood were empirically estimated to be >110 µg a.s./larva, the highest calculated dose tested.

Table 10.3.1.3-4: Pupal survival and adult percent emergence

Mean measured diet concentration (µg a.s./g diet)	**Calculated dose (µg a.s./larva)**	**No.[a]**	**Day 8-22**			**Day 3-22**		
			Cumulative % survival (no. of surviving pupae)	**Cumulative % mortality (no. of dead pupae)**	**Abbott's corrected % mortality[bc]**	**Cumulative % emergence (no. of emerged adults)**	**Cumulative % mortality[d] (no. of dead organisms)**	**Abbott's corrected percent mortality[bc]**
Negative control	Negative control	35	91 (32)	9 (3)	NA	89 (32)	11 (4)	NA
Solvent control	Solvent control	35	97 (34)	3 (1)	-6	94 (34)	6 (2)	-6
36	5.9	35	89 (31)	11 (4)	3	86 (31)	14 (5)	3
67	11	33	91 (30)	9 (3)	1	83 (30)	17 (6)	6
150	24	35	97 (34)	3 (1)	-6	94 (34)	6 (2)	-6
300	49	35	94 (33)	6 (2)	-3	92 (33)	8 (3)	-3
640	110	34	94 (32)	6 (2)	-3	89 (32)	11 (4)	0

a number of larvae transferred
b Per the study guideline, mortality values were also corrected and reported using Abbott's formula (Abbott, 1925).
c Relative to the negative control
d Based on 36 larvae at initiation
NA = not applicable

The mean adult weight at emergence in the negative control, solvent control, 5.9, 11, 24, 49, and 110 µg a.s./larva treatments was 0.1138, 0.1100, 0.1098, 0.1082, 0.0998, 0.1104, and 0.1023 g, respectively. Statistical analysis (Equal Variance Two-Sample t-Test) determined no significant difference in weight between the negative control and solvent control. Therefore, the negative control data was used to determine the treatment weight data effects.

Dunnett's Multiple Comparison Test determined a significant reduction in weight for adults at emergence among honey bees exposed to the 24 and 110 µg a.s./larva treatments, compared to the negative control. Due to the lack of a clear dose response as indicated by no significant reduction in weight at the 49 µg a.s./larva dose rate, the effect at the 24 µg a.s./larva dose rate was not considered toxicant related and was likely a function of biological variability. Therefore, the NOED and LOED values were determined to be 49 and 110 µg a.s./g larva, respectively. Since no concentration tested resulted in ≥10, 20, or 50% reduction, the ED_X values were empirically estimated to be >110 µg a.s./larva, the highest calculated dose tested.

Table 10.3.1.3-5: Adult weight at emergence

Mean measured diet concentration (µg a.s./g diet)	Calculated dose (µg a.s./larva)	No. [a]	Mean adult weight at emergence[b] (g)
Negative control	Negative control	32	0.1138 (0.0118)
Solvent control	Solvent control	34	0.1100 (0.0110)
36	5.9	31	0.1098 (0.0110)
67	11	30	0.1082 (0.0115)
150	24	34	0.0998[c] (0.0148)
300	49	33	0.1104 (0.0089)
640	110	32	0.1023[c] (0.0088)

[a] number of adults weighed
[b] Standard deviations are presented in parentheses.
[c] Significantly reduced, compared to the negative control, based on Dunnett's Multiple Comparison Test. Due to the lack of a clear dose response as indicated by no significant reduction in weight at the 49 µg a.s./larva dose rate, the effect at the 24 µg a.s./larva dose rate was not considered toxicant related and was likely a function of biological variability.

The nominal cumulative dose rate of dimethoate maintained during the reference test was 7.9 µg a.s./larva, equivalent to 48 µg a.s./g diet. Mortality during the larval stage (days 3 to 8) was 69% for honey bee larvae exposed to 7.9 µg a.s./larva nominal cumulative dose. These results confirm the sensitivity of the test system.

All validity criteria were met:
- Larval mortality from days 3 to 8 in the negative control, and solvent control, if present, should be ≤15% prior to pupation (larval mortality was 3 and 3%, respectively).
- Percent emergence in the negative control, and solvent control, if present, should be ≥70% at termination (emergence in the negative control and solvent control was 89 and 94%, respectively).
- Larval mortality in the reference toxicant treatment level (7.9 µg a.s. dimethoate/larva) should be ≥50% on day 8 (larval mortality in the 7.9 µg a.s. dimethoate/larva treatment was 69%.).

Table 10.3.1.3-6: Summary of study endpoints

Endpoint	NOEC / NOED	LOEC / LOED	LC_{10}/EC_{10} / LD_{10}/ED_{10} (95% CI)	LC_{20}/EC_{20} / LD_{20}/ED_{20} (95% CI)	LC_{50}/EC_{50} / LD_{50}/ED_{50} (95% CI)
based on mean measured diet concentrations (µg a.s./g diet)					
3-8-day larval survival	640	> 640	> 640 (NA)	> 640 (NA)	> 640 (NA)
8-22-day pupal survival	640	> 640	> 640 (NA)	> 640 (NA)	> 640 (NA)
3-22-day adult emergence	640	> 640	> 640 (NA)	> 640 (NA)	> 640 (NA)
Adult weight at emergence	300	640	> 640 (NA)	> 640 (NA)	> 640 (NA)

based on calculated dose (µg a.s./larvae)					
3-8-day larval survival	110	> 110	> 110 (NA)	> 110 (NA)	> 110 (NA)
8-22-day pupal survival	110	> 110	> 110 (NA)	> 110 (NA)	> 110 (NA)
3-22-day adult emergence	110	> 110	> 110 (NA)	> 110 (NA)	> 110 (NA)
Adult weight at emergence	49	110	> 110 (NA)	> 110 (NA)	> 110 (NA)

CI = Confidence interval
NA = not applicable. LC_x/EC_x value was empirically estimated; therefore, corresponding 95% confidence intervals could not be calculated

Conclusion

The objective of this study was to evaluate the effect of zoxamide to honey bee larvae and successive pupae survival, adult emergence, and adult weight in an artificial in vitro testing design. The 22-day exposure of larvae to zoxamide treated diet was initiated on day 3 and continued through day 6, resulting in both dermal and oral exposure until pupation.
The results from the stock solution and royal jelly diet analyses indicate appropriate exposure concentrations.
The 8-day NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.s./larva, respectively. The 22-day NOED and LOED values for zoxamide to honey bee pupal percent survival were 110 and >110 µg a.s./larva, respectively. The 22-day percent emergence NOED and LOED values for zoxamide to honey bees were determined to be 110 and >110 µg a.s./larva, respectively. The weight for adults at emergence NOED and LOED values for zoxamide values were 49 and >110 µg a.s./larva, respectively.
The 22-d LD_{50} was > 110 µg a.s/larvae, the 22-d LC_{50} > 640 µg a.s./g diet with regard to larval and pupal survival, adult emergence and adult weight at emergence.
The study is valid.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No new data is submitted with this application.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

No new data is submitted with this application.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No new data is submitted with this application.

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. Following deviation was noted: on day 13 of the test, the recorded temperature and relative humidity dropped below the set ranges for 3 and 4 hours, respectively, due to a power outage that affected the climatic room. The lowest recorded values were 21.1°C and 43.8%. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.3.2.1/01
Report: EFFECTS OF GWN -10616 ON THE PREDATORY MITE *TYPHLODROMUS PYRI* SCHEUTEN (ACARI: PHYTOSEIIDAE) UNDER LABORATORY CONDITIONS, Venturi, S., 2021, report No. BT215/21, Doc. No. 834-004
Guideline(s): Blümel et et. (2000)
Deviations: ~~None~~ Yes
GLP: Yes
Acceptability: Yes

Executive Summary

The potential effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances applied at 5 dose rates (1250 – 20.000 g test item/ha) on the predatory mite *Typhlodromus pyri* were measured in the laboratory via contact on treated glass surfaces compared to a water treated control and to a reference item. An assessment on mortality seen over 7 days of exposure and for sublethal effects recorded at 3 assessment days within one week compared to a water treated control was performed.

Under worst case laboratory conditions, the LR_{50} of GWN-10616 is 6332.2 g test item/ha (corresponding to 2198.9 g Potassium phosphite/ha and 268.9 g Zoxamide/ha. The ER_{50} was estimated to be > 5000 g test item/ha (corresponding to 1736.3 g Potassium phosphite/ha and 212.3 g Zoxamide/ha). The NOER was 1250 g test item/ha (corresponding to 434.1 g Potassium phosphite/ha and 53.1 g Zoxamide/ha) based on mortality.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material: GWN-10616
Active substances: Potassium phosphite and Zoxamide
Description: Beige homogeneous and viscous liquid
Lot/Batch #: P2102669001
Content of a.s.: Potassium phosphite (measured as Phosphonic acid equivalent): 507 g/L, Zoxamide: 62 g/L

2. Vehicle and control:

Control: Deionised water
Positive control: PERFEKTHION TOP (Dimethoate 400 g/L at 12 g Dimethoate/ha)

3. Test animals:

Species:	*Typhlodromus pyri* Scheuten
Taxonomic group:	Predatory mites (Acari: Phytosiidae)
Life Stage:	Protonymphs
Age:	Less than 24 hours
Source:	Katz Biotech AG, Germany

4. Environmental conditions

Temperature:	21.1 – 26.5°C (average = 24.8°C)
Humidity:	43.8 – 78.2 % (average = 72.4 %)
Photoperiod:	16 h light, 8 h dark

B. STUDY DESIGN AND METHODS

1. Experimental phase: 15.10.2021 – 29.10.2021

2. Experimental treatments

Exposure of the predatory mite *Typhlodromus pyri* to GWN-10616 (active substances Potassium phosphite as Phosphonic acid equivalent: 507 g/L, Zoxamide: 62 g/L) was reached via air dried residues on treated glass plates at a spraying volume of 200 L/ha. Seven treatment groups (five test item treatment groups of 1250, 2500, 5000, 10000 and 20000 g test item/ha; water treated control; reference item) were tested with 3 replicates each and each containing 20 mites. Mortality was assessed after 7 days of exposure. For the reproduction assessment the sex of surviving mites from the control and from all test item treatment groups displaying ≤ 50 % corrected mortality were determined and the number of eggs per females was recorded at 3 assessment days (day 10, 12 and 14) within one week. Exposure took place in a controlled environment room at 21.1 – 26.5°C and relative humidity between 43.8 – 78.2 %.

3. Observations

Assessment of the number of living, escaped and dead mites was performed 7 days after application. For the reproduction assessment the number of eggs per female were recorded 10, 12 and 14 days after application.

4. Statistics

The NOER for survival was determined with Step-down Cochran-Armitage Test Procedure (one-sided greater, alpha = 0.05); the LR_{50} was calculated using Weibull analysis (with linear max. likelihood regression). The NOER for reproduction was determined with Step-down Jonckheere-Terpstra Test Procedure (one-sided smaller, alpha = 0.05). The software ToxRatPro 3.3.0 was used for the statistical analysis.

II. RESULTS AND DISCUSSION

Mortality of the control was 20 % (≤ 20% recommended) and the number of laid eggs per female amounted to 8.73 (> 4 recommended), which indicates the validity of the study. Additionally, the mortality in the reference item group was 95 %, which indicates the sensitivity of the test system.

There were significant lethal effects on mortality observed between control and treatment groups except for the lowest treatment group of 1250 g test item/ha. There was no rate response relationship for reproductive parameters, and no significant difference between fecundity in the control and the test item groups up to and including 5000 g test item/ha was observed. The results are summarised in Table 10.3.2.1-1.

Table 10.3.2.1-1: The effects on mortality and reproduction of the predatory mite, *Typhlodromus pyri* exposed to dried residues of GWN-10616 on treated glass plates

Test Group [g test item/ha]	Corrected Mortality [%]	No. of Eggs per Female	Reduction of Reproduction [%][1]
Control	-	8.73	-
1250	6.25	9.47	-8.48
2500	27.09*	10.13	-16.04
5000	43.75*	9.52	-9.05
10000	58.34*	nd	nd
20000	97.91*	nd	nd
Dimethoate 12 g/ha	93.75	nd	nd

* significantly different from the control (Step-down Cochran-Armitage Test Procedure (one-sided greater, alpha = 0.05);
[1] Reproduction relative to control, negative values mean increased reproduction compared to the control
nd = not determined as mortality was > 50 %

The LR_{50} value was calculated to be 6332.2 g test item/ha (95 % CI = 4089.7 to 9804.3 g test item/ha) corresponding to 2198.9 g Potassium phosphite/ha and 268.9 g Zoxamide/ha. Reproduction was not significantly affected at application rates up to and including 5000 g test item/ha, above this application rate more than 50 % mortality was observed. The NOER was 1250 g test item/ha based on mortality.

III. CONCLUSIONS

The toxicity of GWN-10616 to *Typhlodromus pyri* was determined in a laboratory test. The LR_{50} value was calculated to be 6332.2 g test item/ha. Reproduction was not significantly affected at application rates up to and including 5000 g test item/ha, above this application rate more than 50 % mortality was observed. The NOER was 1250 g test item/ha based on mortality.

Comments of zRMS:	The study was conducted to the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.3.2.1/02
Report: EFFECTS OF GWN-10616 ON THE PARASITIC WASP *APHIDIUS RHOPALOSIPHI* UNDER LABORATORY CONDITIONS, Colli, M., 2021, report No. BT209/21, Doc. No. 834-001
Guideline(s): Mead-Briggs et al. (2000), Mead-Briggs et al. (2009)
Deviations: None
GLP: Yes
Acceptability: Yes

Executive Summary

The potential effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, applied at 5 dose rates (1250 – 20.000 g test item/ha) on the parasitoid *Aphidius rhopalosiphi* were measured in the laboratory via contact on treated glass surfaces compared to a water treated control and to a reference item. An assessment on mortality seen over 48 h of exposure and for sublethal effects (parasitation activity) compared to a water treated control was performed.

Under worst case laboratory conditions the LR_{50} and ER_{50} values were determined to be 16491.07 g test item/ha and > 10000 g test item/ha. The respective NOER for mortality and NOER for reproduction were determined to be 5000 g test item/ha and ≥ 10000 g test item/ha, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GWN-10616
Active substances	Potassium phosphite and Zoxamide
Description:	Beige homogeneous and viscous liquid
Lot/Batch #:	P2102669001
Content of a.s.:	Potassium phosphite (measured as Phosphonic acid equivalent): 507 g/L, Zoxamide: 62 g/L

2. Vehicle and control:

Control:	Deionised water
Positive control:	PERFEKTHION TOP (Dimethoate 400 g/L at 0.12 g Dimethoate/ha)

3. Test animals:

Species:	*Aphidius rhopalosiphi* DeStephani-Perez
Taxonomic group:	Parasitic wasp (Hymenoptera: Braconidae)
Life Stage:	Young adults (less than 48 hours old)
Source:	BioTecnologie BT, Italy

4. Environmental conditions

Temperature:	Mortality phase: 20.2 – 22.1°C (average = 21.1°C) Parasitation phase: 18.9 – 19.9°C (average = 19.4°C) Mummies' maturation phase: 18.6 – 21.6°C (average = 19.6°C)
Humidity:	Mortality phase: 59.3 – 78.0 % (average = 65.1 %)
Photoperiod:	16 h light, 8 h dark

B. STUDY DESIGN AND METHODS

1. In life dates: 22.06.2021 – 06.07.2021

2. Experimental treatments

Exposure of the parasitic wasps to GWN-10616 (active substances Potassium phosphite measured as Phosphorous acid equivalent: 507 g/L; Zoxamide: 62 g/L) was reached via air dried residues on treated glass plates at a water amount of 200 L/ha. Seven treatment groups (five test item treatment groups of 1250, 2500, 5000, 10000 and 20000 g test item/ha; water treated control; reference item) were tested with 3 replicates each and each containing 10 wasps (at least 5 females). For the reproduction assessment 15 females each from the control and all test item treatment groups displaying less than 50 % corrected mortality were individually confined over pots of untreated, aphid infested barley plants for 24 h and removed afterwards. Exposure took place in a controlled environment room at 20 ± 2 °C and relative humidity between 60 – 78 %.

3. Observations

Assessment of the number of living, affected, moribund and dead parasitoids was performed at 2, 24 and 48 after application. Number of aphid mummies was counted after 12 days (15 days after introduction in mortality test).

4. Statistics

The software ToxRat Pro 3.3.0 was used for the statistical evaluation of the results. The Step-down

Cochran-Armitage test (α = 0.05, one-sided greater) was used to test for statistical significance the mortality data between the test item treatments vs the control data. Dunnett's Multiple t-test Procedure (α = 0.05, one-sided smaller) was used to determine statistical significance of the reproduction data. The LR_{50} value was calculated by Probit analysis.

II. RESULTS AND DISCUSSION

Adult mortality in the control was 0 % (< 13 % recommended), 22.2 mummies per female were counted (> 5 recommended) and no females produced no mommies in the control (< 2 recommended), thus the validity criteria are fulfilled. Additionally, the mortality in the reference item group was 100 %, which indicates the sensitivity of the test system.

Effects on mortality were observed in treatment groups at 10000 and 20000 g test item/ha. There was no rate response relationship for reproductive parameters up to 10000 g test item/ha. The results are summarised in Table 10.3.2.1-2.

Table 10.3.2.1-2: The effects on mortality and reduction of parasitisation capacity of the aphid parasitoid, *Aphidius rhopalosiphi*, exposed to GWN-10616 on treated glass plates

Test Group [g test item/ha]	Mortality after 48 h [%]	Reproduction (Mummies/Female)	Reduction of Reproduction [%]
Control	0	22.2	-
1250	0	19.7	11.1
2500	0	19.7	11.1
5000	0	20.1	9.3
10000	13.33*	19.3	12.9
20000	66.67*	nd	nd
0.32 (Reference)	100	nd	nd

*significantly different from the control (Step-down Cochran-Armitage test (α = 0.05, one-sided greater)
nd = not determined as mortality was > 50 %

The LR_{50} value for *Aphidius rhopalosiphi* exposed to GWN-10616 was determined to be 16491.07 g test item/ha (95 % CI = 14115.97 to 20127.51 g test item/ha). The reproduction of surviving parasitoids was not statistically significantly affected at rates up to and including 10000 g test item/ha. The NOER was 5000 g test item/ha based on mortality.

III. CONCLUSIONS

Under worst case laboratory conditions, the LR_{50} value for *Aphidius rhopalosiphi* exposed to GWN-10616 was determined to be 16491.07 g test item/ha. The reproduction of surviving parasitoids was not statistically significantly affected at rates up to and including 10000 g test item/ha. The NOER was 5000 g test item/ha based on mortality.

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 222 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.4.1.1/01
Report: EFFECTS OF GWN-10616 ON REPRODUCTION AND GROWTH OF THE EARTHWORM *EISENIA ANDREI* IN ARTIFICIAL SOIL, Pecorari, F., 2021, report No. BT213/21, Doc. No. 833-001
Guideline(s): OECD No. 222 (2016), ISO 11268 (2012)
Deviations: None
GLP: Yes
Acceptability: Yes

Executive Summary

The potential chronic effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, on reproduction and development of earthworms (*Eisenia andrei*) were determined in a laboratory study according to OECD 222 (2016). The test item was tested at concentrations of 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg test item/kg soil dry weight (sdw) and a water control.

In this study, no dose-response related impacts on mortality and biomass were found at tested concentrations up to and including 1000 mg test item/kg sdw. For reproduction, no adverse effects were observed up to and including the concentration of 29.40 mg test item/kg sdw (equivalent to 10.21 mg Potassium phosphite, measured as Phosphonic acid equivalent, and 1.25 mg Zoxamide/kg sdw, respectively). The NOEC for reproduction is therefore 29.4 mg test item/kg sdw. The ECx values for reproduction resulted to be EC_{10}, EC_{20} and EC_{50} values for reproduction were 25.29, 32.50 and 52.54 mg test item/kg sdw, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material: GWN-10616
Active substances: Potassium phosphite and Zoxamide
Description: Beige homogeneous and viscous liquid
Lot/Batch #: P2102669001
Content of a.s.: Potassium phosphite (measured as Phosphonic acid equivalent): 507 g/L, Zoxamide: 62 g/L

2. Vehicle and control:

Control: Untreated artificial soil moistened with deionised water
Vehicle: Deionised water
Reference Item: Boric acid was tested in a separate study at concentrations of 50 to 800 mg test item/kg sdw to confirm the sensitivity of the test organisms.

3. Test animals:

Species:	*Eisenia andrei*
Source:	Breeding stock culture maintained at the test facility
Age:	11 months old with clitellum (difference of age did not deviate by more than 1 month)
Mean weight:	250 to 600 mg
Acclimatisation period:	24 hours under test conditions
Environmental conditions	
Temperature:	18.4 – 20.9°C
Soil water content:	54.55 % of WHC_{max}
Soil:	Artificial soil according to OECD 222, with 10% peat content.
Photoperiod:	16 hours light – 8 hours dark
Light intensity:	670 - 962 lux During the test the light intensity went over the outlined range (400 – 800 lux) for less than 2 hours (max. 962 lux), but this is considered to have a negligible effect due to the short period.
Soil pH	6.19

B. STUDY DESIGN AND METHODS

1. Experimental phase: 02.08.2021 – 30.09.2021

2. Experimental treatments

The potential chronic effects of GWN-10616 (active substances Potassium phosphite measured as Phosphonic acid equivalent: 507 g/L; Zoxamide: 62 g/L) on reproduction and development of earthworms (*Eisenia andrei*) were determined in an 8-week soil exposure laboratory study according to OECD 222 (2016). The test was conducted in a dose-response design with concentrations of 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg test item/kg sdw (mixed into the soil) and a control. Four replicates per treatment group and eight replicates for the control group were used, each containing 10 adult individuals (with clitellum).

After a randomising procedure according to the worm fresh weight, selected groups of 10 acclimatised earthworms were randomly assigned to each treatment group. The test vessels were closed with lids, allowing gas exchange between substrate and atmosphere and access of light, but prevented worms from escaping.

One day after application, an amount of 8 g fresh vegetable mixture (carrots and potatoes sliced and chopped), was spread on the soil surface of each test vessel. The feeding interval was weekly during the first four weeks of the test, food was provided depending on the feeding rate and the density of the earthworm population in the vessels.

After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality), their biomass change and morphological as well as behavioural changes were determined. Subsequently, the soil of each vessel was mixed carefully with 8 g food. The test was then continued for another four weeks. The final assessment included manual counting of juveniles per test vessel, determination of the water content and pH measurements of the artificial soil.

3. Observations

Mortality, feeding activity, development in body weight and behaviour of adult worms were assessed after 4 weeks and reproduction rates after 8 weeks of exposure time.

4. Analytic

The concentration of active substances Zoxamide and Potassium phosphite (measured as Phosphonic acid equivalent) in the stock solution was analysed, in order to verify the effective concentration, together with

the control (deionised water) via LC-MS/MS.

5. Statistics

ToxRat Professional Version 3.3.0 was used as statistical software; the Multiple Sequentially rejective Fisher Test after Bonferroni Holm was selected for mortality significance testing (α=0.05, one-sided greater); the Williams Multiple Sequential rejective Welsh-t-test after Bonferroni-Holm performed by the statistical software for reproduction significance testing (α=0.05, one-sided smaller). Probit analysis was performed to estimate the ECx values.

II. RESULTS AND DISCUSSION

A. Validity criteria

The study was considered valid as each control replicate (containing ten adults) had produced ≥ 30 juveniles at the end of the test (actual: between 90 and 123), the coefficient of variation (% RSD or CV) of reproduction was ≤ 30 % (actual: 10.05 %) and adult mortality over the initial 4 weeks of the test was ≤ 10% (actual: 0.0 %).

In addition, the EC_{50} determined on the reproductive output for the reference item (Boric acid) was evaluated to be 498.95 mg reference item/kg sdw (95 % confidence limits: 456.9 – 544.99 mg reference item/kg sdw), confirming the sensitivity of the test system.

B. Analytical test results

The concentrations of the active substances Zoxamide and Phosphonic acid in the stock solutions used to treat the soil were determined. All measured concentrations of the test item were within ± 20 % of the nominal values. Accordingly, endpoints of the test were calculated with respect to the nominal concentration of the test item.

C. Biological test results

The test item caused no statistically significant mortality compared to the control at test item concentration up to and including 1000 mg test item/kg sdw (Table 10.4.1-1). Thus, no LC_{10} and LC_{20} values could be statistically calculated. The $LOEC_{mortality}$ and the $NOEC_{mortality}$ were determined to be ≥ 1000 mg test item/kg sdw (equivalent to 347.26 mg Potassium phosphite, measured as Phosphonic acid equivalent, and 42.47 mg Zoxamide/kg sdw, respectively), respectively.

No pathological symptoms or changes in the behaviour of adult earthworms were observed during the test. Some unhatched eggs were found at the end of the test starting at test concentration 52.92 mg test item/kg sdw and above.

The test item caused no statistically significant adverse change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group up to and including 1000 mg test item/kg sdw.

Statistically significant effects on earthworm reproduction were observed in the treatment groups of 52.925 mg test item/kg sdw and higher compared to the control. Therefore, the $LOEC_{reproduction}$ and the $NOEC_{reproduction}$ were assessed to be 52.92 and 29.40 mg test item/kg sdw, respectively. The EC_{10} for reproduction was 25.29 mg test item/kg sdw (95 % confidence limits: 20.00 – 29.59 mg test item/kg sdw), the EC_{20} was 32.50 mg test item/kg sdw (95 % confidence limits: 27.38 – 36.68 mg test item/kg sdw) and the EC_{50} was 52.54 mg test item/kg sdw (95 % confidence limits: 47.89 – 57.63 mg test item/kg sdw).

Table 10.4.1-1: Summary of lethal, developmental and reproductive effects of GWN-10616 on earthworm *Eisenia andrei*

Nominal concentration [mg test item/kg soil]	Day 28		Day 56	
	Cumulative mortality [%]	Biomass gain [%]	Mean number of juveniles per treatment	Mean number of juveniles [% of control]
control	0	42.2	107.63	-
16.33	0	49.4	97.0	9.88
29.40	0	56.9	93.5	13.13
52.92	0	60.0	53.25	50.52*
95.26	0	58.1	15.5	85.60*
171.47	2.5	50.9	0.75	99.3*
308.64	0	65.1	0	100*
555.56	5	67.9	0	100*
1000	0	80.3	0	100*

* Statistical Significance (Multiple Sequentially-rejective Welch t-test after Bonferroni-Holm, one-sided smaller, $\alpha = 0.05$) compared to control group.

III. CONCLUSIONS

The chronic toxicity of GWN-10616 to *Eisenia andrei* was tested under laboratory conditions for 56 days. In this study, no dose-response related impacts on mortality and biomass were found at tested concentrations up to and including 1000 mg test item/kg sdw. For reproduction, no adverse effects were observed up to and including the concentration of 29.40 mg test item/kg sdw (equivalent to 10.21 mg Potassium phosphite, measured as Phosphonic acid equivalent, and 1.25 mg Zoxamide/kg sdw, respectively). The NOEC for reproduction is therefore 29.4 mg test item/kg sdw. The EC_{10}, EC_{20} and EC_{50} values for reproduction were 25.29, 32.50 and 52.54 mg test item/kg sdw, respectively.

EFSA (2017) requested further data for the evaluation of the active substance zoxamide. The following study has been performed with an SC formulation containing 240 g/L zoxamide, the representative formulation during AIR. The study endpoints can be used as representative for zoxamide technical. This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

RMS considers the study valid and acceptable.

Agreed endpoints for the earthworm *Eisenia andrei* (5% peat content):

The 56-day NOEC for reproduction = 2.453 mg a.s./kg dw (based on analysed concentrations).

The 56-day EC10, EC20 and EC50 values for reproduction were calculated to be 3.304, 3.907 and 5.382 mg a.s./kg dw based on analysed concentrations, respectively.

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Reference:	KCA 8.4.1
Report	Friedrich, S., 2020: Effects of Zoxium 240 SC on the reproduction of the earthworm *Eisenia andrei* in artificial soil with 5 % peat Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 17 48 TEC 0009, GLP, Not published
Guideline(s):	OECD 222 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (SB 2401)
Active substance content or purity	240 g/L Zoxamide (nominal) 21.08 ± 0.1 % (w/w), 236.4 g/L (analysed) R/S ratio of zoxamide: 50.1:49.9 (analysed)
Species	Earthworm *Eisenia andrei* (BOUCHÉ, 1972)
Age:	adult worms (approximately 4 months old, with clitellum)
Source:	Test facility; original breeding purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany
Weight:	280 - 478 mg/worm
Acclimation period:	at least 24 hours in the artificial substrate (with food)
Food:	air-dried and finely ground horse manure
Test system	plastic vessel (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light, containing 750 g wet weight corresponding to 600 g dry weight of artificial soil with a water content corresponding to 40-60 % of WHC
Soil:	artificial soil, 5% peat
Number of animals:	10 worms/replicate 40 (treatment group) (80 control group)
Number of replicates:	4 replicates for the test item treatments, 8 replicates for the control
Environmental conditions	
Temperature:	19.8 – 20.7 °C
Photoperiod:	light: dark = 16 h: 8 h; 590 lux
Soil moisture:	guideline requirement: 40-60 % of WHC test start: 24.9 – 25.1 (equivalent to 57.8 – 58.2 % of WHC) test end: 24.2 – 24.8 (equivalent to 56.1 – 57.5 % of WHC)
pH:	guideline requirement: 6.0 ± 0.5 test start: 5.97 – 6.03 test end: 5.69 – 5.78

Application rate(s)	0.392, 0.706, 1.271, 2.287, 4.117, 7.410, 13.34, 24.01 mg a.s./kg soil dry weight (nominal) 1.86, 3.35, 6.03, 10.9, 19.5, 35.2, 63.3, 113.9 mg test item/kg soil dry weight based on analysed content of a.s. in the formulated product
Negative control:	deionised water
Positive control:	Carbendazim 500 SC
Post exposure observation period	56 days
Remarks	None

The potential effects of the test item Zoxium 240 SC (containing nominally 240 g/L zoxamide) at application rates of 1.86, 3.35, 6.03, 10.9, 19.5, 35.2, 63.3, 113.9 mg product/kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia andrei* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 5 % organic matter.
One day before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start.
On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60 % of its WHC. The control substrate contained the corresponding amount of deionised water. The test solution was thoroughly mixed into the soil separately for each replicate. Each test vessel was then filled with the treated soil. After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were then randomly assigned to each treatment group. The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. After approximately thirty minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light, but prevented worms from escaping. The test vessels were then set up at random in a controlled-environment test room. One day after application, initially 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity, which was assessed by visual estimation of the food remaining on the surface before addition of new food.
After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. Subsequently, the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion of the experiment. The test was then continued for another four weeks. The final assessment included counting of juveniles per test vessel, determination of the water content and pH measurements of the artificial soil. Juveniles were counted by manual inspection of the substrate.
The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional ToxRat Professional 3.2.1 (Ratte, 2015). The ECx values (number of juveniles) were calculated by Probit analysis using linear max. likelihood regression. For identifying the NOECs, the Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.
The concentration of zoxamide in the artificial soil was analysed at test start (day 0, just after test item application), middle (day 28) and test end (day 56). The nominal initial test item concentrations in the soil were analytically confirmed with a highly specific HPLC-MS/MS method fully validated according to SANCO/3029/99 rev. 4. The active substance zoxamide was analysed in soil specimens by a method developed by Jooß (2013), using extraction with acetonitrile and separation by reverse-phase high-pressure liquid chromatography (HPLC) and tandem mass spectroscopic (MS/MS) determination of zoxamide with matrix-matched external standards. This method was adapted to the expected

concentration range of this study. It was re-validated with artificial soil spiked with test item at concentrations 0.05 mg/kg (moist soil, corresponding to 0.0625 mg/kg dry weight), at 0.5 mg/kg (moist soil, corresponding to 0.625 mg/kg dry weight) and approx. 120% of the highest test concentration (23 mg/kg moist soil, corresponding to 29 mg/kg dry weight)). For analysis, 5 g (± 0.05 g) soil sample were weighed into a 100 mL Erlenmeyer flask. 1.0 mL water and 50 mL acetonitrile were added and the flasks were shaken for 45 minutes on a mechanical shaker. Then 1.0 g sodium chloride was added and the flasks again shaken for 10 minutes. The samples were transferred to centrifuge tubes and centrifuged for 3 minutes. Aliquots of the acetonitrile phase were transferred to autosampler vials and diluted. The analytes were determined after extraction with two mass transitions (zoxamide: m/z 336 -> 187 and 336 -> 159), one for quantification and one for qualification, respectively. The limit of quantification (LOQ) was defined in the context of this phase of the study as the lowest successfully validated fortification level, i.e. 0.051 mg/kg zoxamide in wet soil specimens, equivalent to 0.063 mg/kg zoxamide in dry weight soil.

Results and discussion

Environmental conditions stayed within the recommended ranges.
The analytically verified recoveries of the active substances in the soil substrate were all greater than 80% (i.e. 96-130 % for zoxamide). Thus, confirming sufficiently high (> 80% of nominal) test item concentrations at study start.
At the start of the test, earthworm fresh weight ranged from 280 – 478 mg/worm. The weight change of adult worms ranged between 19.8 % and 23.6 % in the treated groups and was 22.5 % in the control group. The test item caused no statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested.
Mortality rates of 0 % - 5.0 % were recorded in the test item treatment groups and 0 % mortality was observed in the control group. No statistically significant mortality compared to the control was observed at any concentration tested (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater).

Table 10.4.1-2: Effects of the test item on mortality and reproduction of adult earthworms

Treatment group	Zoxium 240 SC Nominal concentration (mg a.s./kg soil d.w.)								
	Control	**0.392**	**0.706**	**1.271**	**2.287**	**4.117**	**7.410**	**13.34**	**24.01**
	Analysed concentration on day 0 (mg a.s./kg soil d.w.)								
	Control	**0.511**	**0.714**	**1.367**	**2.453**	**4.319**	**7.105**	**13.64**	**25.38**
Replicate	**Number of surviving adult worms per replicate (4 weeks after test initiation)**								
1	10	10	10	10	10	10	9	10	9
2	10	10	10	10	10	10	9	9	10
3	10	10	9	9	10	10	10	10	10
4	10	10	10	10	10	10	10	10	9
5	10								
6	10								
7	10								
8	10								
mean	10.0	10.0	9.8	9.8	10.0	10.0	9.5	9.8	9.5
SD	0.0	0.0	0.5	0.5	0.0	0.0	0.6	0.5	0.6
cv %	0.0	0.0	5.1	5.1	0.0	0.0	6.1	5.1	6.1

Treatment group	Zoxium 240 SC Nominal concentration (mg a.s./kg soil d.w.)								
	Control	**0.392**	**0.706**	**1.271**	**2.287**	**4.117**	**7.410**	**13.34**	**24.01**
	Analysed concentration on day 0 (mg a.s./kg soil d.w.)								
	Control	**0.511**	**0.714**	**1.367**	**2.453**	**4.319**	**7.105**	**13.64**	**25.38**
	Mortality (%)								
mean	**0.0**	**0.0**	**2.5**	**2.5**	**0.0**	**0.0**	**5.0**	**2.5**	**5.0**
	Number of juvenile worms per replicate (8 weeks after test initiation)								
1	117	130	125	111	103	87	13	8	0
2	185	149	154	168	145	108	21	10	0
3	160	137	119	131	157	91	38	23	0
4	152	180	173	143	162	119	53	14	0
5	110								
6	133								
7	146								
8	122								
mean	**140.6**	**149.0**	**142.8**	**138.3**	**141.8**	**101.3***	**31.3***	**13.8***	**0.0***
SD	25.1	22.1	25.3	23.8	26.8	14.9	17.9	6.7	0.0
cv %	17.8	14.8	17.7	17.2	18.9	14.7	57.1	48.4	-
	Reduction of reproduction (%)								
% to control	**-**	**-6.0**	**-1.5**	**1.7**	**-0.8**	**28.0**	**77.8**	**90.2**	**100.0**

Not statistically significantly different compared to the control for mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, $\alpha = 0.05$, one-sided greater)
* statistically significantly different compared to the control (Williams-t-test for reproduction, $\alpha = 0.05$, one-sided smaller)
SD: standard deviation, cv %: coefficient of variation, d.w.: dry weight
Negative % values for change of reproduction = increase, relative to control

Statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on the number of juveniles compared to the control group were recorded at nominal concentrations of 4.117, 7.410, 13.34 and 24.01 mg a.s./kg d.w. The NOEC for mortality and biomass was determined to be 24.01 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 25.38 mg/kg zoxamide based on analysed concentrations). The NOEC for reproduction was determined to be 2.287 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 2.453 mg/kg zoxamide based on analysed concentrations). The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 2.987, 3.655 and 5.378 mg a.s./kg soil dry weight based on nominal concentrations, corresponding to 3.304, 3.907 and 5.382 mg/kg zoxamide based on analysed concentrations, respectively.

Table 10.4.1-3: Effects of the test item in a 56-day reproduction study

Endpoint	Treatment group								
	Nominal concentration (mg a.s./kg soil d.w.)								
	Control	0.392	0.706	1.271	2.287	4.117	7.410	13.34	24.01
	Analysed concentration on day 0 (mg a.s./kg soil d.w.)								
	Control	0.511	0.714	1.367	2.453	4.319	7.105	13.64	25.38
Mortality of adult worms after 4 weeks (%)	0.0	0.0	2.5	2.5	0.0	0.0	5.0	2.5	5.0
Mean biomass change after 4 weeks (%)	22.5	23.1	22.5	23.4	21.9	23.6	19.8	20.9	21.2
Mean number of juveniles after 8 weeks	140.6	149.0	142.8	138.3	141.8	101.3*	31.3*	13.8*	0.0*
Reduction of reproduction compared to control (%)	-	-6.0	-1.5	1.7	-0.8	28.0	77.8	90.2	100.0

	Endpoint (mg a.s./kg soil dry weight)	
	Nominal concentration	**Analysed concentration on day 0**
NOEC (mortality)	24.01	25.38
NOEC (biomass)	24.01	25.38
NOEC (reproduction)	2.287	2.453
LC_{50} (mortality)	> 24.01	> 25.38
EC_{10} (reproduction)[1]	2.987 (95 % confidence limits 2.530 - 3.528)	3.304 (95 % confidence limits 2.802 - 3.896)
EC_{20} (reproduction)[1]	3.655 (95 % confidence limits 3.225 - 4.144)	3.907 (95 % confidence limits 3.454 - 4.419)
EC_{50} (reproduction)[1]	5.378 (95 % confidence limits 4.954 - 5.838)	5.382 (95 % confidence limits 4.976 - 5.821)

* statistically significant compared to control (Williams-t-test for biomass and reproduction, $\alpha = 0.05$, one-sided smaller)
1 median values with their 95 % confidence intervals, based on Probit analysis
Negative % values for change of reproduction = increase, relative to control
d.w.: dry weight (of artificial soil)

In the most recent study with Maypon Flow (BioChem project No. 17 48 TEC 0011, dated 20 January 2017) the number of juveniles was reduced by 57 and 100 % at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 46 and 0) after 8 weeks of test duration when compared to control (mean number of juveniles = 107). Thus, confirming the sensitivity of the test system.
All validity criteria were met:
- adult mortality: ≤ 10 % (being 0 % after 4 weeks)
- number of juveniles per replicate: ≥ 30 (being 110 to 185)
- coefficient of variation of reproduction: ≤ 30 % (being 17.8 %)

Conclusion

In a 56-day earthworm reproduction study with Zoxium 240 SC, no statistically significant adverse effects on mortality and biomass of the earthworm *Eisenia andrei* in artificial soil were determined up to and including nominally 24.01 mg a.s./kg soil dry weight, the highest nominal concentration tested (corresponding to 25.38 mg a.s./kg soil d.w. analysed). Therefore, the NOEC for mortality and biomass was determined to be 24.01 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 25.38 mg/kg zoxamide based on analysed concentrations). The NOEC for reproduction was determined to be 2.287 mg a.s./kg soil dry weight based on nominal zoxamide concentrations (corresponding to 2.453 mg/kg zoxamide based on analysed concentrations). The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 2.987, 3.655 and 5.378 mg a.s./kg soil dry weight based on nominal concentrations, corresponding to 3.304, 3.907and 5.382 mg/kg zoxamide based on analysed

concentrations, respectively.
The study is valid.

This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints for *E. fetida* (10% peat content)**:**

The 56-day NOEC for reproduction was empirically determined to be 10 mg a.s./kg dw

The 56-day EC10, EC20 and EC50 values for reproduction were empirically deter-mined to be >10 mg a.i./kg dw

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Reference:	KCA 8.4.1
Report	Gray, J., 2021: RH-127450: Effect on reproduction in the earthworm *Eisenia fetida* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202376, GLP, Not published
Guideline(s):	OECD 222 (2016)
Deviations:	On one occasion the minimum temperature recorded was 17.8°C, below the guideline minimum of 18°C, but within rounding. The number of juveniles in replicates E-H inclusive of the solvent control was not assessed until day 57 as the assessment took longer than expected due to the high numbers of juveniles recorded. This did not affect the number of juveniles present as the adults had been removed at day 28. At day 56 the soil moisture content was equivalent to > 60% MWHC (the guideline maximum, not referenced in the protocol) at 0.53, 0.95, 1.72 and 5.56 mg a.s./kg dry substrate, with a maximum of 61.42% at 1.72 mg a.s./kg dry substrate. However, as all the validity criteria were met, these deviations were not considered to have had any impact on the integrity or outcome of the study. For the application rates where the soil moisture content was equivalent to >60% MWHC at day 56 the mean number of juvenile worms produced at each rate was within the overall range for the study (minimum of 408 in the water control and maximum of 517 at 10 mg a.s./kg dry substrate). As the NOEC for reproduction was determined to be 10 mg a.s./kg dry substrate and the EC_{10}, EC_{20} and EC_{50} values were >10 mg a.s./kg dry substrate the deviations in % MWHC were not considered to have any impact on the number of juveniles produced at the affected application rates or on the integrity or outcome of the study.
GLP:	Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test material (Lot/Batch No.)	RH-127450 (HHGCP002-00-1)
Active substance content or purity	99.51 % (w/w)
Species	Earthworm *Eisenia fetida*
Age:	adult worms, sexually mature (i.e. between 2 and 12 months old with clitellum), weighing between 300 and 600 mg (wet mass)
Source:	Bias Labs Ltd., UK
Weight:	414.8 – 507.0 mg/worm (mean per replicate, day 0)
Acclimation period:	at least 24 hours in the artificial substrate with feed
Food:	finely ground animal faeces *ad libitum*
Test system	1 L glass test vessel containing ten earthworms in the equivalent of approximately 500 g dry weight of artificial soil substrate maintained at nominally 55% maximum water holding capacity (MWHC)
Soil:	artificial soil, 10% peat
Number of animals:	80 (control) 40 (test item treatment)
Number of replicates:	4 replicates per treatment rate, 8 per controls
Environmental conditions	
Temperature:	17.8 – 21.4 °C
Photoperiod:	light: dark = 16 h: 8 h; 484-774 lux
Soil moisture:	guideline requirement: 40-60 % of max. WHC during the study: 49-61 % of max. WHC
pH:	guideline requirement: 6.0 ± 0.5 during the study: 6.1 – 6.4
Application rate(s)	0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate
Negative control:	reverse osmosis (RO) water, solvent (acetone) using a sand carrier
Positive control:	Carbendazim
Post exposure observation period	56 days
Remarks	None

The potential effects of the test item RH-127450 at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia fetida* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 10 % organic matter.
The test item was solved in acetone. After addition of the solvent solution to the sand carrier, the acetone was allowed to evaporate off before the treated sand was added to the bulk test substrate and mixed in. The test substrate was pre-moistened to 27.5 % MWHC five days prior to application of the test substance. Reverse osmosis (RO) water was added to achieve the final weight required at 55% MWHC. After mixing, eight 5 g soil samples were taken from each treatment for chemical analysis (four samples to analyse and four to retain). Two samples were also taken from each treatment and the controls for analysis of water content to enable calculation of the test substance concentration in terms of substrate dry

weight. One sample was taken from each treatment for determination of pH. The remaining soil was then weighed (equivalent to 500 g dry weight) into the test vessels
Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start. After test substrate preparation, pots of 10 worms were randomised using an excel spreadsheet and allocated to the replicate test vessels. The earthworms placed onto the substrate surface. The vessels were re-covered with a perforated clear plastic film to allow for gaseous exchange and penetration of light. After approximately 15 minutes, each vessel was examined to confirm that no worms were remaining on the surface of the substrate. Food in the form of finely ground animal faeces, and RO water (5 g and 5 mL, respectively), were added on day 1 of the test and weekly thereafter up to and including day 28. Uneaten food remaining in the vessel at each feeding interval was removed and replaced with fresh food. Moisture loss due to evaporation was compensated for on a weekly basis. The test was carried out in a temperature-controlled room set at 20 ± 2°C and a 16:8 hour light:dark cycle, with light intensity of 400 – 800 Lux, measured at least weekly throughout the test.
Survival of the worms was assessed 28 days after treatment by removing the soil from the test vessels, spreading it out on a plastic sheet and locating the earthworms, which were classified as dead if they did not respond to gentle mechanical stimulus to the anterior end. Missing earthworms were also considered dead due to rapid decomposition under test conditions. Following observations on day 28 and the removal of samples for chemical analysis and determination of water content, the test substrate was carefully replaced in the test vessels. The adult worms were rinsed in RO water, blotted dry and weighed in replicate groups before being humanely discarded. On day 56, the vessels were placed in a water bath set between 40 and 60ºC, leaving approximately the top 2 cm of substrate above the water level. The vessels were then left for approximately 20 to 30 minutes to allow the juvenile worms to move to the surface of the substrate and away from the heat. The juvenile worms were then removed from the surface of the substrate and counted. When all visible worms had been removed, the substrate was spread out over a tray covered with plastic. Any juvenile worms and cocoons remaining in the substrate were removed and counted, and the total number of juveniles per vessel determined. After removal of the juveniles, soil samples were taken for chemical analysis and determination of water content and pH.
The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). Percentage mortality of the worms in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and LC_{50} were determined for adult mortality. The percentage weight change, based on mean surviving worm weight at day 28 was calculated for each vessel and a treatment no observed effect concentration (NOEC) and EC_{50} were determined empirically as there were no adverse effects. Juvenile worm numbers were determined for each vessel and a treatment mean presented. A treatment no observed effect concentration (NOEC) and EC_{10}, EC_{20} and EC_{50} values were determined empirically as there were no adverse effects on reproduction. Statistical analysis of the survival and reproduction data was not undertaken as there was no effect on survival, adult earthworm weight or the reproductive output.
The analytical procedure (SMV 3202376-01V ff.) was used to confirm the test substance concentration in the test samples. On the day of test item application, after 28 days and at the end of the test samples of 5.0 g soil were dispensed into 50 mL Falcon tubes. They were fortified as required and shaken well by hand to mix. 20 mL of MeCN was added and the sample were extracted by shaking on a rotary shaker at 200 rpm for 10 minutes. They were then sonicated for five minutes before being centrifuged at 2500 rpm for 15 minutes. A portion of the supernatant was transferred to a suitable vial for time-of-flight mass spectrometry (LC-TOF/MS) analysis. If required, samples were diluted with unfortified control extract. Aliquots of the samples were injected onto the 5600 TOF-MS system. The method was validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg mg/kg.

Results and discussion

Environmental conditions stayed within the recommended ranges with few outliers (see deviations).
Post application the mean test item concentrations were 0.1287, 0.2455, 0.4599, 0.8628, 1.6655, 3.4432, 5.54763 and 10.7197 mg a.s./kg dry substrate at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate respectively. This is equivalent to 80.43, 84.66, 86.78, 90.82, 96.83, 111.43, 98.49 and 107.20% of nominal at 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate respectively. Thus, the analytically verified recoveries of the test item in the soil substrate were

all greater than 80% (i.e. 80-111 %), confirming sufficiently high (> 80% of nominal) test item concentrations at study start.
At day 28, there was a mean gain in weight 68.0, 68.1, 72.0, 71.6, 63.5, 67.9, 63.8 and 58.1% in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups, respectively in comparison to 66.3 and 63.4% in the water and solvent controls respectively. This was equivalent to increases of 2.6, 2.7, 8.6, 8.0 and 2.6% in the 0.16, 0.29, 0.53, 0.95 and 3.09 mg a.s./kg dry substrate groups, respectively in comparison to the water control with decreases of 4.2, 3.6 and 12.4% in the 1.71, 5.56 and 10 mg a.s./kg dry substrate groups, respectively. This was equivalent to increases of 7.3, 7.4, 13.6, 12.9, 0.2, 7.1 and 0.6% in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.s./kg dry substrate groups, respectively in comparison to the solvent controls with a decrease of 8.4% in the 10 mg a.s./kg dry substrate group. The NOEC and EC_{50} for overall weight change were determined to be 10 mg a.s./kg dry substrate and >10 mg a.s./kg dry substrate, respectively.

Table 10.4.1-4: Mean live weight of individual *E. fetida* per treatment and percentage weight change from days 0 to 28

Nominal concentration (mg a.s./kg dry substrate	Live weight (mg)		Change (% increase)	% Effect in comparison to the water control	% Effect in comparison to the solvent control
	Day 0	Day 28			
Water control	448.6	745.5	66.3	N/A	+4.4
Solvent control	457.6	747.8	63.4	-4.4	N/A
0.16	442.8	743.9	68.0	+2.6	+7.3
0.29	459.0	771.3	68.1	+2.7	+7.4
0.53	454.4	781.6	72.0	+8.6	+13.6
0.95	436.7	749.3	71.6	+8.0	+12.9
1.72	464.6	759.8	63.5	-4.2	+0.2
3.09	465.4	781.3	67.9	+2.4	+7.1
5.56	473.0	774.9	63.8	-3.8	+0.6
10	485.5	767.6	58.1	-12.4	-8.4

N/A = not applicable

After 28 days of exposure, 1.25% mortality was recorded in the water control and 2.5% mortality was recorded at 0.53 and 3.09 mg a.s./kg dry substrate. Based on these results, the NOEC for 28-day adult survival was determined to be 10 mg a.s./kg dry substrate, and the LC_{50} for 28-day survival was empirically determined to be >10 mg a.s./kg dry substrate. The NOEC and EC_{50} for overall weight change were determined to be 10 mg a.s./kg dry substrate and >10 mg a.s./kg dry substrate, respectively.

Table 10.4.1-5: Mean treatment mortality for adult *E. fetida*

Nominal concentration (mg a.s./kg dry substrate	Mortality		
	Number of *E. fetida* exposed	Day 28 Number of mortalities	Total (%)
Water control	80	1	1.25
Solvent control	80	0	0
0.16	40	0	0
0.29	40	0	0
0.53	40	1	2.50
0.95	40	0	0

Nominal concentration (mg a.s./kg dry substrate	Mortality		
	Number of *E. fetida* exposed	Day 28 Number of mortalities	Total (%)
1.72	40	0	0
3.09	40	1	2.50
5.56	40	0	0
10	40	0	0

The mean number of juveniles per vessel was 463, 476, 434, 470, 475, 503, 466 and 517 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups respectively in comparison to 408 and 444, in the water and solvent controls respectively. This corresponded to increases in juvenile production of 13, 17, 6, 15, 16, 23, 14 and 27%, in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups, respectively, when compared to the water control. In comparison to the solvent control there were increases of 4, 7, 6, 7, 13, 5 and 16% 0.16, 0.29, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups, respectively, and a reduction of 2% at 0.53 mg a.s./kg dry substrate.
The number of cocoons was not included in the calculations as the mean number of cocoons per treatment rate ranged from 0 – 5.3 and there was no evidence of a dose response. The mean number of cocoons in the water and solvent control replicates was 1.0 and 0.5, respectively.
No other observations were noted.
Based on these results, the NOEC for reproduction was empirically determined to be 10 mg a.s./kg dry substrate. The EC_{10}, EC_{20} and EC_{50} values for reproduction were empirically determined to be >10 mg a.s./kg dry substrate.

Table 10.4.1-6: Mean number of juvenile worms at day 56

Nominal concentration (mg a.s./kg dry substrate	Mean number of juveniles	% Difference when compared to the water control	% Difference when compared to the solvent control	Mean Number of cocoons
Water control	408	N/A	-8.1	1.0
Solvent control	444	+8.8	N/A	0.5
0.16	463	+13.5	+4.3	0.0
0.29	476	+16.7	+7.2	0.8
0.53	434	+6.4	-2.3	0.3
0.95	470	+15.2	+5.9	0.5
1.72	475	+16.4	+7.0	1.0
3.09	503	+23.3	+13.3	0.0
5.56	466	+14.2	+5.0	5.3
10	517	+26.7	+16.4	0.3

N/A = not applicable
Coefficient of variance for water and solvent controls = 12.05 and 8.8% respectively

Earthworms from the same source culture as those used in the reproductive test were used in a reference toxicity test with Carbendazim, a known toxic substance (performed under in-house GLP Smithers Viscient Study Number 3202329 from December 2018 to February 2019). The EC_{50} value was estimated to be 2.05 mg a.s./kg dry soil. This is within the given toxicity range of 1 to 5 mg a.s./kg dry soil, indicating that the worms were responding as expected in the test system.

All validity criteria were met:
- The mortality of adults in the control group was ≤ 10% over the initial 28 days (actual adult mortality

= 1.3 and 0% in the water and solvent controls respectively).
- The rate of production of juveniles was ≥ 30 per control container containing 10 adults by the end of test (mean actual rate of production of juveniles = 408 and 444 in the water and solvent controls respectively).
- The coefficient of variance of reproduction in the control was 30% or less (actual coefficient of variance = 12.05 and 8.8% in the water and solvent controls respectively).

Table 10.4.1-7: Effects of the test item: summary of statistical analysis

Endpoint	mg test item/kg soil dry weight
28-day NOEC for adult survival [b]	10 mg a.s./kg dry substrate
28-day LC_{50} adult survival	>10 mg a.s./kg dry substrate
28-day NOEC for adult weight change [b]	10 mg a.s./kg dry substrate
28-day LC_{50} for adult weight change [b]	>10 mg a.s./kg dry substrate
56-day NOEC for reproduction [b]	10 mg a.s./kg dry substrate
56-day EC_{10} reproduction [b]	>10 mg a.s./kg dry substrate
56-day EC_{20} reproduction [b]	>10 mg a.s./kg dry substrate
56-day EC_{50} reproduction [b]	>10 mg a.s./kg dry substrate

[a] Rounded figure
[b] Empirically determined

Conclusion

A laboratory test was conducted in which mature *E. fetida* were exposed to RH-127450 for a period of 28 days, after which the adult worms were removed. Any cocoons produced were then allowed to hatch and the juveniles given time to mature for a further 28 days. There were no adverse effects on adult worm survival or weight over the 28-day exposure period. There were no adverse effects on reproductive capacity at day 56. The study endpoints were determined as follows:
- 28-day NOEC value for adult *E. fetida* survival = 10 mg a.s./kg dry substrate
- 28-day EC_{50} value for adult *E. fetida* survival ≥10 mg a.s./kg dry substrate
- NOEC value based on reproduction = 10 mg a.s./kg dry substrate
- EC_{10} value based on reproduction ≥10 mg a.s./kg dry substrate
- EC_{20} value based on reproduction ≥10 mg a.s./kg dry substrate
- EC_{50} value based on reproduction ≥10 mg a.s./kg dry substrate

The study is valid.

This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints for *E. fetida* (10% peat content)**:**

The 56-day NOEC value based on reproduction = 10 mg a.s./kg dw

The 56-day EC10 value based on reproduction = 7.449 mg a.s./kg dw

The 56-day EC20 value based on reproduction = 9.882 mg a.s./kg dw

The 56-day EC50 value based on reproduction ≥10 mg a.s./kg dw

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Reference:	KCA 8.4.1
Report	Gray, J., 2021: RH-24549: Effect on reproduction in the earthworm *Eisenia fetida* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202395, GLP, Not published
Guideline(s):	The study was designed in accordance with OECD Guideline for Testing of Chemicals, Earthworm Reproduction Test, No. 222, Adopted 29 July 2016
Deviations:	The light intensity deviated from the guideline times of 16:8 hours light:dark on one occasion during the reproduction phase of the test, when the timer was still recording a dark period after 15.05 hours. The soil used in the study had a peat content of 8.1% and not 10% as stated in the protocol. The pipette used to adjust the soil moisture content on day 35 (06 August), day 42 (13 August 2019) and day 49 (20 August 2019) was found to have failed to meet the required criteria when calibrated on 27 August 2019. At day 56 the soil moisture content was equivalent to > 60% MWHC (the guideline maximum, not referenced in the protocol) in the water control and at 0.16, 0.29, 0.53, and 1.72 mg a.s./kg dry substrate, with a maximum of 62.80 % at 0.53 mg a.s./kg dry substrate. Chemical analysis at day 0 – recovery in one of the eight treatment rates was below the 80% minimum requirement of the analytical procedure documentation (actual recovery for the 5.56 mg a.s./kg dry substrate was 73.05 % of nominal, equivalent to 4.06 mg a.s./kg). As there was 100.14% recovery at the maximum rate of application and the applied dose of 4.06 mg a.s./kg dry substrate was higher than for the 3.09 mg a.s./kg dry substrate a dose sequence was maintained. These deviations were not considered to have had an adverse impact on the study as all the validity criteria were met. For the application rates where the soil moisture content was equivalent to > 60 % MWHC at day 56 the mean number of juvenile worms produced at each rate was within the overall range for the study (minimum of 131 at 10 mg a.s./kg dry substrate and maximum of 185 at 5.56 mg a.s./kg dry substrate). As the NOEC for reproduction was determined to be 10 mg a.s./kg dry substrate and the EC_{50} value was >10 mg a.s./kg dry substrate the deviations in % MWHC were not considered to have any impact on the number of juveniles produced. In addition, the EC_{10} and

EC_{20} were >1.72 mg a.s./kg dry substrate the maximum rate at which the % MWHC exceeded the guideline maximum. Therefore, the increase in % MWHC is not considered to have had an adverse impact on the integrity or outcome of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test material (Lot/Batch No.)	RH-24549 (FCC25806)
Active substance content or purity	99.59 % (w/w)
Species	Earthworm *Eisenia fetida*
Age:	adult worms, sexually mature (i.e. between 2 and 12 months old with clitellum), weighing between 300 and 600 mg (wet mass)
Source:	Bias Labs Ltd., UK
Weight:	356-453 mg/worm (mean, day 0)
Acclimation period:	at least 24 hours in the artificial substrate with feed
Food:	finely ground animal faeces *ad libitum*
Test system	1 L glass test vessel containing ten earthworms in the equivalent of approximately 500 g dry weight of artificial soil substrate maintained at nominally 55% maximum water holding capacity (MWHC)
Soil:	artificial soil, 10% peat
Number of animals:	80 (control) 40 (test item treatment)
Number of replicates:	4 replicates per treatment rate, 8 per controls
Environmental conditions	
Temperature:	18.6 - 22°C
Photoperiod:	light: dark = 16 h: 8 h; 427 to 778 lux
Soil moisture:	guideline requirement: 40-60 % of max. WHC during the study: 48-63 % of max. WHC
pH:	guideline requirement: 6.0 ± 0.5 during the study: 6.5 – 7.66

Application rate(s)	0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate
Negative control:	reverse osmosis (RO) water, sand carrier (The test substance was applied dry using a sand carrier.)
Positive control:	Carbendazim
Post exposure observation period	56 days
Remarks	None

The potential effects of the test item RH-24549 at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia fetida* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 10 % organic matter.
The test substance was applied dry using a sand carrier before the treated sand was added to the bulk test substrate and mixed in. The test substrate was pre-moistened to 27.5% MWHC four days prior to application of the test substance. Reverse osmosis (RO) water was added to achieve the final weight required at 55% MWHC. After mixing, eight 5 g soil samples were taken from each treatment for chemical analysis (four samples to analyse and four to retain). Two samples were also taken from each treatment and the controls for analysis of water content to enable calculation of the test substance concentration in terms of substrate dry weight. One sample was taken from each treatment for determination of pH. The remaining soil was then weighed (equivalent to 500 g dry weight) into the test vessels
Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start. After test substrate preparation, pots of 10 worms were randomised using an excel spreadsheet and allocated to the replicate test vessels. The earthworms placed onto the substrate surface. The vessels were re-covered with a perforated clear plastic film to allow for gaseous exchange and penetration of light. After approximately 15 minutes, each vessel was examined to confirm that no worms were remaining on the surface of the substrate. Food in the form of finely ground animal faeces, and RO water (5 g and 5 mL, respectively), were added on day 1 of the test and weekly thereafter up to and including day 28. Uneaten food remaining in the vessel at each feeding interval was removed and replaced with fresh food. Moisture loss due to evaporation was compensated for on a weekly basis. The test was carried out in a temperature-controlled room set at 20 ± 2°C and a 16:8 hour light:dark cycle, with light intensity of 400 – 800 Lux, measured at least weekly throughout the test.
Survival of the worms was assessed 28 days after treatment by removing the soil from the test vessels, spreading it out on a plastic sheet and locating the earthworms, which were classified as dead if they did not respond to gentle mechanical stimulus to the anterior end. Missing earthworms were also considered dead due to rapid decomposition under test conditions. Following observations on day 28 and the removal of samples for chemical analysis and determination of water content, the test substrate was carefully replaced in the test vessels. The adult worms were rinsed in RO water, blotted dry and weighed in replicate groups before being humanely discarded. On day 56, the vessels were placed in a water bath set between 40 and 60ºC, leaving approximately the top 2 cm of substrate above the water level. The vessels were then left for approximately 20 to 30 minutes to allow the juvenile worms to move to the surface of the substrate and away from the heat. The juvenile worms were then removed from the surface of the substrate and counted. When all visible worms had been removed, the substrate was spread out over a tray covered with plastic. Any juvenile worms and cocoons remaining in the substrate were removed and counted, and the total number of juveniles per vessel determined. After removal of the juveniles, soil samples were taken for chemical analysis and determination of water content and pH.
The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). Percentage mortality of the worms in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and LC_{50} were determined for adult mortality. The percentage weight change, based on mean surviving worm weight at day 28 was calculated for each vessel and a treatment no observed effect concentration (NOEC) and EC_x were determined. Due to the low effect of the test substance on adult survival and weight change, the endpoints for these data have been empirically

determined. Juvenile worm numbers were determined for each vessel and a treatment mean presented. A treatment no observed effect concentration (NOEC) and EC_{10}, EC_{20} and EC_{50} values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. The NOEC for number of juveniles was determined using a Bonferroni Adj t Test and the ECx values were determined using Linear Interpolation (ICPIN).
The analytical procedure (SMV 3202395-05V) was used to confirm the test substance concentration in the test samples on the day of test item application, after 28 days and at the end of the test. Concentrations of RH-24549 were determined by extracting soil samples with acetonitrile containing 1% formic acid, then diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS) system or liquid chromatography triple quadrupole mass spectrometry (LC-TQMS). The method was validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg mg/kg.

Results and discussion

Environmental conditions stayed within the recommended ranges (with the deviations reported above).
Post application the mean soil concentrations were 0.1728, 0.3006, 0.4737, 0.8424, 1.5507, 2.8239, 4.0618 and 10.0142 mg a.s./kg dry substrate at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate respectively. This is equivalent to 107.99, 103.65, 89.37, 88.68, 90.16, 91.39, 73.05 and 100.14% of nominal at 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate respectively. RH-24549 was not detected in the water control samples. Thus, the analytically verified recoveries of the test item in the soil substrate were 73.05-108 %, confirming sufficiently high (> 80% of nominal) test item concentrations at study start (besides 1 treatment, for which only 73.05 % of nominal could be recovered).
At day 28, there was a mean gain in weight of 38.4, 38.8, 44.3, 39.4, 36.5, 33.8, 37.2, 35.8 and 34.2% in the 0 (water control), 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups, respectively. This was equivalent to changes of +1.0, +15.4, +2.6, -5.0, -12.0, -3.1, -6.8 and -10.9% in comparison to the water control. The NOEC and EC_{50} for overall weight change were determined empirically to be 10 mg a.s./kg dry substrate and >10 mg a.s./kg dry substrate, respectively.

Table 10.4.1-8: Mean live weight of individual *E. fetida* per treatment and percentage weight change from days 0 to 28

Nominal concentration (mg a.s./kg dry substrate)	Live weight (mg)		Increase in mean weight (%)	% Effect in comparison to the water control
	Day 0	Day 28		
Water control	416.8	576.9	38.4	-
0.16	403.9	560.7	38.8	+1.0
0.29	408.9	590.1	44.3	+15.4
0.53	406.3	566.5	39.4	+2.6
0.95	392.1	535.1	36.5	-5.0
1.72	420.1	562.2	33.8	-12.0
3.09	420.0	576.3	37.2	-3.1
5.56	420.0	570.4	35.8	-6.8
10	413.8	555.5	34.2	-10.9

N/A = not applicable

After 28 days of exposure, no adult mortality or sub-lethal effects were recorded in the controls or in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 or 10 mg a.s./kg dry substrate groups. Based on these results, the NOEC for 28-day adult survival was determined to be 10 mg a.s./kg dry substrate, and the LC_{50} for 28-day survival was empirically determined to be >10 mg a.s./kg dry substrate.

Table 10.4.1-9: Mean treatment mortality for adult *E. fetida*

Nominal concentration (mg a.s./kg dry substrate)	Mortality		
	Number of *E. fetida* Exposed	Day 28 Number of mortalities	Total (%)
Water control	80	0	0
0.16	40	0	0
0.29	40	0	0
0.53	40	0	0
0.95	40	0	0
1.72	40	0	0
3.09	40	0	0
5.56	40	0	0
10	40	0	0

The mean number of juveniles per vessel was 165, 173, 155, 168, 158, 165, 185 and 131 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups respectively in comparison to 149 in the water control. This corresponded to an increase in juveniles of 10.7, 16.1, 4.0, 12.8, 6.0, 10.7 and 24.2%, in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.s./kg dry substrate groups, respectively, when compared to the water control. There was a reduction of 12.1% in the number of juveniles at 10 mg a.s./kg dry substrate in comparison to the water control.
The number of cocoons was not included in the calculations as the mean number of cocoons per treatment rate ranged from 0.3 – 3.3 and there was no evidence of a dose response. The mean number of cocoons in the water control replicates was 1.3.
No adverse behavioural observations or morphological symptoms were recorded.

Table 10.4.1-10: Mean number of juvenile worms at day 56

Nominal concentration (mg a.s./kg dry substrate	Mean number of juveniles	% Difference when compared to the control	Mean Number of cocoons
Water control	149	-	1.8
0.16	165	+10.7	0.3
0.29	173	+16.1	2.0
0.53	155	+4.0	1.5
0.95	168	+12.8	1.5
1.72	158	+6.0	3.3
3.09	165	+10.7	1.0
5.56	185	+24.2	2.5
10	131	-12.1	1.5

N/A = not applicable
Coefficient of variance for controls = 28.6%

Based on these results, the NOEC for reproduction was statistically determined to be 10 mg a.s./kg dry substrate. Based on these results, the NOEC for reproduction was statistically determined to be 10 mg a.s./kg dry substrate. The ECx values for reproductive performance were determined as follows:

Table 10.4.1-11: EC_{10}, EC_{20} and EC_{50} values for reproduction

Parameter	Value (mg a.s./kg dry substrate)	95% confidence limits (mg a.s./kg dry substrate)
EC_{10}	7.449	4.664– 9.113
EC_{20}	9.882	6.752 – N/A
EC_{50}	>10	N/A

N/A= not available

Earthworms from the same source culture as those used in the reproductive test were used in a reference toxicity test with Carbendazim, a known toxic substance (performed under in-house GLP Smithers Viscient Study Number 3202329 from December 2018 to February 2019). The EC_{50} value was estimated to be 2.05 mg a.s./kg dry soil. This is within the given toxicity range of 1 to 5 mg a.s./kg dry soil, indicating that the worms were responding as expected in the test system.

All validity criteria were met:
- The mortality of adults in the control group was ≤ 10% over the initial 28 days (actual adult mortality = 0%).
- The rate of production of juveniles was ≥ 30 per control container containing 10 adults by the end of test (mean actual rate of production of juveniles = 149).
- The coefficient of variance of reproduction in the control was 30% or less (actual coefficient of variance = 28.6%).

Conclusion

A laboratory test was conducted in which mature *E. fetida* were exposed to RH-24549 for a period of 28 days, after which the adult worms were removed. Any cocoons produced were then allowed to hatch and the juveniles given time to mature for a further 28 days. The study endpoints were determined as follows:
- 28-day NOEC value for adult *E. fetida* survival = 10 mg a.s./kg dry substrate
- 28-day LC_{50} value for adult *E. fetida* survival ≥10 mg a.s./kg dry substrate
- NOEC value based on adult weight change = 10 mg a.s./kg dry substrate
- EC_{50} value based on adult weight change ≥10 mg a.s./kg dry substrate
- NOEC value based on reproduction = 10 mg a.s./kg dry substrate
- EC_{10} value based on reproduction = 7.449 mg a.s./kg dry substrate
- EC_{20} value based on reproduction = 9.882 mg a.s./kg dry substrate
- EC_{50} value based on reproduction ≥10 mg a.s./kg dry substrate

The study is valid.

This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints for *E. fetida* (10% peat content)**:**

The 56-day NOEC (reproduction) determined to be = 10 mg a.s./kg dw

The 56-day EC10, EC20, EC50 (reproduction) determined to be >10 mg a.s./kg dw

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Reference:	KCA 8.4.1
Report	Gray, J., 2021: RH-163353: Effect on reproduction in the earthworm *Eisenia fetida* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202389, GLP, Not published
Guideline(s):	OECD 222 (2016)
Deviations:	At day 28 the soil moisture content was equivalent to 37.59% MWHC (below the guideline minimum of 40%, not referenced in the protocol) at 10 mg a.s./kg dry substrate. Any moisture loss was made up when the soil was returned to the test vessel. The data shows that the Day 56 the moisture content was >40% MWHC and there was no effect on survival (all LCx values >10 mg a.s./kg dry substrate) or the percentage gain in adult weight over the 28-day exposure period. In addition, the number of juveniles produced was greater than in both controls with all the ECx values being >10 mg a.s./kg dry substrate. Therefore, this deviation was considered to have no impact on the integrity or outcome of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-163353 (HHGCP001-00-2)
Active substance content or purity	99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)
Species	Earthworm *Eisenia fetida*
Age:	adult worms, sexually mature (i.e. between 2 and 12 months old with clitellum), weighing between 300 and 600 mg (wet mass)
Source:	Bias Labs Ltd., UK
Weight:	380 - 488 mg/worm (mean, day 0)
Acclimation period:	at least 24 hours in the artificial substrate with feed
Food:	finely ground animal faeces *ad libitum*
Test system	1 L glass test vessel containing ten earthworms in the equivalent of approximately 500 g dry weight of artificial soil substrate maintained

	at nominally 45% maximum water holding capacity (MWHC)
Soil:	artificial soil, 10% peat
Number of animals:	80 (control) 40 (test item treatment)
Number of replicates:	4 replicates per treatment rate, 8 per controls
Environmental conditions	
Temperature:	18 – 21.5°C
Photoperiod:	light: dark = 16 h: 8 h; 441 to 790 lux
Soil moisture:	guideline requirement: 40-60 % of max. WHC during the study: 38 - 49 % of max. WHC
pH:	guideline requirement: 6.0 ± 0.5 during the study: 6.4 – 6.95
Application rate(s)	0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg soil dry weight
Negative control:	reverse osmosis (RO) water, solvent (acetone) using a sand carrier
Positive control:	Carbendazim
Post exposure observation period	56 days
Remarks	None

The potential effects of the test item RH-163353 at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg soil dry weight on the reproduction, mortality and growth of the earthworm *Eisenia fetida* has been studies, considering dermal and alimentary uptake after mixing of the test item with an artificial soil containing 10 % organic matter.
The test substance was diluted in acetone and applied over a sand carrier. The test substrate was pre-moistened to 22.5% MWHC three days prior to application of the test substance. The soil for the water control vessels was mixed with the same ratio of untreated sand and the solvent control was dosed with the same sand: solvent: soil ratio as the RH-163353 treatments. Reverse osmosis (RO) water was added to achieve the final weight required at 45% MWHC. After mixing, eight 5 g soil samples were taken from each treatment for chemical analysis (four samples to analyse and four to retain). Two samples were also taken from each treatment and the controls for analysis of water content to enable calculation of the test substance concentration in terms of substrate dry weight. One sample was taken from each treatment for determination of pH. The remaining soil was then weighed (equivalent to 500 g dry weight) into the test vessels.
Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for at least 24 hours before test start. After test substrate preparation, pots of 10 worms were randomised using an excel spreadsheet and allocated to the replicate test vessels. The earthworms placed onto the substrate surface. The vessels were re-covered with a perforated clear plastic film to allow for gaseous exchange and penetration of light. After approximately 15 minutes, each vessel was examined to confirm that no worms were remaining on the surface of the substrate. Food in the form of finely ground animal faeces, and RO water (5 g and 5 mL, respectively), were added on day 1 of the test and weekly thereafter up to and including day 28. Uneaten food remaining in the vessel at each feeding interval was removed and replaced with fresh food. Moisture loss due to evaporation was compensated for on a weekly basis. The test was carried out in a temperature-controlled room set at 20 ± 2°C and a 16:8 hour light:dark cycle, with light intensity of 400 – 800 Lux, measured at least weekly throughout the test.
Survival of the worms was assessed 28 days after treatment by removing the soil from the test vessels, spreading it out on a plastic sheet and locating the earthworms, which were classified as dead if they did not respond to gentle mechanical stimulus to the anterior end. Missing earthworms were also considered dead due to rapid decomposition under test conditions. Following observations on day 28 and the removal of samples for chemical analysis and determination of water content, the test substrate was carefully replaced in the test vessels. The adult worms were rinsed in RO water, blotted dry and weighed in

replicate groups before being humanely discarded. On day 56, the vessels were placed in a water bath set between 40 and 60ºC, leaving approximately the top 2 cm of substrate above the water level. The vessels were then left for approximately 20 to 30 minutes to allow the juvenile worms to move to the surface of the substrate and away from the heat. The juvenile worms were then removed from the surface of the substrate and counted. When all visible worms had been removed, the substrate was spread out over a tray covered with plastic. Any juvenile worms and cocoons remaining in the substrate were removed and counted, and the total number of juveniles per vessel determined. After removal of the juveniles, soil samples were taken for chemical analysis and determination of water content and pH.
The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). Percentage mortality of the worms in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and LC_{50} were determined for adult mortality. The percentage weight change, based on mean surviving worm weight at day 28 was calculated for each vessel and a treatment no observed effect concentration (NOEC) and EC_x were determined. Juvenile worm numbers were determined for each vessel and a treatment mean presented. A treatment no observed effect concentration (NOEC) and EC_{10}, EC_{20} and EC_{50} values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. The NOEC for survival was determined using a Wilcoxon/ Bonferroni Adj Test and the LCx values were determined using Linear Interpolation (ICPIN). The NOEC for number of juveniles was determined using a Bonferroni Adj t Test for comparison to the water control and a Wilcoxon Bonferroni Adj Test for comparison with the solvent control. The ECx values were determined using Linear Interpolation (ICPIN).
The analytical procedure (SMV 3202389-02V) was used to confirm the test substance concentration in the test samples on the day of test item application, after 28 days and at the end of the test. Concentrations of RH-163353 were determined by extracting soil samples with acetonitrile/acetonitrile 3:1 (v/v) containing 1% formic acid, then diluting further with unfortified control sample extract as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS). The method was validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg mg/kg.
The enantiomeric ratio of RH-163353 was assessed on day 56 soil samples. The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure SMV 3202586-01V). A combination of the analytical procedures, SMV 3202389-02V and SMV 3202586-01V were used to assess the enantiomeric ratio of the test substance.

Results and discussion

Environmental conditions stayed within the recommended ranges (with the deviations above).
On day 28 the mean soil concentrations were 0.0918, 0.1951, 0.3716, 0.6788, 1.2634, 2.3723, 4.1252 and 6.9682 mg a.s./kg dry substrate at application rates of 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate respectively. This is equivalent to 57.38, 67.27, 70.11, 71.45, 73.46, 76.77, 74.19 and 69.68% of nominal at 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate respectively. RH-163353 was not detected in the water or solvent control samples.
At day 28, there was a mean gain in weight of 61.1, 67.5, 64.7, 66.0, 67.2, 72.4, 72.8 and 68.2% in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups, respectively in comparison to 63.3 and 74.2% in the water and solvent controls respectively. This was equivalent to increases of 6.6, 2.2, 4.3, 6.2, 14.4, 15.0 and 7.7% in the 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups, respectively in comparison to the water control with a reduction of 3.5% at 0.16 mg a.s./kg dry substrate. This was equivalent to reductions of 17.7, 9.0, 12.8, 11.1, 9.4, 2.5, 1.9 and 8.1% the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups, respectively in comparison to the solvent control. The NOEC and EC_{50} for overall weight change were determined empirically to be 10 mg a.s./kg dry substrate and >10 mg a.s./kg dry substrate, respectively.

Table 10.4.1-12: Mean live weight of individual *E. fetida* per treatment and percentage weight change from days 0 to 28

Nominal concentration (mg a.s./kg dry substrate)	Live weight (mg)		Increase in mean weight (%)	% Effect in comparison to the water control	% Effect in comparison to the solvent control
	Day 0	Day 28			
Water control	438.8	716.5	63.3	-	-14.7
Solvent control	409.6	713.3	74.2	+17.2	-
0.16	446.7	719.6	61.1	-3.5	-17.7
0.29	436.0	730.1	67.5	+6.6	-9.0
0.53	440.3	724.9	64.7	+2.2	-12.8
0.95	532.4	717.9	66.0	+4.3	-11.1
1.72	415.8	695.0	67.2	+6.2	-9.4
3.09	436.2	752.2	72.4	+14.4	-2.5
5.56	404.2	698.4	72.8	+15.0	-1.9
10	425.4	715.3	68.2	+7.7	-8.1

N/A = not applicable

After 28 days of exposure, no adult mortality or sub-lethal effects were recorded in the controls or in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 or 10 mg a.s./kg dry substrate groups. Based on these results, the NOEC for 28-day adult survival was determined to be 10 mg a.s./kg dry substrate, and the LC_{10}, LC_{20} and LC_{50} for 28-day survival was empirically determined to be >10 mg a.s./kg dry substrate.

Table 10.4.1-13: Mean treatment mortality for adult *E. fetida*

Nominal concentration (mg a.s./kg dry substrate)	Mortality		
	Number of *E. fetida* exposed	Day 28 Number of mortalities	Total (%)
Water control	80	0	0
Solvent control	80	0	0
0.16	40	0	0
0.29	40	0	0
0.53	40	0	0
0.95	40	0	0
1.72	40	0	0
3.09	40	0	0
5.56	40	0	0
10	40	0	0

The mean number of juveniles per vessel was 211, 266, 199, 160, 219, 185, 190 and 276 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups respectively in comparison to 257 in the water control and 188 in the solvent control. This corresponded to a decrease in the number of juveniles of 10.6, 17.9, 22.6, 38.0, 15.1, 28.2 and 26.1%, in the 0.16, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.s./kg dry substrate groups, respectively, when compared to the water control. There were increases of 3.2 and 7.0% in the number of juveniles at 0.29 and 10 mg a.s./kg dry substrate respectively in comparison to the water control. When compared to the solvent control there were increases of 12.4, 41.3, 6.1, 16.3, 1.3 and 46.6% at 0.16, 0.29, 0.53, 1.72, 5.56 and 10 mg a.s./kg dry substrate groups respectively and decreases of 15.1 and 1.7% at 0.95 and 3.09 mg a.s./kg dry substrate groups respectively.

As the mean number of cocoons per treatment rate ranged from 24.8 – 43.5 with no evidence of a dose response, the effects on reproduction have been evaluated below using the mean number of juveniles plus cocoons in each treatment. The mean number of cocoons in the water and solvent control replicates was 30.0 and 44.3 respectively.

The mean number of juveniles plus cocoons per vessel was 255, 294, 234, 199, 246, 215, 215 and 300 in the 0.16, 0.29, 0.53, 0.95, 1.72, 3.09, 5.56 and 10 mg a.s./kg dry substrate groups respectively in comparison to 285 in the water control and 232 in the solvent control. This corresponded to a decrease in the number of juveniles plus cocoons of 10.6, 17.9, 30.1, 13.6, 24.5 and 24.4%, in the 0.16, 0.53, 0.95, 1.72, 3.09 and 5.56 mg a.s./kg dry substrate groups, respectively, when compared to the water control. There were increases of 3.2 and 5.3% in the number of juveniles at 0.29 and 10 mg a.s./kg dry substrate respectively in comparison to the water control. When compared to the solvent control there were increases of 9.9, 26.8, 0.9, 6.1 and 29.3% at 0.16, 0.29, 0.53, 1.72 and 10 mg a.s./kg dry substrate groups respectively and decreases of 14.1, 7.3 and 7.3% at 0.95, 3.09 and 5.56 mg a.s./kg dry substrate groups respectively. Each cocoon has been included as representative of one juvenile worm.

Table 10.4.1-14: Mean number of juvenile worms at day 56

Nominal concentration (mg a.s./kg dry substrate	Mean number of juveniles including cocoons	% Difference when compared to the water control	% Difference when compared to the solvent control	Mean number of cocoons
Water control	285	-	+22.8	30.0
Solvent control	232	-18.6	-	44.3
0.16	255	-10.6	+9.9	43.5
0.29	294	+3.2	+26.8	28.5
0.53	234	-17.9	+0.8	34.5
0.95	199	-30.1	+14.1	39.8
1.72	246	-13.6	6.1	27.5
3.09	215	-24.5	-7.3	30.3
5.56	215	-24.4	-7.2	25.0
10	300	+29.5	+29.5	24.8

N/A = not applicable
Coefficient of variance for controls = 18.88 and 18.68% for the water and solvent controls respectively

Although it appears the solvent control had an effect on the production of juveniles, in comparison to the water control, this is not considered to be a genuine effect as no solvent would have been present at the time the adult earthworms were introduced into the test vessels. In addition, the number of juveniles produced in the solvent control and at each treatment rate was significantly higher than the validity criterion of 30, indicating a healthy test system. Comparison of the treatment data with the solvent control also shows that the potential presence of the solvent had no additional adverse impact on the number of juveniles produced as for the majority of treatment rates the mean number of juveniles was greater than in the solvent control.
Based on these results, the NOEC for reproduction was statistically determined to be 10 mg a.s./kg dry substrate. The EC_{10}, EC_{20} and EC_{50} values for reproduction in comparison to both water and solvent controls were statistically determined to be >10 mg a.s./kg dry substrate:
No adverse behavioural observations or morphological symptoms were recorded.
Earthworms from the same source culture as those used in the reproductive test were used in a reference toxicity test with Carbendazim, a known toxic substance (performed under in-house GLP Smithers Viscient Study Number 3202329 from December 2018 to February 2019). The EC_{50} value was estimated to be 2.05 mg a.s./kg dry soil. This is within the given toxicity range of 1 to 5 mg a.s./kg dry soil, indicating that the worms were responding as expected in the test system.

All validity criteria were met:
- The mortality of adults in the control group was ≤ 10% over the initial 28 days (actual adult mortality = 0% in both water and solvent controls).
- The rate of production of juveniles was ≥ 30 per control container containing 10 adults by the end of test (mean actual rate of production of juveniles including cocoons = 285 and 232 in the water and solvent controls respectively).
- The coefficient of variance of reproduction in the control was 30% or less (actual coefficient of variance = 18.88 and 18.68% in the water and solvent controls respectively).

Conclusion

A laboratory test was conducted in which mature *E. fetida* were exposed to RH-163353 for a period of 28 days, after which the adult worms were removed. Any cocoons produced were then allowed to hatch and the juveniles given time to mature for a further 28 days. There were no adverse effects on adult worm weight over the 28-day exposure period. The study endpoints were determined as follows:

Table 10.4.1-15: Effects of the test item: summary of statistical analysis

Endpoint	mg test item/kg soil dry weight
28-day NOEC for adult survival [b]	10 mg a.s./kg dry substrate
28-day LC_{50} adult survival	>10 mg a.s./kg dry substrate
28-day NOEC for adult weight change [b]	10 mg a.s./kg dry substrate
28-day LC_{50} for adult weight change [b]	>10 mg a.s./kg dry substrate
56-day NOEC for reproduction [b]	10 mg a.s./kg dry substrate
56-day EC_{10} reproduction [b]	>10 mg a.s./kg dry substrate
56-day EC_{20} reproduction [b]	>10 mg a.s./kg dry substrate
56-day EC_{50} reproduction [b]	>10 mg a.s./kg dry substrate

[a] Rounded figure
[b] Empirically determined
N/A = not applicable

The validity criteria were met, and therefore the study was considered valid.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

This active substance related study has already been provided to the RMS Latvia. Thus, the sum-mary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

It can be concluded that Zoxium 240 SC (containing nominally 240 g/L zoxamide) tested to bare soil at application rates of 5 x 140 g a.s./ha (0.5833 L test item/ha), 5 x 180 g a.s./ha (0.75 L test item/ha) and 5 x 280 g a.s./ha (1.1667 L test item/ha) with an interval of 7-8 days had no adverse effects on single species, ecological groups and total earthworm abundance and biomass one year after the first application.

Reference:	KCA 8.4.1
Report	Schulz, L., 2020: Effects of Zoxium 240 SC on earthworms under field conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 18 48 FEW 0001, GLP, Not published
Guideline(s):	ISO 11268-3 (2014) Technical recommendations to ISO 11268-3 (Kula et al. 2006) Guidance for summarising earthworm field studies (De Jong et al. 2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (18011201-72-52)
Active substance content or purity	zoxamide 240 g/L (nominal), 21.7 % w/w (analysed) isomeric ratio of R-/S-zoxamide = 49.8 / 50.2
Test organisms	Naturally occurring earthworm (Annelida: *Oligochaeta*) population with an abundance greater 60 ind./m² and two dominant species representing different life forms (*Aporrectodea caliginosa* and *Lumbricus terrestris*) present at 10 % or at least 10 to 15 ind./m², with a relative homogenous distribution on a typical arable field.
Test system	20 plots, each 10 m x 10 m, were arranged in a randomised block design. The test item applications were performed on bare soil. About 1 month after the last application, the test field was seeded with the fodder crop “Landsberger Gemenge” (clover grass mixture) which stayed on the field until the end of the study.
Field:	Typical arable field near Dornreichenbach in Saxony, Germany. Soil textural class: sandy-loamy silt (DIN 4220) / silt loam (USDA), mean pH (CaCl2) 6.0, mean total organic carbon content 1.30 % and mean maximum water holding capacity 42.8 g/100 g soil dry weight.
Number of replicates:	Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion.
Surface monitoring:	Assessment of alive, moribund and dead earthworms on the soil surface in a monitoring area of 20.0 m² per plot in all control and test item

	replicates from day 1 to day 3 after each application.
Earthworm sampling:	Hand sorting combined with formalin extraction.
Environmental conditions	
Air temperature during application:	12.3 – 27.8 °C
Soil moisture during applications:	8.60 – 19.3 % w/w
Application rate(s)	5 applications at each: 1) 140 g a.s./ha (0.5833 L test item/ha) = total of 700 g a.s./ha/season 2) 180 g a.s./ha (0.75 L test item/ha) = total of 900 g a.s./ha/season 3) 280 g a.s./ha (1.1667 L test item/ha) = total of 1400 g a.s./ha/season
Reference item:	20 L Maypon Flow/ha in 600 L water/ha (equivalent to nominally 10 kg carbendazim/ha)
Post exposure observation period	1st sampling about 1 month after 1st application 2nd sampling about 6 months after 1st application 3rd sampling about 12 months after 1st application
Remarks	None

Potential effects and potential recovery of field populations of earthworms after the spray application of Zoxium 240 (containing nominally 240 g/L zoxamide) to bare soil (silt loam according to USDA) at a pattern of 5x 140 g a.s./ha (0.5833 L test item/ha) = total of 700 g a.s./ha/season, 5x 180 g a.s./ha (0.75 L test item/ha) = total of 900 g a.s./ha/season and 5x 280 g a.s./ha (1.1667 L test item/ha) = total of 1400 g a.s./ha/season with an interval of 7-8 days were investigated on a typical arable field located near Dornreichenbach in Saxony, Germany. The results with regard to earthworm species composition, biomass and abundance were compared to an untreated control and a reference item (Maypon Flow, containing nominally 50 % w/v carbendazim) applied at a rate of 20 L/ha, corresponding to 10 kg a.s./ha, in parallel to the 1st test item application. The correct test item application has been verified by analysis of soil samples taken immediately after applications with a method validated according to SANCO/3029/99 rev. 4 (11/07/2000).
Twenty plots, each 10 m x 10 m, were arranged in a 5 x 4 formation, each plot surrounded by a 2 m wide path - also between the plots. The set-up was a randomised block design. The assignment of the treatment groups to the plots was based on the results of a pre-sampling. The pre-sampling was conducted to determine the density, diversity and homogeneity of earthworm populations at the site.
Defined areas were sampled to assess earthworm populations before application and three times after application, i.e., about 1, 6 and 12 months after the 1st test item application. Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion by combining hand sorting with formalin extraction in the excavated hole (according to DIN ISO 23611-1, 2006).
Directly after digging out the soil, 2.5 L of a 0.2 % formaldehyde solution were uniformly poured into the hole in 2 or 3 portions according to seepage capacity. The total duration of the formaldehyde extraction was at least 30 minutes. All earthworms coming to the surface were collected and placed into a vessel containing water, one for each sample. Earthworms found by hand sorting were collected in separate vessels containing water, one for each sample. Vessels with earthworms were stored in a cold room in the dark for approximately 48 hours. The abundance of earthworms was recorded and the weight (biomass) determined with a precision balance for each sample separately. Before the worms were weighed each single worm was placed on a dry filter paper to free the worm body from excess water. Animals were counted, weighed, and species identified in a period of approximately 48 hours after sampling.
Environmental conditions (air temperature, relative air humidity, soil temperature (10 cm height), wind speed, wind direction, cloudiness, rainfall, and soil moisture) were assessed during the test item applications. Furthermore, soil moisture, soil temperature, vegetation coverage (in % covered soil surface) and vegetation height were evaluated during each sampling occasion.
Statistical analysis was carried out with the statistical software package ToxRat Professional. Data were analysed for normal distribution with the Shapiro-Wilk's-test or Kolmogorov-Smirnoff-test and for homogeneity in variance with the Levene's test. Afterwards, pre-sampling data were analysed with a two-factorial analysis of variance (ANOVA, 5% significance level) with treatment as fixed factor and block as

random factor. Post-treatment sampling data were analysed for monotone dose-response using trend analysis by contrasts. Afterwards, the data were analysed by a one-sided Williams-t-test, Dunnett's-t-test or Welch-t-test after Bonferroni-Holm with test item treatment group < control as well as Student-t-test or Welch-t-test with reference item treatment group < control at the 5 % significance level. Test item and reference item effects were analysed in separate analyses.

Results and discussion

The test item has been applied on days with low wind speed and no rain during and after application. Due to warm and dry weather conditions during 2018, irrigation of the test field was required to support the exposure of the test organisms. The test field was irrigated on day 2 after the 1st application, day 1 after 2nd application, day 1 after 3rd application, day 2 after 4th application and day 1 after 5th application with each 10 mm.

No measurable residues (< LOQ) of zoxamide were determined in any of the soil samples of the control plots taken immediately after each application.

The calculated zoxamide concentrations in a 5 cm deep soil layer based on a soil bulk density of 1.5 g/cm³ amount to 0.187, 0.241 and 0.374 mg/kg soil dry weight for a single application at the low, middle and high test rate, respectively. The residue levels analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the low test item treatment group (5 x 140 g a.s./ha) were 0.247, 0.434, 0.254, 0.424 and 0.342 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.187 mg/kg just after application. The residue levels analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the middle test item treatment group (5 x 180 g a.s./ha) were 0.264, 0.472, 0.367, 0.564, and 0.454 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.241 mg/kg just after application. The residue levels analysed in the field soil samples just after each application (sampling depth: of 0 - 5 cm) of the high test item treatment group (5 x 280 g a.s./ha) were 0.403, 0.641, 0.687, 1.065 and 0.793 mg a.s./kg soil d.w. after 1st, 2nd, 3rd, 4th and 5th application – and thus always above the nominal soil concentration of 0.374 mg/kg just after application. After each test item application to the soil, zoxamide degrades with a geometric mean DT_{50} value of 5.5 days (20°C, pF2; see EFSA, 2017).

Earthworm species found in the plots of the field site at pre-sampling were the endogeic species *Aporrectodea chlorotica* (2.7 % of total earthworms), *Aporrectodea caliginosa* (80.3 % of total earthworms) and *Aporrectodea rosea* (5.3 % of total earthworms) as well as the anecic species *Aporrectodea longa* (<0.1 % of total earthworms) and *Lumbricus terrestris* (11.0 % of total earthworms). The presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris*, representing different ecological groups, indicated the suitability of the field site.

The mean earthworm abundance in the control plots was 337.0 ind./m² at pre-sampling, 49.5 ind./m² at 1st sampling, 89.5 ind./m² at 2nd sampling and 271.0 ind./m² at 3rd sampling.

The surface monitoring on days 1 - 3 after each application showed that there were no acute primary effects on earthworms by Zoxium 240 SC. No alive, moribund or dead earthworms were found on the soil surface, neither in the test item treated nor in the untreated control areas.

No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rates of 5 x 140 g a.s./ha (nominal), 5 x 180 g a.s./ha (nominal) and 5 x 280 g a.s./ha (nominal) about 1, 6 and 12 months after 1st application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species (*Aporrectodea chlorotica*, *Aporrectodea caliginosa*, *Aporrectodea rosea* and *Lumbricus terrestris*) and ecological groups (endogeic and anecic earthworms) could be observed for the tested application rates about 1, 6 and 12 months after the 1st application.

The toxic reference item reduced the total earthworm abundance by 58.6 % at 1st sampling, 37.4 % at 2nd sampling and 29.9 % at 3rd sampling. *Lumbricus terrestris* was the most sensitive species and was reduced in total abundance by 87.5 % at 1st sampling, 100 % at 2nd sampling and 63.6 % at 3rd sampling. The total earthworm biomass was reduced by the reference item by 66.6 % at 1st sampling, 47.7 % at 2nd sampling and 41.3 % at 3rd sampling. *Lumbricus terrestris* was the most sensitive species and was reduced in total biomass by 96.2 % at 1st sampling, 100 % at 2nd sampling and 98.8 % at 3rd sampling. These results clearly indicated the effect of the toxic reference item and thus the suitability of the test system and the validity of the field study.

Table 10.4.1-16: Mean abundance of the earthworm populations

	Treatment group	Abundance (ind./m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
Total abundance	Control	337.0	49.5	89.5	271.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	363.5	50.0	121.0	302.0
		(107.9%)	(101.0%)	(135.2%)	(111.4%)
	Test item (middle rate)	359.5	28.5	81.0	306.5
		(106.7%)	(57.6%)	(90.5%)	(113.1%)
	Test item (high rate)	347.0	36.5	90.0	294.0
		(103.0%)	(73.7%)	(100.6%)	(108.5%)
	Reference item	333.0	**20.5**	**56.0**	190.0
		(98.8%)	**(41.4%)**	**(62.6%)**	(70.1%)
Total adult abundance	Control	155.5	11.0	16.0	75.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	150.5	6.5	17.0	87.0
		(96.8%)	(59.1%)	(106.3%)	(116.0%)
	Test item (middle rate)	157.5	6.0	11.5	91.0
		(101.3%)	(54.5%)	(71.9%)	(121.3%)
	Test item (high rate)	134.5	5.0	10.0	82.5
		(86.5%)	(45.5%)	(62.5%)	(110.0%)
	Reference item	132.0	4.5	0.5	**34.0**
		(84.9%)	(40.9%)	(3.1%)	**(45.3%)**
Total juvenile abundance	Control	170.0	38.5	73.5	177.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	201.5	43.0	104.0	192.5
		(118.5%)	(111.7%)	(141.5%)	(108.5%)
	Test item (middle rate)	182.5	22.0	69.5	193.5
		(107.4%)	(57.1%)	(94.6%)	(109.0%)
	Test item (high rate)	192.0	30.0	79.5	190.0
		(112.9%)	(77.9%)	(108.2%)	(107.0%)
	Reference item	182.5	**16.0**	55.5	141.5
		(107.4%)	**(41.6%)**	(75.5%)	(79.7%)
***Allolobophora chlorotica* (total)**	Control	19.0	12.0	8.0	13.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	19.5	9.0	10.5	9.5
		(102.6%)	(75.0%)	(131.3%)	(70.4%)
	Test item (middle rate)	9.0	1.5	1.0	5.5
		(47.4%)	(12.5%)	(12.5%)	(40.7%)
	Test item (high rate)	0.0	0.0	0.0	0.0
		-	-	-	-
	Reference item	0.0	0.0	0.0	0.5
		-	-	-	(3.7%)
***Allolobophora chlorotica* (adults)**	Control	16.5	5.0	0.0	0.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	14.0	2.5	0.5	0.0
		(84.8%)	(50.0%)	-	-
	Test item (middle rate)	8.5	1.0	0.0	0.0
		(51.5%)	(20.0%)	-	-
	Test item (high rate)	0.0	0.0	0.0	0.0
		-	-	-	-
	Reference item	0.0	0.0	0.0	0.0
		-	-	-	-
***Allolobophora chlorotica* (juveniles)**	Control	2.5	7.0	8.0	13.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item	5.5	6.5	10.0	9.5

	Treatment group	**Abundance (ind./m²)**			
		pre-sampling	**1st sampling**	**2nd sampling**	**3rd sampling**
	(low rate)	(220.0%)	(92.9%)	(125.0%)	(70.4%)
	Test item (middle rate)	0.5	0.5	1.0	5.5
		(20.0%)	(7.1%)	(12.5%)	(40.7%)
	Test item (high rate)	0.0	0.0	0.0	0.0
		-	-	-	-
	Reference item	0.0	0.0	0.0	0.5
		-	-	-	(3.7%)
***Aporrectodea caliginosa* (total)**	Control	255.0	30.0	78.0	238.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	299.0	35.5	102.5	266.0
		(117.3%)	(118.3%)	(131.4%)	(111.8%)
	Test item (middle rate)	279.5	20.5	74.0	272.5
		(109.6%)	(68.3%)	(94.9%)	(114.5%)
	Test item (high rate)	282.5	29.0	87.0	271.5
		(110.8%)	(96.7%)	(111.5%)	(114.1%)
	Reference item	280.5	19.0	55.5	178.0
		(110.0%)	(63.3%)	(71.2%)	(74.8%)
***Aporrectodea caliginosa* (adults)**	Control	113.5	4.0	14.5	64.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	118.5	2.5	13.5	76.0
		(104.4%)	(62.5%)	(93.1%)	(118.8%)
	Test item (middle rate)	133.0	3.0	9.5	79.0
		(117.2%)	(75.0%)	(65.5%)	(123.4%)
	Test item (high rate)	115.0	3.5	9.0	78.0
		(101.3%)	(87.5%)	(62.1%)	(121.9%)
	Reference item	117.5	4.0	0.0	**31.0**
		(103.5%)	(100.0%)	-	**(48.4%)**
***Aporrectodea caliginosa* (juveniles)**	Control	138.0	26.0	63.5	155.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	172.5	32.5	89.0	170.5
		(125.0%)	(125.0%)	(140.2%)	(109.6%)
	Test item (middle rate)	139.5	17.0	64.5	173.0
		(101.1%)	(65.4%)	(101.6%)	(111.3%)
	Test item (high rate)	155.5	24.5	78.0	173.0
		(112.7%)	(94.2%)	(122.8%)	(111.3%)
	Reference item	154.5	15.0	55.5	133.0
		(112.0%)	(57.7%)	(87.4%)	(85.5%)

	Treatment group	Abundance (ind./m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
***Aporrectodea rosea* (total)**	Control	20.5	2.5	0.5	13.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	17.0	3.5	1.0	15.0
		(82.9%)	(140.0%)	(200.0%)	(111.1%)
	Test item (middle rate)	21.0	1.5	2.5	16.0
		(102.4%)	(60.0%)	(500.0%)	(118.5%)
	Test item (high rate)	20.0	1.5	1.0	12.5
		(97.6%)	(60.0%)	(200.0%)	(92.6%)
	Reference item	14.5	1.0	0.0	8.0
		(70.7%)	(40.0%)	-	(59.3%)
***Aporrectodea rosea* (adults)**	Control	11.5	0.5	0.0	9.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	8.5	1.0	0.0	8.5
		(73.9%)	(200.0%)	-	(89.5%)
	Test item (middle rate)	10.5	0.0	1.0	7.5
		(91.3%)	-	-	(78.9%)
	Test item (high rate)	9.5	0.0	0.5	2.5
		(82.6%)	-	-	(26.3%)
	Reference item	7.0	0.5	0.0	**3.0**
		(60.9%)	(100.0%)	-	**(31.6%)**
***Aporrectodea rosea* (juveniles)**	Control	9.0	2.0	0.5	4.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	8.5	2.5	1.0	6.5
		(94.4%)	(125.0%)	(200.0%)	(162.5%)
	Test item (middle rate)	10.5	1.5	1.5	8.5
		(116.7%)	(75.0%)	(300.0%)	(212.5%)
	Test item (high rate)	10.5	1.5	0.5	10.0
		(116.7%)	(75.0%)	(100.0%)	(250.0%)
	Reference item	7.5	0.5	0.0	4.5
		(83.3%)	(25.0%)	-	(112.5%)
***Aporrectodea longa* (total)**	Control	0.0	0.0	1.0	0.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	0.0	0.0	1.5	0.0
		-	-	(150.0%)	-
	Test item (middle rate)	0.0	0.0	0.5	1.5
		-	-	(50.0%)	-
	Test item (high rate)	0.5	0.0	0.0	0.5
		-	-	-	-
	Reference item	0.0	0.0	0.5	0.0
		-	-	(50.0%)	-
***Aporrectodea longa* (adults)**	Control	0.0	0.0	1.0	0.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	0.0	0.0	0.5	0.0
		-	-	(50.0%)	-
	Test item (middle rate)	0.0	0.0	0.5	1.0
		-	-	(50.0%)	-
	Test item (high rate)	0.0	0.0	0.0	0.0
		-	-	-	-
	Reference item	0.0	0.0	0.5	0.0
		-	-	(50.0%)	-

	Treatment group	Abundance (ind./m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
Aporrectodea longa **(juveniles)**	Control	0.0	0.0	0.0	0.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	0.0	0.0	1.0	0.0
		-	-	-	-
	Test item (middle rate)	0.0	0.0	0.0	0.0
		-	-	-	-
	Test item (high rate)	0.5	0.0	0.0	0.0
		-	-	-	-
	Reference item	0.0	0.0	0.0	0.0
		-	-	-	-
Lumbricus terrestris **(total)**	Control	42.5	4.0	2.0	5.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	26.0	1.5	5.5	8.5
		(61.2%)	(37.5%)	(275.0%)	(154.5%)
	Test item (middle rate)	45.5	4.5	2.5	11.0
		(107.1%)	(112.5%)	(125.0%)	(200.0%)
	Test item (high rate)	41.0	5.0	1.5	8.5
		(96.5%)	(125.0%)	(75.0%)	(154.5%)
	Reference item	36.0	**0.5**	**0.0**	2.0
		(84.7%)	**(12.5%)**	**-**	(36.4%)
Lumbricus terrestris **(adults)**	Control	14.0	1.5	0.5	1.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	9.5	0.5	2.5	2.5
		(67.9%)	(33.3%)	(500.0%)	(166.7%)
	Test item (middle rate)	5.5	2.0	0.5	3.5
		(39.3%)	(133.3%)	(100.0%)	(233.3%)
	Test item (high rate)	10.0	1.5	0.5	2.0
		(71.4%)	(100.0%)	(100.0%)	(133.3%)
	Reference item	7.5	0.0	0.0	**0.0**
		(53.6%)	-	-	**-**
Lumbricus terrestris **(juveniles)**	Control	20.5	2.5	1.5	4.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	13.0	1.0	3.0	5.5
		(63.4%)	(40.0%)	(200.0%)	(137.5%)
	Test item (middle rate)	31.0	2.5	2.0	6.5
		(151.2%)	(100.0%)	(133.3%)	(162.5%)
	Test item (high rate)	22.5	3.5	1.0	6.5
		(109.8%)	(140.0%)	(66.7%)	(162.5%)
	Reference item	18.5	0.5	**0.0**	2.0
		(90.2%)	(20.0%)	**-**	(50.0%)

pre-sampling on 03.05.2018 (1 weeks before 1st application)
1st sampling on 04.06.2019 (about 1 month after 1st application)
2nd sampling on 12.11.2018 (about 6 months after 1st application)
3rd sampling on 08.05.2019 (about 12 months after 1st application)
Statistic: comparisons of test item treatments *vs.* control and reference *vs*. control: one-sided t-test
Bold values indicate statistically significant differences to control ($p \leq 0.05$)
In brackets: the percentages from control

Table 10.4.1-17: Mean biomass of the total earthworm populations

	Treatment group	Biomass (g/m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
Total biomass	Control	239.39	14.60	44.12	140.34
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	244.83	8.45	67.96	161.94
		(102.3%)	(57.9%)	(154.0%)	(115.4%)
	Test item (middle rate)	244.29	14.67	41.37	178.45
		(102.0%)	(100.5%)	(93.8%)	(127.2%)
	Test item (high rate)	255.88	12.00	45.29	168.66
		(106.9%)	(82.2%)	(102.6%)	(120.2%)
	Reference item	224.51	**4.87**	**23.07**	**82.37**
		(93.8%)	**(33.4%)**	**(52.3%)**	**(58.7%)**
Total adult biomass	Control	160.07	8.90	13.36	60.22
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	165.30	3.45	19.17	76.94
		(103.3%)	(38.7%)	(143.5%)	(127.8%)
	Test item (middle rate)	162.14	9.06	9.09	88.36
		(101.3%)	(101.8%)	(68.0%)	(146.7%)
	Test item (high rate)	156.97	6.33	9.28	75.58
		(98.1%)	(71.1%)	(69.5%)	(125.5%)
	Reference item	144.63	1.84	0.54	**25.34**
		(90.4%)	(20.7%)	(4.0%)	**(42.1%)**
Total juvenile biomass	Control	76.55	5.69	30.77	75.78
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	76.27	4.90	48.79	81.39
		(99.6%)	(86.0%)	(158.6%)	(107.4%)
	Test item (middle rate)	75.66	5.55	32.28	83.61
		(98.8%)	(97.5%)	(104.9%)	(110.3%)
	Test item (high rate)	91.95	5.38	35.96	87.70
		(120.1%)	(94.5%)	(116.9%)	(115.7%)
	Reference item	75.56	3.03	22.53	54.54
		(98.7%)	(53.2%)	(73.2%)	(72.0%)
***Allolobophora chlorotica* (total)**	Control	7.06	2.44	1.73	2.59
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	6.43	1.63	2.43	2.34
		(91.0%)	(66.7%)	(140.6%)	(90.3%)
	Test item (middle rate)	3.86	0.39	0.19	1.30
		(54.6%)	(15.9%)	(11.0%)	(50.0%)
	Test item (high rate)	0.00	0.00	0.00	0.00
		-	-	-	-
	Reference item	0.00	0.00	0.00	0.06
		-	-	-	(2.2%)
***Allolobophora chlorotica* (adults)**	Control	6.43	1.37	0.00	0.00
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	5.23	0.68	0.12	0.00
		(81.3%)	(49.6%)	-	-
	Test item (middle rate)	3.75	0.34	0.00	0.00
		(58.3%)	(24.5%)	-	-
	Test item (high rate)	0.00	0.00	0.00	0.00
		-	-	-	-
	Reference item	0.00	0.00	0.00	0.00
		-	-	-	-

	Treatment group	Biomass (g/m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
***Allolobophora chlorotica* (juveniles)**	Control	0.64	1.07	1.73	2.59
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	1.20	0.95	2.31	2.34
		(188.9%)	(88.6%)	(133.5%)	(90.3%)
	Test item (middle rate)	0.11	0.05	0.19	1.30
		(16.6%)	(5.0%)	(11.0%)	(50.0%)
	Test item (high rate)	0.00	0.00	0.00	0.00
		-	-	-	-
	Reference item	0.00	0.00	0.00	0.06
		-	-	-	(2.2%)
***Aporrectodea caliginosa* (total)**	Control	167.34	4.43	36.74	125.03
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	185.67	4.61	53.20	145.46
		(111.0%)	(104.1%)	(144.8%)	(116.3%)
	Test item (middle rate)	182.67	2.93	36.62	152.67
		(109.2%)	(66.1%)	(99.7%)	(122.1%)
	Test item (high rate)	188.76	3.54	43.29	153.86
		(112.8%)	(79.9%)	(117.8%)	(123.1%)
	Reference item	186.11	4.52	22.53	**80.25**
		(111.2%)	(102.0%)	(61.3%)	**(64.2%)**
***Aporrectodea caliginosa* (adults)**	Control	111.08	2.26	10.19	51.38
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	115.41	1.09	10.58	65.70
		(103.9%)	(48.1%)	(103.8%)	(127.9%)
	Test item (middle rate)	126.83	1.13	6.63	70.09
		(114.2%)	(50.2%)	(65.0%)	(136.4%)
	Test item (high rate)	113.87	1.37	8.10	65.61
		(102.5%)	(60.9%)	(79.5%)	(127.7%)
	Reference item	116.58	1.78	0.00	**24.40**
		(105.0%)	(78.9%)	-	**(47.5%)**
***Aporrectodea caliginosa* (juveniles)**	Control	55.47	2.17	26.55	69.31
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	67.42	3.41	42.62	76.41
		(121.5%)	(157.4%)	(160.5%)	(110.2%)
	Test item (middle rate)	54.10	1.74	29.99	77.35
		(97.5%)	(80.1%)	(113.0%)	(111.6%)
	Test item (high rate)	71.23	1.92	35.19	83.44
		(128.4%)	(88.8%)	(132.6%)	(120.4%)
	Reference item	67.33	2.73	22.53	53.42
		(121.4%)	(126.1%)	(84.9%)	(77.1%)
***Aporrectodea rosea* (total)**	Control	4.62	0.22	0.06	2.99
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	3.83	0.74	0.05	3.10
		(82.9%)	(340.7%)	(84.4%)	(103.8%)
	Test item (middle rate)	4.79	0.05	0.36	3.45
		(103.7%)	(22.7%)	(591.8%)	(115.5%)
	Test item (high rate)	4.10	0.05	0.15	2.11
		(88.7%)	(21.1%)	(245.9%)	(70.6%)
	Reference item	3.23	0.07	0.00	1.91
		(69.9%)	(33.9%)	-	(64.0%)

	Treatment group	Biomass (g/m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
***Aporrectodea rosea* (adults)**	Control	3.52	0.11	0.00	2.41
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	2.81	0.38	0.00	2.17
		(79.9%)	(329.3%)	-	(89.8%)
	Test item (middle rate)	3.73	0.00	0.25	2.24
		(106.0%)	-	-	(92.9%)
	Test item (high rate)	2.89	0.00	0.10	0.77
		(82.2%)	-	-	(32.1%)
	Reference item	2.30	0.06	0.00	0.94
		(65.4%)	(52.4%)	-	(38.8%)
***Aporrectodea rosea* (juveniles)**	Control	1.10	0.10	0.06	0.57
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	1.02	0.37	0.05	0.94
		(92.4%)	(353.4%)	(84.4%)	(162.9%)
	Test item (middle rate)	1.07	0.05	0.11	1.21
		(96.6%)	(47.6%)	(182.8%)	(210.6%)
	Test item (high rate)	1.21	0.05	0.05	1.33
		(109.4%)	(44.2%)	(82.0%)	(232.1%)
	Reference item	0.93	0.01	0.00	0.91
		(84.1%)	(13.5%)	-	(159.1%)
***Aporrectodea longa* (total)**	Control	0.00	0.00	2.18	0.00
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	0.00	0.00	2.16	0.00
		-	-	(99.0%)	-
	Test item (middle rate)	0.00	0.00	0.97	3.07
		-	-	(44.6%)	-
	Test item (high rate)	0.84	0.00	0.00	0.53
		-	-	-	-
	Reference item	0.00	0.00	0.54	0.00
		-	-	(24.7%)	-
***Aporrectodea longa* (adults)**	Control	0.00	0.00	2.18	0.00
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	0.00	0.00	1.41	0.00
		-	-	(64.6%)	-
	Test item (middle rate)	0.00	0.00	0.97	2.69
		-	-	(44.6%)	-
	Test item (high rate)	0.00	0.00	0.00	0.00
		-	-	-	-
	Reference item	0.00	0.00	0.54	0.00
		-	-	(24.7%)	-
***Aporrectodea longa* (juveniles)**	Control	0.00	0.00	0.00	0.00
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	0.00	0.00	0.75	0.00
		-	-	-	-
	Test item (middle rate)	0.00	0.00	0.00	0.00
		-	-	-	-
	Test item (high rate)	0.84	0.00	0.00	0.00
		-	-	-	-
	Reference item	0.00	0.00	0.00	0.00
		-	-	-	-

	Treatment group	Biomass (g/m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
***Lumbricus terrestris* (total)**	Control	60.36	7.47	3.41	9.71
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	48.80	1.46	10.12	10.85
		(80.9%)	(19.5%)	(296.5%)	(111.7%)
	Test item (middle rate)	52.46	11.30	3.21	17.97
		(86.9%)	(151.3%)	(94.1%)	(185.1%)
	Test item (high rate)	61.75	8.35	1.80	12.13
		(102.3%)	(111.8%)	(52.7%)	(124.9%)
	Reference item	35.06	**0.28**	**0.00**	**0.12**
		(58.1%)	**(3.8%)**	**-**	**(1.2%)**
***Lumbricus terrestris* (adults)**	Control	39.05	5.16	0.98	6.43
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	41.85	1.30	7.06	9.08
		(107.2%)	(25.3%)	(717.3%)	(141.2%)
	Test item (middle rate)	27.83	7.59	1.24	13.35
		(71.3%)	(147.2%)	(125.7%)	(207.6%)
	Test item (high rate)	40.21	4.95	1.09	9.20
		(103.0%)	(96.1%)	(110.4%)	(143.2%)
	Reference item	25.74	0.00	0.00	**0.00**
		(65.9%)	-	-	**-**
***Lumbricus terrestris* (juveniles)**	Control	19.34	2.31	2.43	3.28
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	6.52	0.15	3.06	1.69
		(33.7%)	(6.6%)	(125.9%)	(51.4%)
	Test item (middle rate)	20.36	3.71	1.97	3.75
		(105.2%)	(160.3%)	(81.2%)	(114.3%)
	Test item (high rate)	18.29	3.40	0.71	2.93
		(94.5%)	(146.9%)	(29.3%)	(89.2%)
	Reference item	7.18	0.28	**0.00**	0.12
		(37.1%)	(12.1%)	**-**	(3.7%)

pre-sampling on 03.05.2018 (1 weeks before 1st application)
1st sampling on 04.06.2019 (about 1 month after 1st application)
2nd sampling on 12.11.2018 (about 6 months after 1st application)
3rd sampling on 08.05.2019 (about 12 months after 1st application)
Statistic: comparisons of test item treatments *vs.* control and reference *vs*. control: one-sided t-test
Bold values indicate statistically significant differences to control ($p \leq 0.05$).
In brackets: the percentages from control

All validity criteria were met:
- The mean abundance of earthworms of the test field at trial start was 348 ind./m², thus fulfilling the guideline recommendation (60 ind./m² for arable soils).
- At least one representative of endogeic and anecic earthworms was present at the field site in a sufficient number (>10 % of total earthworms or 10 - 15 ind./m²), with abundances of 279.3 ind./m² for *Aporrectodea caliginosa* (endogeic) and 38.2 ind./m² for Lumbricus terrestris (anecic; pre-sampling values).
- In the reference item treatment group total earthworm abundance and biomass were reduced by 58.6 % and 66.6 % at 1st sampling (about 1 month after 1st application), respectively, fulfilling the guideline recommendation (reduction of the earthworm abundance and / or biomass of > 50 % compared to the control).

Conclusion

Zoxium 240 SC (containing 240 g/L zoxamide) tested at application rates of 5 x 140 g a.s./ha (nominal), 5 x 180 g a.s./ha (nominal) and 5 x 280 g a.s./ha had no adverse effects on single species, ecological groups and total earthworm abundance and biomass about one year after application.
The study meets all validity criteria required by the available guidance for earthworm field studies (ISO 11268-3, 1999; KULA et al., 2006).

This active substance related study has already been provided to the RMS Latvia. Thus, the sum-mary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Zoxium 240 SC (containing 240 g/L zoxamide) tested at application rates of 3 x 0.5833 L/h, 3 x 0.75 L/ha and 3 x 1.5 L/ha (corresponding to 3 x 140 g a.s./ha, 3 x 180 g a.s./ha and 3 x 360 g a.s./ha) to bare soil with an interval of 7±1 days had no adverse effects on single species, ecological groups and total earthworm abundance and biomass about one year after the first application.

Reference:	KCA 8.4.1
Report:	Schulz, L., 2021: Effects of Zoxium 240 SC on earthworms under field conditions Gowan Crop Protection Ltd., UK BioChem agrar, Germany, Report No. 19 48 FEW 0002, GLP, Not published
Guideline(s):	ISO 11268-3 (2014) Technical recommendations to ISO 11268-3 (Kula et al. 2006) Guidance for summarising earthworm field studies (De Jong et al. 2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (18011201-72-52)
Active substance content or purity	zoxamide 240 g/L (nominal), 21.49 % w/w (analysed) isomeric ratio of R-/S-zoxamide = 49.8 / 50.2
Test organisms	Naturally occurring earthworm (Annelida: *Oligochaeta*) population with an abundance greater 60 ind./m² and two dominant species representing different life forms (*Aporrectodea caliginosa* and *Lumbricus terrestris*) present at 10 % or at least 10 to 15 ind./m², with a relative homogenous distribution on a typical arable field.
Test system	20 plots, each 10 m x 10 m, were arranged in a randomised block design. The test item applications were performed on bare soil. About 1 month after the last application, the test field was seeded with the fodder crop “Landsberger Gemenge” (clover grass mixture) which stayed on the field until the end of the study.
Field:	Typical arable field near Dornreichenbach in Saxony, Germany. Soil textural class: sandy-loamy silt (DIN 4220) / silt loam (USDA), mean pH ($CaCl_2$) 6.3, mean total organic carbon content 1.83 % and mean maximum water holding capacity 37.9 g/100 g soil dry weight.
Number of replicates:	Earthworms were sampled from four 0.125 m² sampling areas per plot

	per sampling occasion.
Surface monitoring:	Assessment of alive, moribund and dead earthworms on the soil surface in a monitoring area of 20.0 m² per plot in all control and test item replicates from day 1 to day 3 after each application.
Earthworm sampling:	Hand sorting combined with formalin extraction.
Environmental conditions	
Air temperature during application:	2.1 – 17.4 °C
Soil moisture during applications:	19.1 – 20.2 % w/w
Application rate(s)	3 applications at each: 1) 140 g a.s./ha (0.5833 L test item/ha) 2) 180 g a.s./ha (0.75 L test item/ha) 3) 360 g a.s./ha (1.5 L test item/ha)
Reference item:	20 L Maypon Flow/ha in 600 L water/ha (equivalent to nominally 10 kg carbendazim/ha)
Post exposure observation period	1st sampling about 1 month after 1st application 2nd sampling about 6 months after 1st application 3rd sampling about 12 months after 1st application
Remarks	None

Potential effects and potential recovery of field populations of earthworms after the spray application of Zoxium 240 (containing nominally 240 g/L zoxamide) to bare soil (silt loam according to USDA) at a pattern of 3x 140 g a.s./ha (0.5833 L test item/ha), 3x 180 g a.s./ha (0.75 L test item/ha) and 3x 360 g a.s./ha (1.5 L test item/ha) with an interval of 7±1 days were investigated on a typical arable field located near Dornreichenbach in Saxony, Germany. The results with regard to earthworm species composition, bio-mass and abundance were compared to an untreated control and a reference item (Maypon Flow, containing nominally 50 % w/v carbendazim) applied at a rate of 20 L/ha, corresponding to 10 kg a.s./ha, in parallel to the 1st test item application. The correct test item application has been verified by analysis of soil samples taken immediately after applications with a method validated according to SANCO/3029/99 rev. 4 (11/07/2000).
Twenty plots, each 10 m x 10 m, were arranged in a 5 x 4 formation, each plot surrounded by a 2 m wide path, between the plots. The set-up was a randomised block design. The assignment of the treatment groups to the plots was based on the results of a pre-sampling. The pre-sampling was conducted to determine the density, diversity and homogeneity of earthworm populations at the site. Defined areas were sampled to assess earthworm populations before application and three times after application, i.e. about 1, 6 and 12 months after 1st application. Earthworms were sampled from four 0.125 m² sampling areas per plot per sampling occasion by combining hand sorting with formalin extraction in the excavated hole.
Directly after digging out the soil, 2.5 L of a 0.2 % formaldehyde solution were uniformly poured into the hole in 2 or 3 portions according to seepage capacity. The total duration of the formaldehyde extraction was at least 30 minutes. All earthworms coming to the surface were collected and placed into a vessel containing water, one for each sample. Earthworms found by hand sorting were collected in separate vessels containing water, one for each sample. Vessels with earthworms were stored in a cold room in the dark for approximately 48 hours. The abundance of earthworms was recorded and the weight (biomass) determined with a precision balance for each sample separately. Before the worms were weighed each single worm was placed on a dry filter paper to free the worm body from excess water. Animals were counted, weighed, and species identified in a period of approximately 48 hours after sampling.
Environmental conditions (air temperature, relative air humidity, soil temperature (10 cm height), wind speed, wind direction, cloudiness, rainfall, and soil moisture) were assessed during the test item applications. Furthermore, soil moisture, soil temperature, vegetation coverage (in % covered soil surface) and vegetation height were evaluated during each sampling occasion.
Statistical analysis was carried out with the statistical software package ToxRat Professional 3.3.0 (2018). Data were analysed for normal distribution with the Shapiro-Wilk's-test or Kolmogorov-Smirnoff-test

and for homogeneity in variance with the Levene's test. Afterwards, pre-sampling data were analysed with a two-factorial analysis of variance (ANOVA, 5 % significance level) with treatment as fixed factor and block as random factor. Post-treatment sampling data were analysed for monotone dose-response using Trend analysis by Contrasts. Afterwards, the data were analysed by a one-sided Williams-t-test or Dunnett-t-test or Welch-t-test after Bonferroni-Holm with test item treatment group < control as well as Student-t-test or Welch-t-test with reference item treatment group < control at the 5 % significance level. Test item and reference item were analysed in separate analyses.

Results and discussion

The test item has been applied on days with low wind speed and no rain during and after application. Due to warm and dry weather conditions during 2019, irrigation of the test field was required to support the exposure of the test organisms. The test field was irrigated with 10 mm tap water after each application.
No measurable residues (< LOQ) of zoxamide were determined in any of the soil samples of the control plots taken immediately after each application.
The mean recoveries of total zoxamide residues in the soil samples (sampling depth: of 0 - 5 cm) taken from the plots treated with 3 x 140 g a.s./ha (3 x 0.5833 L test item/ha) at an interval of 6 to 8 days immediately after each application were 162 %, 128 % and 113 % after the 1st, 2nd and 3rd application, respectively, of the corresponding nominal levels of 0.187, 0.374 and 0.562 mg a.s./kg soil - assuming an application rate of 140 g/ha zoxamide, a bulk density of 1.5 g/cm³, a soil depth of 5 cm, and no degradation of the active substance between the applications.
The mean recoveries of total zoxamide residues in the soil samples (sampling depth: of 0 - 5 cm) taken from the plots treated with 3 x 180 g a.s./ha (3 x 0.75 L test item/ha) at an interval of 6 to 8 days immediately after each application were 149 %, 166 % and 135 % after the 1st, 2nd and 3rd application, respectively, of the corresponding nominal levels of 0.241, 0.481 and 0.722 mg a.s./kg soil - assuming an application rate of 180 g/ha zoxamide, a bulk density of 1.5 g/cm³, a soil depth of 5 cm, and no degradation of the active substance between the applications.
The mean recoveries of total zoxamide residues in the soil samples (sampling depth: of 0 - 5 cm) taken from the plots treated with 3 x 360 g a.s./ha (3 x 1.5 L test item/ha) at an interval of 6 to 8 days immediately after each application were 135 %, 127 % and 93 % after the 1st, 2nd and 3rd application, respectively, of the corresponding nominal levels of 0.481, 0.963 and 1.444 mg a.s./kg soil - assuming an application rate of 360 g/ha zoxamide, a bulk density of 1.5 g/cm³, a soil depth of 5 cm, and no degradation of the active substance between the applications.
Since the average residue levels of zoxamide in the soil samples taken immediately after the 3rd application were within the recommended range of 50 % to 150 % of the nominal values, the correct applications were verified according to KULA et al.: Technical recommendations for the update of the ISO earthworm field test guideline (ISO 11268-3).
The mean earthworm abundance in the control plots was 101.5 ind./m² at pre-sampling, 85.0 ind./m² at 1st sampling, 69.5 ind./m² at 2nd sampling and 162.5 ind./m² at 3rd sampling.
Earthworm species found in the plots of the field site at pre-sampling were the endogeic species *Aporrectodea caliginosa* (83.6 % of total earthworms) and *Aporrectodea rosea* (1.3 % of total earthworms) as well as the anecic species *Lumbricus terrestris* (15.1 % of total earthworms). The presence of the dominant species *Aporrectodea caliginosa* and *Lumbricus terrestris* representing different ecological groups indicated the reliability of the field site.
The toxic reference item reduced total earthworm abundance by 50.6 % at 1st sampling, 50.4 % at 2nd sampling and 35.4 % at 3rd sampling. *Lumbricus terrestris* was the most sensitive species and was reduced in total abundance by 66.7 % at 1st sampling, 80.8 % at 2nd sampling and 55.6 % at 3rd sampling.
The total earthworm biomass was reduced by the reference item by 64.1 % at 1st sampling, 65.1 % at 2nd sampling and 24.1 % at 3rd sampling. The total biomass of *Lumbricus terrestris* was reduced by 79.3 % at 1st sampling, 94.1 % at 2nd sampling and 24.3 % at 3rd sampling. These results clearly indicated the effect of the toxic reference item and thus the validity of the test system.
The surface monitoring on days 1 - 3 after each application showed that there were no acute primary effects on earthworms by Zoxium 240 SC. No alive, moribund or dead earthworms were found on the soil surface, neither in the test item nor in the control monitoring areas.
No statistically significant reductions in total earthworm abundance and biomass could be observed for the tested application rates of 3 x 140 g a.s./ha, 3 x 180 g a.s./ha and 3 x 360 g a.s./ha about 1, 6 and 12

months after 1st application. Furthermore, no statistically significant reductions in abundance and biomass of the different earthworm species (*Aporrectodea caliginosa* and *Lumbricus terrestris*) and ecological groups (endogeic and anecic earthworms) could be observed for the tested application rates about 1, 6 and 12 months after the 1st test item treatment.

Table 10.4.1-18: Mean abundance of the earthworm populations

	Treatment group	Abundance (ind./m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
Total abundance	Control	101.5	85.0	69.5	162.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	109.0	94.5	81.0	120.0
		(107.4%)	(111.2%)	(116.5%)	(73.8%)
	Test item (middle rate)	102.0	109.5	75.0	169.0
		(100.5%)	(128.8%)	(107.9%)	(104.0%)
	Test item (high rate)	105.5	72.5	58.0	159.5
		(103.9%)	(85.3%)	(83.5%)	(98.2%)
	Reference item	103.5	**42.0**	**34.5**	105.0
		(102.0%)	**(49.4%)**	**(49.6%)**	(64.6%)
Total adult abundance	Control	46.0	44.5	50.0	58.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	42.5	55.5	38.0	30.5
		(92.4%)	(124.7%)	(76.0%)	(52.1%)
	Test item (middle rate)	38.0	72.0	41.5	55.5
		(82.6%)	(161.8%)	(83.0%)	(94.9%)
	Test item (high rate)	46.5	44.0	33.5	61.5
		(101.1%)	(98.9%)	(67.0%)	(105.1%)
	Reference item	40.5	27.0	**16.0**	42.5
		(88.0%)	(60.7%)	**(32.0%)**	(72.6%)
Total juvenile abundance	Control	43.0	37.5	18.0	100.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	48.0	38.5	39.5	88.0
		(111.6%)	(102.7%)	(219.4%)	(87.6%)
	Test item (middle rate)	54.5	37.0	29.5	109.5
		(126.7%)	(98.7%)	(163.9%)	(109.0%)
	Test item (high rate)	47.5	27.5	22.5	97.0
		(110.5%)	(73.3%)	(125.0%)	(96.5%)
	Reference item	48.5	15.0	14.5	**59.5**
		(112.8%)	(40.0%)	(80.6%)	**(59.2%)**
Aporrectodea caliginosa (total)	Control	86.0	68.5	56.0	129.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	92.5	74.0	63.5	89.0
		(107.6%)	(108.0%)	(113.4%)	(68.7%)
	Test item (middle rate)	81.5	88.5	56.5	126.5
		(94.8%)	(129.2%)	(100.9%)	(97.7%)
	Test item (high rate)	89.0	60.0	42.0	114.5
		(103.5%)	(87.6%)	(75.0%)	(88.4%)
	Reference item	87.0	35.5	**31.5**	90.5
		(101.2%)	(51.8%)	**(56.3%)**	(69.9%)

	Treatment group	Abundance (ind./m²)			
		pre-sampling	**1st sampling**	**2nd sampling**	**3rd sampling**
Aporrectodea caliginosa (adults)	Control	43.0	38.5	41.0	52.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	39.0	45.0	34.5	24.5
		(90.7%)	(116.9%)	(84.1%)	(47.1%)
	Test item (middle rate)	34.0	60.5	35.5	49.0
		(79.1%)	(157.1%)	(86.6%)	(94.2%)
	Test item (high rate)	42.0	37.5	27.0	52.0
		(97.7%)	(97.4%)	(65.9%)	(100.0%)
	Reference item	38.5	24.5	**15.5**	37.5
		(89.5%)	(63.6%)	**(37.8%)**	(72.1%)
Aporrectodea caliginosa (juveniles)	Control	31.5	27.0	13.5	75.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	35.0	28.5	25.5	63.0
		(111.1%)	(105.6%)	(188.9%)	(84.0%)
	Test item (middle rate)	39.0	27.5	17.0	73.5
		(123.8%)	(101.9%)	(125.9%)	(98.0%)
	Test item (high rate)	37.0	21.5	13.0	61.5
		(117.5%)	(79.6%)	(96.3%)	(82.0%)
	Reference item	35.5	11.0	12.5	50.0
		(112.7%)	(40.7%)	(92.6%)	(66.7%)
Lumbricus terrestris (total)	Control	15.0	16.5	13.0	27.0
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	15.5	20.0	17.5	29.0
		(103.3%)	(121.2%)	(134.6%)	(107.4%)
	Test item (middle rate)	18.5	20.5	18.5	38.5
		(123.3%)	(124.2%)	(142.3%)	(142.6%)
	Test item (high rate)	15.0	10.0	16.0	38.0
		(100.0%)	(60.6%)	(123.1%)	(140.7%)
	Reference item	14.5	**5.5**	2.5	12.0
		(96.7%)	**(33.3%)**	(19.2%)	(44.4%)
Lumbricus terrestris (adults)	Control	3.0	6.0	8.5	4.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	3.0	10.0	3.5	5.5
		(100.0%)	(166.7%)	(41.2%)	(122.2%)
	Test item (middle rate)	4.0	11.0	6.0	6.0
		(133.3%)	(183.3%)	(70.6%)	(133.3%)
	Test item (high rate)	4.5	5.0	6.5	7.0
		(150.0%)	(83.3%)	(76.5%)	(155.6%)
	Reference item	1.5	2.5	**0.0**	4.0
		(50.0%)	(41.7%)	**-**	(88.9%)

	Treatment group	Abundance (ind./m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
Lumbricus terrestris (juveniles)	Control	11.0	10.5	4.5	22.5
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	12.5	10.0	14.0	23.5
		(113.6%)	(95.2%)	(311.1%)	(104.4%)
	Test item (middle rate)	13.5	9.5	12.5	32.5
		(122.7%)	(90.5%)	(277.8%)	(144.4%)
	Test item (high rate)	9.0	5.0	9.5	31.0
		(81.8%)	(47.6%)	(211.1%)	(137.8%)
	Reference item	11.5	3.0	2.0	8.0
		(104.5%)	(28.6%)	(44.4%)	(35.6%)

pre-sampling on 25.03.2019 (about 2 weeks before 1st application)
1st sampling on 13.05.2019 (about 1 month after 1st application)
2nd sampling on 24.10.2019 (about 6 months after 1st application)
3rd sampling on 07.04.2020 (about 12 months after 1st application)
Statistic: comparisons of test item treatments vs. control and reference vs. control: one-sided t-test
Bold values indicate statistically significant differences to control ($p \leq 0.05$).
In brackets: the percentages from control

Table 10.4.1-19: Mean biomass of the total earthworm populations

	Treatment group	Biomass (g/m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
Total biomass	Control	104.17	115.30	99.28	156.02
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	99.28	150.28	81.94	106.38
		(95.3%)	(130.3%)	(82.5%)	(68.2%)
	Test item (middle rate)	110.89	181.34	91.94	162.22
		(106.4%)	(157.3%)	(92.6%)	(104.0%)
	Test item (high rate)	111.20	103.44	88.65	186.19
		(106.7%)	(89.7%)	(89.3%)	(119.3%)
	Reference item	100.55	**41.43**	**34.65**	118.35
		(96.5%)	**(35.9%)**	**(34.9%)**	(75.9%)
Total adult biomass	Control	66.36	87.36	92.43	101.10
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	58.65	124.13	66.43	63.55
		(88.4%)	(142.1%)	(71.9%)	(62.9%)
	Test item (middle rate)	64.84	154.58	74.79	104.73
		(97.7%)	(176.9%)	(80.9%)	(103.6%)
	Test item (high rate)	70.98	88.37	77.59	127.84
		(107.0%)	(101.2%)	(83.9%)	(126.4%)
	Reference item	59.24	**35.92**	**24.58**	87.40
		(89.3%)	**(41.1%)**	**(26.6%)**	(86.5%)

	Treatment group	Biomass (g/m²)			
		pre-sampling	**1st sampling**	**2nd sampling**	**3rd sampling**
Total juvenile biomass	Control	32.90	26.55	6.49	53.85
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	34.60	26.01	13.64	42.56
		(105.2%)	(98.0%)	(210.3%)	(79.0%)
	Test item (middle rate)	42.57	26.58	15.70	55.72
		(129.4%)	(100.1%)	(242.0%)	(103.5%)
	Test item (high rate)	35.49	14.77	10.38	58.11
		(107.9%)	(55.6%)	(160.0%)	(107.9%)
	Reference item	36.15	**5.51**	6.79	**30.11**
		(109.9%)	**(20.8%)**	(104.7%)	**(55.9%)**
Aporrectodea caliginosa (total)	Control	73.51	67.99	65.18	96.37
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	71.29	75.60	55.31	44.95
		(97.0%)	(111.2%)	(84.9%)	(46.6%)
	Test item (middle rate)	74.08	99.91	60.58	84.77
		(100.8%)	(146.9%)	(92.9%)	(88.0%)
	Test item (high rate)	77.80	66.83	47.88	95.41
		(105.8%)	(98.3%)	(73.5%)	(99.0%)
	Reference item	76.57	**31.47**	**32.51**	72.84
		(104.2%)	**(46.3%)**	**(49.9%)**	(75.6%)
Aporrectodea caliginosa (adults)	Control	51.50	53.17	59.83	72.63
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	47.75	61.65	47.35	29.68
		(92.7%)	(115.9%)	(79.1%)	(40.9%)
	Test item (middle rate)	47.45	86.59	49.60	66.76
		(92.1%)	(162.9%)	(82.9%)	(91.9%)
	Test item (high rate)	52.24	56.82	42.82	77.13
		(101.4%)	(106.9%)	(71.6%)	(106.2%)
	Reference item	50.02	**27.23**	**24.44**	54.87
		(97.1%)	**(51.2%)**	**(40.8%)**	(75.5%)
Aporrectodea caliginosa (juveniles)	Control	17.37	13.43	4.99	22.98
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	17.50	13.82	6.09	14.99
		(100.8%)	(102.9%)	(122.1%)	(65.2%)
	Test item (middle rate)	23.42	13.12	9.53	16.24
		(134.8%)	(97.7%)	(191.1%)	(70.7%)
	Test item (high rate)	21.47	9.72	4.37	18.05
		(123.6%)	(72.3%)	(87.7%)	(78.5%)
	Reference item	21.76	**4.24**	5.97	17.12
		(125.3%)	**(31.6%)**	(119.7%)	(74.5%)
Lumbricus terrestris (total)	Control	30.57	47.31	33.97	57.93
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	27.65	74.57	26.63	60.98
		(90.5%)	(157.6%)	(78.4%)	(105.3%)
	Test item (middle rate)	36.57	81.29	31.35	76.73
		(119.7%)	(171.8%)	(92.3%)	(132.5%)
	Test item (high rate)	33.14	35.90	40.77	88.56
		(108.4%)	(75.9%)	(120.0%)	(152.9%)
	Reference item	23.61	9.78	**2.00**	43.85

	Treatment group	Biomass (g/m²)			
		pre-sampling	1st sampling	2nd sampling	3rd sampling
		(77.3%)	(20.7%)	**(5.9%)**	(75.7%)
Lumbricus terrestris (adults)	Control	14.86	34.19	32.47	27.41
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	10.65	62.38	19.08	33.63
		(71.7%)	(182.4%)	(58.8%)	(122.7%)
	Test item (middle rate)	17.39	67.84	25.19	37.72
		(117.0%)	(198.4%)	(77.6%)	(137.6%)
	Test item (high rate)	18.74	31.04	34.77	48.95
		(126.1%)	(90.8%)	(107.1%)	(178.6%)
	Reference item	9.05	8.69	**0.00**	31.35
		(60.9%)	(25.4%)	-	(114.4%)
Lumbricus terrestris (juveniles)	Control	15.43	13.12	1.50	30.52
		(100.0%)	(100.0%)	(100.0%)	(100.0%)
	Test item (low rate)	17.00	12.19	7.55	27.35
		(110.1%)	(92.9%)	(503.6%)	(89.6%)
	Test item (middle rate)	18.91	13.45	6.17	39.01
		(122.5%)	(102.5%)	(411.3%)	(127.8%)
	Test item (high rate)	13.77	4.86	6.01	39.61
		(89.2%)	(37.0%)	(400.6%)	(129.8%)
	Reference item	14.19	1.09	0.83	**12.50**
		(92.0%)	(8.3%)	(55.0%)	**(41.0%)**

pre-sampling on 25.03.2019 (about 2 weeks before 1st application)
1st sampling on 13.05.2019 (about 1 month after 1st application)
2nd sampling on 24.10.2019 (about 6 months after 1st application)
3rd sampling on 07.04.2020 (about 12 months after 1st application)
Statistic: comparisons of test item treatments vs. control and reference vs. control: one-sided t-test
Bold values indicate statistically significant differences to control ($p \leq 0.05$).
In brackets: the percentages from control

All validity criteria were met:

- Earthworm abundance on arable land (average): ≥ 60/m² at test initiation (pre-sampling)
- Each of two dominant species representing different life forms (anecic, endogeic) present in a sufficiently high density of 10 to 15 individuals per m² or 10 % of total earthworm population
- Significant reduction of the earthworm abundance and / or biomass by the reference item: at least 50 %

Conclusion

Zoxium 240 SC (containing 240 g/L zoxamide) tested at application rates of 3 x 0.5833 L/h, 3 x 0.75 L/ha and 3 x 1.5 L/ha (corresponding to 3 x 140 g a.s./ha, 3 x 180 g a.s./ha and 3 x 360 g a.s./ha) had no adverse effects on single species, ecological groups and total earthworm abundance and biomass about one year after application.

The current study meets all criteria required for a valid earthworm field study as requested by the available guidance for earthworm field studies (ISO 11268-3, 1999; KULA et al., 2006).

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 232 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4.2.1/01
Report:	EFFECTS OF GWN-10616 ON REPRODUCTION OF THE COLLEMBOLAN *FOLSOMIA CANDIDA* IN ARTIFICIAL SOIL, Grandolini, G., 2021, report No. BT211/21, Doc. No. 834-002
Guideline(s):	OECD No. 232 (2016), ISO 11267 (2014)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Executive Summary

The potential chronic effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phos-phite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, on the springtail *Folsomia candida* were investigated in a laboratory experiment in artificial soil over a time period of 4 weeks. The test was performed in a limit test design at a concentration of 1000 mg test item/kg soil dry weight (sdw) corresponding to 347.26 mg Potassium phosphite/kg sdw and 42.47 Zoxamide mg a.s./kg sdw, respectively.

No significant adult mortality was observed in the treatment group during the test. The NOEC for survival and reproduction was determined to be equal to or higher than the limit test concentration of 1000 mg test item/kg sdw corresponding to 347.26 mg Potassium phosphite /kg sdw and 42.47 mg Zoxamide/kg sdw, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GWN-10616
Active substances	Potassium phosphite and Zoxamide
Description:	Beige homogeneous and viscous liquid
Lot/Batch #:	P2102669001
Content of a.s.:	Potassium phosphite (measured as Phosphonic acid equivalent): 507 g/L, Zoxamide: 62 g/L

2. Vehicle and control:

Control:	Untreated artificial soil moistened with deionised water
Vehicle:	Deionised water
Reference item:	Boric acid tested in a separate study

3. Test animals:

Species:	*Folsomia candida* (L.)
Source:	In-house culture
Age:	9 days old
Test unit:	Glass beakers (100 mL) filled with artificial soil (artificial soil according to OECD 232 with 5 % organic matter)

4. Environmental conditions

Temperature:	19.7 – 21.2 °C
Moisture:	at test initiation about 50 % of water holding capacity, at test termination about 50 % of water holding capacity
pH:	6.05 to 6.13 at test initiation and at test termination
Photoperiod:	16 hours light – 8 hours dark with a light intensity of 641 – 941 lux

5. Experimental phase: 01.09.2021 to 30.09.2021

B. STUDY DESIGN AND METHODS

Experimental treatments

The test was performed with a single concentration of GWN-10616 (active substances Potassium phosphite measured as Phosphonic acid equivalent: 507 g/L; Zoxamide: 62 g/L), 1000 mg test item/kg soil dry weight (sdw), corresponding to 347.26 mg Potassium phosphite/kg sdw and 42.47 Zoxamide mg a.s./kg sdw, respectively. Eight replicates for the water control and test item group were tested, each containing 10 collembolans.

Six days before the start of the test, dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. An amount of solution necessary to reach the final moisture content of the soil was prepared. The solutions were thoroughly mixed with the soil substrate before introducing it into the test vessels.

The test chambers consisted of glass beakers (100 mL) covered with lids (designed to reduce water evaporation). Collembola were carefully transferred into each test vessel (allocated randomly) and placed onto the surface of the soil. Springtails were fed with 2 mg granulated dry yeast per replicate at test start and again with 2 mg granulated dry yeast per replicate after 14 days.

Observations

Synchronous juvenile springtails were exposed for 28 days until offspring (F1) emerged from eggs laid by mature adults. The springtail survival and the number of juveniles were assessed. Mortality and reproduction data of the test item treatment group were compared to the results of the control group.

Tullgren funnels were used to extract the collembolans from the soil. This apparatus creates a temperature gradient of approximately 14 °C in a soil sample, stimulating downward movement of soil arthropods into a collecting vessel. The juveniles were then counted with a stereomicroscope.

Statistics

Appropriate statistical methods were used to analyse mortality and fecundity data for significance (e.g. Student-t test for Homogeneous Variances, one-sided smaller, α=0.05 for reproduction and Fisher's Exact Binomial Test, one sided greater, α = 0.05 for mortality). For the statistical analysis the software ToxRat Pro. 3.3.0. was used.

II. RESULTS AND DISCUSSION

Validity criteria

The study was considered valid because less than 20 % mortality was observed in the control group (actual 6.25 %), the coefficient of variation of reproduction in the water controls did not exceed 30 % (actual 16.31 %) and the mean number of juveniles in each replicate of the control treatments was at least 100 (actual 659.25). In addition, application of the toxic reference substance Boric acid resulted in substantial and unequivocal effects.

Biological results
A mortality of 6.25 % was observed in the control and the test item treated group. Please refer to Table 10.4.2-1 for further details.

Reproduction of the springtails exposed to GWN-10616 was not statistically significantly different compared to the control at 1000 mg test item/kg sdw (Student-t test for Homogeneous Variances, one sided smaller, $\alpha = 0.05$, one-sided smaller).

Table 10.4.2-1: Summary of survival data and reproductive effects of GWN-10616 on *Folsomia candida*

Test item concentration [mg/kg sdw]	Mortality [%]	Mean number of juveniles	Reduction of reproduction [%] *
0	6.25	659.25	-
1000	6.25	846.50	-28.4

sdw = soil dry weight
*Student-t test for Homogeneous Variances, one sided smaller, α=0.05; negative values indicate higher reproductive output compared to the Control group

The toxic reference item Boric Acid was tested in a separate study. The EC_{50} for reproduction was determined as 101.47 mg/kg sdw (95 %-confidence interval: 73.45 – 139.74 mg/kg sdw). This is within the target effect range of about 100 mg/kg sdw given by the OECD guideline 232 (2016).

III. CONCLUSIONS

In a 28-day laboratory study with *Folsomia candida*, GWN-10616 caused no statistically significant effects on mortality and reproduction in a limit test design. The NOEC for mortality and reproduction was estimated to be 1000 mg test item/kg sdw, respectively.

Comments of zRMS:	The study was conducted to OECD 226 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.4.2.1/02
Report: EFFECTS OF GWN-10616 ON REPRODUCTION OF THE PREDATORY MITE HYPOASPIS ACULEIFER IN SOIL, Grandolini, G., 2021, report No. BT212/21, Doc. No. 834-003
Guideline(s): OECD No. 226 (2016)
Deviations: Light intensity was above target range for less than 2 h. This short deviation is not assumed to have any influence on the outcome of the study
GLP: Yes
Acceptability: Yes

Executive Summary
The potential chronic effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, on survival and reproduction on the predatory mite *Hypoaspis aculeifer* were determined in a laboratory study according to OECD 226 (2016) over a 14-day period. GWN-10616 was tested at concentrations of 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg test item/kg soil dry weight (sdw), mixed into artificial soil containing 5 % peat.

Effects on mortality and reproduction were all ≤ 17.5 % and not statistically significant. Thus, EC_{10} and EC_{20} values were estimated to be > 1000 mg test item/kg sdw and the NOEC with regard to mortality and reproduction was determined to be 1000 mg test item/kg sdw (corresponding to 347.26 mg Potassium phosphite (measured as Phosphonic acid equivalent) and 42.47 mg Zoxamide/kg sdw, respectively).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

Test Material:	GWN-10616
Active substances	Potassium phosphite and Zoxamide
Description:	Beige homogeneous and viscous liquid
Lot/Batch #:	P2102669001
Content of a.s.:	Potassium phosphite measured as Phosphonic acid equivalent: 507 g/L, Zoxamide: 62 g/L

2. Vehicle and reference item

Control:	Untreated artificial soil moistened with deionised water
Vehicle:	Deionised water
Reference item:	Dimethoate was tested in a separate study

3. Test animals

Species	*Hypoaspis aculeifer*
Source:	In-house culture
Age:	Adult females, 33 days old after start of egg laying period.
Test unit:	Glass vessels (100 mL) filled with artificial soil (artificial soil according to OECD 226 with 5 % organic matter)

4. Environmental conditions

Temperature:	19.7-21.2 °C
Photoperiod:	16 h light
Moisture:	approximately 20.51 mL of water/100 g of dry soil (50% of the maximum WHC)
pH:	6.09 to 6.76 at test initiation and at test termination
Light intensity	641.2-941.2 lux

B. STUDY DESIGN AND METHODS

1. Experimental phase 01.09.2021 – 30.09.2021

2. Experimental Treatments

The test was performed with GWN-10616 (active substances Potassium phosphite measured as Phosphonic acid equivalent: 507 g/L; Zoxamide: 62 g/L) in a dose response design. The treatments consisted of a control and 8 treatment levels at concentrations of 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000 mg test item/kg sdw. A reference item (Dimethoate) was tested in a separate study at concentrations between 1 and 10.5 mg/kg sdw. Eight replicates for the control and 4 replicates for each test item treatment group were investigated. Additional replicates were included in the study for the determination of the water content, soil pH value and soil temperature.

The test item was dissolved in deionised water and mixed with the pre-moistened artificial soil substrate with a 5 % organic matter content, before introduction of the test organisms. The control was treated in an identical way but without test item. 10 adult females per replicate were inserted, thus a total of 80 female mites were used per control and 40 female mites were used per treatment. Cheese mites were provided as food source *ad libitum*.

3. Observations

Synchronous adult female mites were exposed for 14 days until offspring (F1) emerge from laid eggs. The mite survival and the number of juveniles were assessed. Mortality and reproduction data of the test

item treatment groups were compared to the results of the control group. In addition, the behaviour and the morphology of the mites in the control and the treated vessels were observed.

4. Analytics

At test start, the concentrations of active substances Zoxamide and Potassium phosphite (measured as Phosphonic acid equivalent) in the stock solutions were confirmed by analytical verification (LC-MS/MS method).

5. Statistics

Appropriate statistical methods were used to analyse mortality and fecundity data for significance (e.g. Dunnett' Multiple t-test Procedure, one-sided smaller, α=0.05 for reproduction performances and Chi^2 2x2 Table Test with Bonferroni Correction, one sided greater, α=0.05 for mortality). For the statistical analysis the software ToxRat Pro. 3.3.0. was used.

II. RESULTS AND DISCUSSION

A. Validity criteria

The study was considered valid because ≤ 10 % mortality was observed in the control group (actual: 10 %), the mean number of juveniles in each replicate of the control treatments was at least 30 (actual: 195.6) and the coefficient of variation of reproduction in the control did not exceed 30 % (actual: 15.05 %).

In addition, the EC_{50} determined on the reproductive output for the reference item (Dimethoate) was evaluated to be 5.77 mg reference item/kg sdw (95 % confidence limits: 3.49 – 9.36 mg reference item/kg sdw), confirming the sensitivity of the test system.

B. Analytical test results

The concentrations of the active substances Zoxamide and Phosphonic acid in the stock solutions used to treat the soil were determined. All measured concentrations of the test item were within ± 20 % of the nominal values. Accordingly, endpoints of the test were calculated with respect to the nominal concentration of the test item.

C. Biological test results

In the test item treatment groups, 2.5 to 17.5 % mortality was observed (Table 10.4.2-2). No statistically significant difference concerning mortality between the control and any test item treatment group was detected. Therefore, the $NOEC_{Mortality}$ was determined to be ≥ 1000 mg test item/kg sdw corresponding to 347.26 mg Potassium phosphite (measured as Phosphonic acid equivalent) and 42.47 mg Zoxamide/kg sdw, respectively.

For reproduction (Table 10.4.2-2), the mean number of juvenile mites in the control was 195.6. The mean number of juvenile mites in the different treatment groups ranged between 177 and 217.5. Statistical analysis showed no significant difference concerning the number of juveniles between the control and any test item concentration. Therefore, the $NOEC_{Reproduction}$ was determined to be ≥ 1000 mg test item/kg sdw corresponding to 347.26 mg Potassium phosphite (measured as Phosphonic acid equivalent) and 42.47 mg Zoxamide/kg sdw, respectively.

No ECx and LCx values can be reported since no statistically significant results were obtained from this data set and no concentration-response was observed.

Table 10.4.2-2: Summary of mortality and reproductive effects of GWN-10616 on *Hypoaspis aculeifer* (after 14 days)

Treatment group [mg test item/kg sdw]	Mortality [%]	Mean Number of Juveniles ± SD	Reduction of Reproduction* [%]
Control	10.0	195.6	-
16.33	2.5	179.5	8.24
29.40	5.0	214.0	-9.39
52.92	15.0	206.8	-5.69
95.26	7.5	217.5	-11.18
171.47	10.0	181.8	7.09
308.64	12.5	215.0	-9.90
555.56	5.0	177.0	9.52
1000	17.5	204.3	-4.41

* negative values refer to an increase in reproduction compared to the control.

No differences in the behaviour and the morphology of the mites in the control and the treated vessels were observed.

III. CONCLUSIONS

After 14 days of exposure to GWN-10616 no statistically significant effects were detectable on mortality and reproduction of the adult predatory mite *Hypoaspis aculeifer* up to and including the highest tested concentration of 1000 mg test item/kg soil dry weight (sdw).

Therefore, the NOEC for mortality and reproduction was determined to be ≥ 1000 mg test item/kg sdw. No ECx calculations were possible and the EC_{10} and EC_{20} values were estimated to be > 1000 mg test item/kg sdw.

According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_XXXX_LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, concerning deviations from the study protocol and test results the study is not considered to be valid and is not considered suitable for the risk assessment.

Reference:	KCA 8.4.2
Report	Gray, J., 2021: RH-163353: Collembolan reproduction test in soil – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., U.K., Report No. 3202390, GLP, Not published
Guideline(s):	OECD 232 (2016)

Deviations:	Each test vessel was weighed and the weight adjusted to the day 0 level by addition of RO water on day 14. In this way individual vessel moisture loss was made up to the correct level whereas the use of a single moisture control vessel as stated in the protocol does not account for the variation in loss between vessels. The process was repeated at Day 21 to ensure optimum environmental conditions were maintained as far as possible throughout the exposure period. The light period was less than 16 hours on one occasion over the 28-day exposure period. The mean % MWHC was below the minimum of 40% stated in the protocol for all treatments and the control throughout the study. As the % MWHC to obtain the correct soil structure had been determined to be 40%MWHC, the lowest level specified, any moisture lost during the mixing process would have resulted in the final % MWHC being below the selected level. These deviations are considered to have no impact on the study integrity. In addition, the OECD 232 guideline states 'The moisture content of the testing soil should be optimised to obtain a loose porous structure allowing the collembolans to enter into the pores. This is usually between 40-60 % maximum WHC.' Therefore, the % MWHC that provides the correct soil structure may be outside the 40-60 % range. It is considered that as the validity criterion of ≥100 juveniles per replicate was achieved in the controls and at all rates of application the lower % MWHC has had no impact on the reproductive output.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-163353 (HHGCP001-00-2)
Purity	99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)
Species	*Folsomia candida*
Age:	9-12 days old
Source:	Bias Labs Ltd., UK
Acclimation period:	Not relevant
Food:	dried yeast (~2 -10 mg) was added to each test vessel on day 0 after introduction and again on days 14 and 21
Test system	50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum. Each vessel contained ten juvenile *F. candida* in 30 g dry weight equivalent of substrate.
Soil:	OECD artificial soil, 5% peat
Environmental conditions	
Temperature:	19.8 – 21.0°C
Photoperiod:	16:8-hour light: dark cycle at a range of 428 to 639 Lux
Soil moisture:	guideline requirement: 40-60 % of max. WHC during the test: 34-39 % max. WHC
pH:	5.89 - 6.60
Application rate(s)	0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate

Negative control:	reverse osmosis (RO) water solvent control (acetone)
Positive control:	boric acid
Post exposure observation period	28 days
Remarks	None

The toxicity of the zoxamide metabolite RH-163353 has been tested under laboratory test conditions on the springtail *Folsomia candida* (Willem) (Collembola, Isotomidae). Based on the results of a non-GLP range-finder, RH-163353 was evaluated in a bioassay at five concentrations of 0.08, 0.4, 2.0, 10 and 50 mg a.s./kg dry artificial soil substrate. These were compared to a solvent control and an untreated (water only) control. A toxic reference item (boric acid) was included in a separate validation study performed by the laboratory.
The test item was applied in acetone to a sand carrier. The test substrate was pre-moistened to 20.0% MWHC three days prior to application of the test substance. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water to achieve a soil moisture of 40% max. WHC. A control and a solvent control were included in the test.
Ten juvenile 9 to 12-day old *F. candida* were introduced into eight replicate test vessels for the controls and four vessels at each RH-163353 treatment rate. The test organisms were fed with dried yeast (~2 -10 mg) added to each test vessel (containing *F. candida*) on day 0, 14 and 21 of the bioassays. The moisture content was adjusted to 40 % max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 28 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light/dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.
After 28 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. RO water was added to the test substrate in each vessel to allow the collembola to rise to the surface. Enough water was added to achieve standing water above the test substrate and was stirred gently to allow any collembola present in air pockets to float to the surface. A small amount of liquid black dye was added to each test chamber to create a contrast between the collembola and the flotation liquid. *Ca*. 5 mL ethanol were added to anaesthetise the collembola. The number of surviving adult (parental Collembola) and juvenile *F. candida* were counted using a magnifying sheet.
Percent mortality of the introduced parental collembola was determined for each vessel and a treatment mean was calculated. A no observed effect concentration (NOEC) and LC_{10}, LC_{20} and LC_{50} values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on nominal test concentrations. The NOEC for adult survival was determined using a Bonferroni Adj t Test and the LC_x values were determined using Linear Interpolation (ICPIN). The NOEC for number of juveniles was determined using a Bonferroni Adj t Test and the EC_x values were determined using Linear Interpolation (ICPIN). Corrected mortality, in comparison to both the water and solvent controls, was calculated using Abbott's formula (Abbott, 1925) The reproductive output of the collembola is presented as the number of juveniles present in each vessel at the end of the test. A mean for each treatment was calculated from the vessel counts. No observed effect concentration (NOEC) and EC_{10}, EC_{20} and EC_{50} values were determined.
On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of RH-163353 (racemate) soil concentrations and the ratio of the enantiomers of exposed RH-163353. The methods were validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg (racemate). Concentrations of RH-163353 were determined by extracting soil samples with 1% formic acid in acetonitrile/acetone 3:1 v/v, then diluting further with unfortified control sample extract as required to bring the response within the calibration range. Samples were analysed by injection onto a liquid chromatography-time of flight mass spectrometry (LC-TOF/MS) system. The analytical procedure SMV 3202390-01V (SMV 3202390-02V to include stability data) was used to determine RH-166353 and

SMV 3202586-01V (a combination of the analytical procedures SMV 3202390-02V and SMV 3202586-01V) to assess the enantiomeric ratio the test substance.

Results and discussion

Environmental conditions stayed within the recommended ranges (besides the deviations given above).
Post application the mean test item concentrations were 0.6917, 1.1829, 2.0970, 3.9415, 6.8889, 13.3316, 23.4319 and 39.9660 mg a.s./kg dry substrate at application rates of 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. This is equivalent to 84.36, 80.47, 79.13, 82.80, 80.38, 86.40, 84.35 and 79.99% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate, respectively.
On day 28 the mean test item concentrations were 0.5624, 0.9504, 1.6736, 3.0910, 5.8986, 10.8197, 19.4087 and 35.1938 mg a.s./kg dry substrate at application rates 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. This is equivalent to 68.59, 64.65, 63.15, 64.94, 68.83, 70.12, 69.87 and 70.39% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate, respectively.
The mean enantiomeric ratio of the incubated RH-163353 in the 28-day samples was 47.93 : 52.07 for isomer A and B, respectively. This is in the range of the enantiomeric ratio of 48.6 : 51.4 given on the Certificate of Analysis for the test item batch, confirming its chiral stability during incubation.
As the mortality in all the treatment rates was less than in the solvent control, it indicates that the mortality in the treatments was not directly attributable to the presence or action of the solvent. In addition, mortality in the treatments reached a maximum of 20% in one rate only and this was therefore not significantly higher than in the water control (16.25%) and was also within the control validity criterion. In the treatments mortality was in the range was 5-20% with five of the rates having lower mortality than the water control. In this circumstance it is considered acceptable to use the valid water control as reference.
Mortality of 12.5, 17.5, 17.5, 10.0, 10.0, 20.0, 15.0 and 5.0% was recorded at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. Corrected mortality in comparison to the water control was 0, 1.49, 1.49, 0, 0, 4.48, 0 and 0% at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. Although the data is variable, and there is no clear dose response, the corrected mortality indicates that there is no overall adverse effect on survival, with lower levels of mortality in five of the eight treatment rates than in the water control and a maximum corrected mortality of 4.48%. This also indicates that the mortality in the treatments was not directly attributable to the presence or action of the solvent used in the treatment preparations.

Table 10.4.2-3: Percentage mortality of adult mites after 28 days

Treatment (mg a.s./kg dry soil)	Initial number of introduced Collembola	Mean mortality per treatment (%)	% reduction in comparison to the water control (%)
Water control	80	16.25	-
Solvent control	80	35.00	22.39
0.82	40	12.50	0
1.47	40	17.50	1.49
2.65	40	17.50	1.49
4.76	40	10.00	0
8.57	40	10.00	0
15.43	40	20.00	4.48
27.78	40	15.00	0
50	40	5.00	0

After 28 days of exposure there was no relevant mortality compared to the control. Based on mortality, the LC_{10}, $LC_{20,}$ LC_{50} and LOEC were > 50 mg a.s./kg dry substrate, the NOEC was determined at 50 mg

a.s./kg dry substrate.

Table 10.4.2-4: Mean number of juvenile mites produced after 28 days

Treatment (mg a.s./kg dry soil)	Mean number of juveniles	Reduction compared to the water control (%)
Water control	223.8	-
Solvent control	129.8	42.0
0.82	164.4	26.5
1.47	152.5	31.8
2.65	173.1	22.6
4.76	200.5	10.4
8.57	128.3	42.7
15.43	113.4	49.3
27.78	118.0	47.3
50	117.3	47.6

The mean number of juveniles produced was 164.4, 152.5, 173.1, 200.5, 128.3, 113.4, 118.0 and 117.3 at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively in comparison to 223.8 in the water control. Reductions in reproduction in comparison to the water control were 26.5, 31.8, 22.6, 10.4, 42.7, 49.3, 47.3 and 47.6% at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. As the potential presence or action of the solvent used in the treatment preparations had no effect on first generation survival it is considered unlikely that there would have been an effect on the reproductive capacity and therefore the results, although variable, indicate that RH-163353 has an adverse impact on reproductive capacity.

For RH-163353, the EC_{10} EC_{20} and EC_{50} values, LOEC and NOEC based on reproductive performance were:

EC_{50}	> 50	mg a.s./kg dry substrate
EC_{20}	0.6992	mg a.s./kg dry substrate (0.2183 - 10.65)
EC_{10}	0.3009	mg a.s./kg dry substrate (0.11 - 8.52)
LOEC	8.57	mg a.s./kg dry substrate
NOEC	4.76	mg a.s./kg dry substrate

The 95% confidence levels are given in parentheses.

All validity criteria were met:
- The mortality of the parental collembola in the water control group did not exceed 20% (actual adult mortality = 16.25 %). Mortality in the solvent control group was 36.25%, effects have been calculated in relation to the water control.
- The rate of production of juveniles was at least 100 per control vessel (mean actual rate of production of juveniles = 224 and 130 in the water and solvent controls, respectively).
- The coefficient of variance of reproduction in the water control treatment was 30% or less (actual coefficient of variance = 28.8% in the water control). The coefficient of variance of reproduction in the solvent control treatment was 55.1%, effects have been calculated in relation to the water control.

The sensitivity of the test system was confirmed in a separate study with boric acid as test item. The EC_{50} value was calculated to be 52.1 mg boric acid/kg artificial soil dry weight (ERS Study Number 3202464).

Conclusion

In a laboratory bioassay with zoxamide metabolite RH-163353 and the springtail *Folsomia candida,* assessments of survival and reproductive performance were made over 28 days. Included were data for a solvent control and an untreated (water only) control.

For RH-163353 the LC_{50}/EC_{50}, LOEC and NOEC values - based on comparison of the effect data to the (water only) control data – were determined as follow:

Endpoint	LC_{50} / EC_{50}	LOEC	NOEC
	(mg a.s./kg dry soil)		
Parental mortality	>50	>50	50
Reproductive output	>50	8.57	4.76

The following validity criteria as specified in OECD guideline 232 were achieved during the study and therefore the study data were considered valid: Mortality in the water control treatment was ≤ 20% and there were ≥ 100 juveniles per vessel. The co-efficient of variance for the reproductive output in the water control treatment was ≤ 30%.

According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

In overall, concerning deviations from the study protocol and test results the study is not considered to be valid and is not considered suitable for the risk assessment.

Reference:	KCA 8.4.2
Report	Gray, J., 2021: RH-141455: Collembola reproduction test in soil – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Limited, UK., Report No. 3202382, GLP, Not published
Guideline(s):	OECD 232 (2016)
Deviations:	During the main test at day 28 the mean % MWHC was below the minimum of 40% stated in the protocol in both the controls and in the 1.47 and 4.76 mg a.s./kg dry substrate treatment rates. Moisture content over the exposure period varied by >10% in both controls and at 0.82, 1.47, 2.65 and 4.75 mg a.s./kg dry substrate. However, these deviations were not considered to have had an adverse impact on the conduct or outcome of the study as the validity criteria were met. In addition, the OECD 232 guideline states 'The moisture content of the testing soil should be optimised to obtain a loose porous structure allowing the collembolans to enter into the pores. This is usually between 40-60% maximum WHC.' Therefore, the % MWHC that provides the correct soil structure may be outside the 40 – 60 % range. It is considered that as the validity criterion of ≥ 100 juveniles per replicate was achieved in the controls and at all rates of application the lower % MWHC has had no impact on the reproductive output.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (A19X08291)
Purity	92.77% (w/w)
Species:	*Folsomia candida*
Age:	10-12 days old
Source:	Bias Labs Ltd., UK
Acclimation period:	Not relevant
Food:	dried yeast (~2 -10 mg) was added to each test vessel on day 0 after introduction and again on days 14 and 21
Test system	50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum. Each vessel contained ten juvenile *F. candida* in 30 g dry weight equivalent of substrate.
Soil	OECD artificial soil, 5% peat
Environmental conditions	
Temperature:	19.3 – 20.6 °C
Photoperiod:	16:8-hour light: dark cycle at a range of 405 to 669 Lux
Soil moisture:	50 % max. WHC (nominally)
pH:	7.22 - 7.41
Application rate(s)	0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate
Negative control:	reverse osmosis (RO) water solvent control (acetone)
Positive control:	boric acid
Post exposure observation period	28 days
Remarks	None

The toxicity of the zoxamide metabolite RH-141455 has been tested under laboratory test conditions on the springtail *Folsomia candida* (Willem) (Collembola, Isotomidae). Based on the results of a non-GLP range-finder (with no test item related effects up to and inclusive 50 mg a.s./kg dry soil), RH-141455 was evaluated in a bioassay at the same five concentrations of 0.08, 0.4, 2.0, 10 and 50 mg a.s./kg dry artificial soil substrate. These were compared to a solvent control and an untreated (water only) control. A toxic reference item (boric acid) was included in a separate validation study performed by the laboratory.
The maximum water holding capacity (MWHC) of the substrate was 55.46% and the correct moisture content for the study was determined to be 50% MWHC, equivalent to 27.73% water content. The test substrate was pre-moistened to 25.0% MWHC two days prior to application of the test substance.
The test item was applied in acetone to a sand carrier. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water to achieve a soil moisture of 50% max. WHC. A control and a solvent control were included in the test.
Ten juvenile 10 to 12-day old *F. candida* were introduced into eight replicate test vessels for the controls and four vessels at each RH-141455 treatment rate. The test organisms were fed with dried yeast (~2 -10 mg) added to each test vessel (containing *F. candida*) on day 0, 14 and 21 of the bioassays. The moisture

content was adjusted to 50 % max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 28 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light:dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.
After 28 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. RO water was added to the test substrate in each vessel to allow the collembola to rise to the surface. Enough water was added to achieve standing water above the test substrate and was stirred gently to allow any collembola present in air pockets to float to the surface. A small amount of liquid black dye was added to each test chamber to create a contrast between the collembola and the flotation liquid. *Ca*. 5 mL ethanol were added to anaesthetise the collembola. The number of surviving adult (parental Collembola) and juvenile *F. candida* were counted using a magnifying sheet.
Percent mortality of the introduced parental collembola was determined for each vessel and a treatment mean was calculated. A no observed effect concentration (NOEC) and LC_{10}, LC_{20} and LC_{50} values were determined. Statistical analysis of the reproduction data was undertaken using CETIS version 1.8.6.8, based on nominal test concentrations. The NOEC for adult survival was determined using a Bonferroni Adj t Test and the LC_x values were determined using Linear Interpolation (ICPIN). The NOEC for number of juveniles was determined using a Bonferroni Adj t Test and the EC_x values were determined using Linear Interpolation (ICPIN). Corrected mortality, in comparison to both the water and solvent controls, was calculated using Abbott's formula (Abbott, 1925) The reproductive output of the collembola is presented as the number of juveniles present in each vessel at the end of the test. A mean for each treatment was calculated from the vessel counts. No observed effect concentration (NOEC) and EC_{10}, EC_{20} and EC_{50} values were determined.
On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of RH-141455 soil concentrations. The methods were validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.2 mg/kg. Concentrations of RH-141455 were determined by extracting soil samples with an extraction solvent (acetonitrile/acetone 2:1 v:v containing 0.1% formic acid), then diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography Fourier-transform mass spectrometry (LC-FT/MS). The analytical procedure (SMV 3202383-01V and its revisions) was used to determine RH-141455.

Results and discussion

Environmental conditions stayed within the recommended ranges.
Post-application the mean test item concentrations were 0.7724, 1.3638, 2.5931, 4.4097, 7.6760, 14.2632, 24.7952 and 41.0196 mg a.s./kg dry soil at application rates of 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry soil, respectively. This is equivalent to 94.19, 92.78, 97.85, 92.64, 89.57, 92.44, 89.26 and 82.04% of nominal at 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry soil, respectively. Due to recoveries of 82.04-97.85 % of nominal (calculated) values, the correct dosing of the test item has been confirmed.
On day 28 the mean substrate concentrations were 0.6979, 1.4805, 2.5892, 5.0642, 8.8461, 15.1094, 29.1776 and 57.9505 mg a.s./kg dry soil at application rates 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry soil, respectively. This is equivalent to 85.11, 100.72, 97.71, 106.39, 103.22, 97.92, 105.03 and 115.90% of nominal at 0.82, 1.47, 2.65, 476, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry soil, respectively. Recoveries of 85.11-115.9 % of nominal (calculated) values at the end of the test confirm the stability of the test item over 28 days in soil under the test conditions.

Table 10.4.2-5: Percentage mortality of adult mites after 28 days

Treatment (mg a.s./kg dry soil)	Initial no. of introduced collembola	Mean mortality per treatment (%)	Effects in comparison to the water control (%)	Effects in comparison to the water control (%)	Corrected mortality in comparison to both controls (%)
Water control	80	20.0	N/A	0	N/A
Solvent control	80	20.0	0	N/A	N/A
0.82	40	17.5	-3.1	-3.1	0
1.47	40	22.5	3.1	3.1	3.1
2.65	40	30.0	12.5	12.5	12.5
4.76	40	25.0	6.3	6.3	6.3
8.57	40	17.5	-3.1	-3.1	0
15.43	40	30.5	12.5	12.5	12.5
27.78	40	17.5	-3.1	-3.1	0
50	40	15.0	-6.3	-6.3	0

N/A = not applicable.

After 28 days of exposure there was no clear dose response and no relevant test item related mortality up to and inclusive the highest test rate of 50 mg a.s./kg dry soil compared to the control.
Mortality of 17.5, 22.5, 30.0, 25.0, 17.5, 30.0, 17.5 and 15.0% was recorded at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. Corrected mortality in comparison to both water and solvent controls was 0, 3.1, 12.5, 6.3, 0, 12.5, 0 and 0% at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. Although the data is variable, and there is no clear dose response, the corrected mortality indicates that there is no overall adverse effect on survival, with lower levels of mortality in the highest two rates than in both controls. As mortality in both the water and solvent controls was the same, there is no evidence to suggest that the use of solvent had an adverse impact.

The mean number of juveniles produced was 143.9, 156.6, 170.6, 176.3, 193.0, 160.3, 184.6 and 175.1 at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively in comparison to 130.3 and 102.7 in the water and solvent controls respectively. As more juveniles were produced at all rates of application than in either of the controls, the reductions in survival of 3.1, 12.5, 6.3 and 12.5% at 1.47, 2.65, 4.76 and 15.43 mg a.s./kg dry substrate respectively had no impact on overall reproductive capacity.

Table 10.4.2-6: Mean number of juvenile mites produced after 28 days

Treatment (mg a.s./kg dry substrate)	Mean number of juveniles	Effect in comparison to the water control (%)	Effect in comparison to the solvent control (%)
Water control	130.3	--	+26.8
Solvent control	102.7	-21.0	--
0.82	143.9	+10.5	+40.1
1.47	156.6	+20.2	+52.5
2.65	170.6	+31.0	+66.2
4.76	176.3	+35.3	+71.6
8.57	193.0	+48.2	+88.0
15.43	160.3	+23.0	+56.1
27.78	184.6	+41.7	+79.8
50	175.1	+34.5	+70.5

After 28 days of exposure there was up to and inclusive the highest rate of 50 mg a.s./kg dry soil no negative impact of the test item on the reproductive performance and no dose response notable.
As a result, the EC_{10}, EC_{20}, EC_{50}, LOEC and NOEC based on reproductive output were determined at:

- EC_{10}, EC_{20} and EC_{50} > 50 mg a.s./kg dry substrate
- LOEC >50 mg a.s./kg dry substrate
- NOEC 50 mg a.s./kg dry substrate

All validity criteria were met:

- The mortality of the original parental collembola in the control group(s) did not exceed 20% (actual adult mortality = 20 % in both the water and solvent controls).
- The mean rate of production of juveniles was at least 100 per control vessel (mean actual rate of production of juveniles = 130.3 and 102.7 in the water and solvent controls respectively).
- The coefficient of variance of reproduction in the water control treatment was 30% or less (actual coefficient of variance = 27.7%).
- The coefficient of variance of reproduction in the solvent control treatment exceeded 30% (actual coefficient of variance = 36.8%), however as no treatment related effects were recorded at any rate of application of RH-141455 the study is considered valid.

The sensitivity of the test system was confirmed in a separate study with boric acid as test item. The EC_{50} value was calculated to be 52.1 mg boric acid/kg artificial soil dry weight (ERS Study Number 3202464).

Conclusion

In a laboratory bioassay with zoxamide metabolite RH-141455 and the springtail *Folsomia candida,* assessments of survival and reproductive performance were made over 28 days. Included were data for a solvent control and an untreated (water only) control.
For RH-141455 the LC_{50}/EC_{50}, LOEC and NOEC values - based on comparison of the effect data to the (water only) control data – were determined as follow:

Endpoint	LC_{50} / EC_{50}	LOEC	NOEC
	(mg a.s./kg dry soil)		
Parental mortality	>50	>50	50
Reproductive output	>50	>50	50

The following validity criteria as specified in OECD guideline 232 were achieved during the study and therefore the study data were considered valid: Mortality in the water and solvent control treatment was ≤ 20% and there was a mean of ≥ 100 juveniles; per vessel. The co-efficient of variance for the reproductive output in the water control treatment was ≤ 30%.

In addition to the further zoxamide metabolite studies on soil macro-organisms (other than earthworms) requested by EFSA (2017), studies on *Folsomia* and *Hypoaspis* have been performed with the parent compound zoxamide. The zoxamide studies have been conducted with a SC formulation containing 240 g/L zoxamide, the representative formulation during AIR. The studies are regarded representative to address the potential risk from the use of zoxamide technical. This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints for *Folsomia candida* (5% peat content):

The 28-days NOEC value for Zoxium 240 SC for both survival and reproduction = 1000 mg test item/kg soil dry weight, equivalent* to 217 mg a.s./kg soil dry weight.

The 28-days EC20 and EC10 values for Zoxium 240 SC >1000 mg test item/kg soil dry weight, equivalent* to >217 mg a.s./kg soil dry weight (based on the numbers of offspring produced).

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

*based on analysed a.s. content of 21.7% w/w in the test item.

Reference:	KCA 8.4.2
Report	Parsons, Ch. 2020: Zoxium 240 SC - A laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae) in artificial soil substrate Gowan Crop Protection Ltd., UK Mambo-Tox Ltd., UK, Report No. GOW-17-13, GLP, Not published
Guideline(s):	OECD 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (18011201-72-52)
Content of a.s.:	240 g/L Zoxamide (nominal), 21.7 % (w/w) (analysed)
Species:	*Folsomia candida* (Collembola, Isotomidae)
Age:	11-12 days old
Source:	In-house culture since 2015; original source: Bias Labs Ltd., UK
Acclimation period:	2 days

Food:	6 mg of dried granulated yeast on days 0 and 14 during the bioassay
Test system	Glass jars (approximately 125 mL capacity and 4.5 cm internal diameter), secured with close-fitting, screw-top lids. A 5-mm-diameter hole was made in the lid for ventilation and this was covered with fine nylon netting (80 µm mesh). The arenas were filled with test substrate to a depth of 2-4 cm.
Soil	OECD artificial soil, 5% peat
Environmental conditions	
Temperature:	19.5 – 21.5°C
Photoperiod:	16-hour photoperiod (580-650 lux)
Soil moisture:	50 % max. WHC
pH:	6 ±0.5
Application rate(s)	1000 mg f.p./kg soil dry weight (217 mg a.s./kg soil dry weight)
Negative control:	purified water
Positive control:	boric acid
Post exposure observation period	28 days
Remarks	None

The toxicity of the test item Zoxium 240 SC (product code GWN-9790EU), a suspension concentrate (SC) formulation containing the active substance zoxamide at nominally 240 g/L, has been tested under laboratory test conditions on the springtail *Folsomia candida* (Willem) (Collembola, Isotomidae). Based on the results of a non-GLP range-finder, Zoxium 240 SC was evaluated in a limit test bioassay at a single concentration of 1000 mg test item/kg soil dry weight (217 mg a.s./kg soil dry weight). This was compared to an untreated (water only) control. A toxic reference item (boric acid) was included in a separate validation study performed by the laboratory.
The control treatment and test item treatment, diluted in purified water, were thoroughly mixed into an artificial soil substrate (containing 5% w/w peat). Ten juvenile *F. candida* (11-12 days old) were introduced into small, ventilated, glass jar arenas (8 per treatment). Dry granulated yeast was provided on the soil surface as food and this was re-plenished 14 days after treatment (DAT). The soil was kept at 50% max. WHC. The bioassay was carried out under environmentally controlled conditions.
At 28 DAT, the numbers of both surviving adults and F1 progeny (i.e. juvenile springtails) in each test arena were recorded at 28 DAT. The test substrate from each arena was tipped into a tray (approximately 11 cm x 17 cm in area and 6 cm in depth). Water (approx. 200 mL) was then added to the substrate and stirred gently, so that the soil sank and the springtails floated to the surface. Any adult springtails floating on the water were counted and removed. Black ink was then added to the water and the numbers of any nymphs (smaller in size to adults) left in each arena were assessed. The ink darkened the water so that it contrasted with the light-coloured springtails floating on the water surface. A grill was held over the surface to aid with counting using a binocular microscope. It was assumed that any adult springtails that were recovered would have been those originally introduced and that any shortfall in the original number was an indication that they had died during the bioassay.
Statistical analyses were performed using validated computer software, namely SPSS (IBM Corp., 2016). The percentage mortality of the springtails originally introduced was calculated for the test item treatment, both before and after correction for any control treatment losses using Abbott's formula. The 28-day mortality data for the individual test-item treatment were compared to those for the control using Fisher's Exact Test ($\alpha = 0.05$). The results were used to determine the no observed effect concentration (NOEC) with respect to mite survival. The mean number of offspring produced per replicate and the standard deviation were calculated for the control and test item treatment. In addition, the percentage difference in reproductive performance in the test-item treatment group, compared to the control group, was calculated. The data from the reproduction assessments in the individual treatments were checked for

normality (Shapiro-Wilk test, α = 0.05) and for equality of variances (Levene's test, α = 0.05). As normality and homogeneity could be assumed, the numbers of F1 progeny produced in the test-item treatment was compared to numbers in the control, using t-test for independent samples (α = 0.05). A NOEC with respect to springtail reproduction was determined.
Three separate samples of approximately 25-30 g of soil were preserved from both the test-item treatment and the control treatment for analytical verification of the concentration of test item present in the treated soil. The 0 DAT samples were frozen immediately after preparation and the remaining samples were kept alongside the test arenas until 14 DAT or 28 DAT when they were made up to 50% WHC before being frozen. The samples were shipped frozen to Biochem Agrar for analytical verification of the test item concentrations in the soil with a method fully validated according to SANCO/3029/99 rev. 4 under project number 18 35 CRX 0024.

Results and discussion

Environmental conditions stayed within the recommended ranges.
The nominal initial test item concentration in the freshly prepared soil specimen was analytically confirmed (112% recovery for the active substance). Moreover, the zoxamide concentrations stayed within the range of 98 – 112% of the nominal initial concentration throughout the whole study period.
At 28 days, there was 5% mortality in the control treatment, compared with 8% mortality (3% corrected mortality) in the 1000 mg test item/kg soil dry weight treatment concentration of Zoxium 240 SC. The result from the test item treatment was not statistically significantly different from the control (Fisher's Exact Test, α = 0.05). The NOEC value was therefore 1000 mg test item/kg soil dry weight.

Table 10.4.2-7: Percentage mortality of adult mites at 28 days

Treatment (mg f.p./kg soil dry weight)	Initial number of introduced collembola	Mean mortality per treatment (%)	Effect in comparison to the control (%)
Control	80	5	-
1000	80	8	3

At 28 days of exposure, based on the numbers of offspring produced, the EC_{20} and EC_{10} values for Zoxium 240 SC were >1000 mg test item/kg soil dry weight. Statistical comparisons with the control indicated that the overall NOEC with respect to both springtail survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item).
The mean number of progeny produced per replicate was 469 in the control and 439 (6% reduction compared to the control) in the 1000 mg test item/kg soil dry weight treatment. The result from the test item treatment was not statistically significantly different from the control, the NOEC value was therefore 1000 mg test item/kg soil dry weight. The reproduction data was not appropriate for regression analysis to calculate effect concentrations (ECx) from a dose-response curve. The EC_{20} and EC_{10} values were therefore extrapolated from the available data as > 1000 mg test item/kg soil dry weight.

Table 10.4.2-8: Mean number of juveniles produced after 28 days

Treatment (mg f.p./kg soil dry weight)	Mean number of juveniles	Effect in comparison to the control (%)
Control	469	-
1000	439	6

All validity criteria were met:
- The mortality of the parental collembola in the water control group did not exceed 20% (actual value of control in test = 5%).
- The mean number of juveniles recorded in the control treatment should exceed 100 per replicate at the end of the test (actual value of control in test = 469).
- The coefficient of variation of reproduction in the control should not exceed 30% (actual value of control in test = 15.1%).

The efficiency of the method used to extract the springtails in this test was > 95%. This was confirmed in a separate test carried out by the test facility in October 2014. Here, the efficiency of the extraction method was 100% for adult and 98.3% for juvenile springtails.
The sensitivity of the test system was confirmed in a separate study with boric acid as test item, in which the EC_{50} was calculated to be 111 mg boric acid/kg artificial soil dry weight.

Conclusion

In a laboratory bioassay with Zoxium 240 SC and the springtail *Folsomia candida*, assessments of survival and reproductive performance were made over 28 days.
For Zoxium 240 SC the lowest observed effect concentration (LOEC), the $EC_{50,}$ EC_{20} and EC_{10} values were >1000 mg test item/kg soil dry weight. Statistical comparisons with the control indicated that the overall NOEC with respect to both springtail survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item) – the highest test item rate investigated (limit test).

In addition to the further zoxamide metabolite studies on soil macro-organisms (other than earthworms) requested by EFSA (2017), studies on *Folsomia* and *Hypoaspis* have been performed with the parent compound zoxamide. The zoxamide studies have been performed with a SC formulation containing 240 g/L zoxamide, the representative formulation during AIR. The studies are regarded representative to address the potential risk from the use of zoxamide. This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints for *Hypoaspis aculeifer* (5% peat content):

The 14-days LOEC, the EC50, EC20 and EC10 values were >1000 mg test item/kg soil dry weight (equivalent* to 217 mg a.s./kg soil dry weight).

14-days NOEC with respect to both soil mite survival and reproduction = 1000 mg test item/kg soil dry weight (equivalent* to 217 mg a.s./kg soil dry weight) – the highest test item rate investigated (limit test).

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

*based on analysed a.s. content of 21.7% w/w in the test item

Reference:	KCA 8.4.2
Report	Parsons, Ch., 2020: Zoxium 240 SC – A laboratory test to determine the effects of fresh residues on the predatory soil mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate Gowan Crop Protection Ltd., UK Mambo-Tox Ltd., UK, Report No. GOW-17-14, GLP, Not published
Guideline(s):	OECD 232 (2016)
Deviations:	The temperature range at which the test mite culture was intended to be maintained was 18-22°C. The actual temperature range recorded was 20.2-

22.6°C. A total of 76 out of 696 readings exceeded 22°C on 6 separate occasions. This deviation from the intended range was due high ambient temperatures in the laboratory, which caused a malfunction in the controlled-environment facilities. With regard to performance of the test organisms in the control, this deviation was considered to not cause adverse effects on the bioassay and to not affect the integrity of the study.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test material (Lot/Batch No.)	Zoxium 240 SC (18011201-72-52)
Content of a.s.:	240 g/L Zoxamide (nominal), 21.7 % (w/w) (analysed)
Species:	*Hypoaspis aculeifer*
Age:	Adult mites
Source:	In-house culture since 2016; original source: Bias Labs Ltd., UK
Acclimation period:	At least 2 days
Food:	Cheese mites (*Tyrophagus putrescentiae* (Schrank)) *ad libitum* and springtails (*Folsomia candida* (Willem))
Test system	60 mL capacity glass jars (5.5 cm tall x 5.2 cm outer diameter, 4.4 cm inner diameter), with screw-top lids. An 8-mm-diameter hole was made in the lid for ventilation and this was covered with fine nylon netting (80 µm mesh size).
Soil	OECD artificial soil, 5% peat
Environmental conditions	
Temperature:	19.2 – 21.3°C
Photoperiod:	16-hour photoperiod (600-690 lux)
Soil moisture:	50 % max. WHC
pH:	6 ±0.5
Application rate(s)	1000 mg f.p./kg soil dry weight (217 mg a.s./kg soil dry weight)
Negative control:	purified water
Positive control:	Dimethoate
Post exposure observation period	14 days
Remarks	None

The test item Zoxium 240 SC (product codes GWN-9790EU and GWN-10624), a suspension concentrate (SC) formulation containing the active substance zoxamide at nominally 240 g/L, has been studied under laboratory conditions whether it has harmful effects on the predatory soil mite *Hypoaspis aculeifer* (Canestrini) (Acari, Laelapidae).
Based on the results of a range-finding study, Zoxium 240 SC was evaluated in a bioassay at a single treatment concentration, equivalent to 1000 mg test item/kg soil dry weight (limit test). This was compared to an untreated (water only) control. A toxic reference item (dimethoate) was included in a separate validation study.

The control treatment and test item treatment, diluted in purified water, were thoroughly mixed into an artificial soil substrate (containing 5% w/w peat), aliquots of which were then transferred into small, ventilated, glass jar arenas (n = 8 per treatment). Ten female soil mites (approximately 7-14 days after becoming adult) were then introduced into each arena. There were eight replicate arenas for the control treatment and for the test-item treatment concentration. Cheese mites (*Tyrophagus putrescentiae* (Schrank)) and springtails (*Folsomia candida* (Willem)) were provided as food for the predatory soil mites and were replenished *ad libitum*. The moisture content of the soil was adjusted to 50 % max. WHC. The pH of soil was measured according to ISO 10390 in abiotic replicate vessels at the start and end of the study. The test was carried out under environmentally controlled conditions.
At 14 days after treatment (DAT), both the number of surviving adult predatory soil mites and of their offspring were recorded following extraction from the soil via Tullgren funnel. Over a two-day period, the heat of a light-bulb (25 Watt, with a 24 h photoperiod) slowly dried the soil from the top, forcing the *H. aculeifer* to move downwards through the soil until they fell from the base of the funnels into collecting vials with a 70% (v/v) methyl alcohol in which they were preserved.
Statistical analyses were performed using validated computer software, namely SPSS (IBM Corp., 2016). The percentage mortality of the mites originally introduced was calculated for the test item treatment, both before and after correction for any control treatment losses using Abbott's formula. The 14-day mortality data were compared to those for the control using Fisher's Exact Test ($\alpha = 0.05$). The results were used to determine the no observed effect concentration (NOEC) with respect to mite survival. The mean number of offspring produced per replicate and the standard deviation were calculated for the control and test item treatment. In addition, the percentage difference in reproductive performance in the test-item treatment group, compared to the control group, was calculated. The data from the reproduction assessments in the individual treatments were checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for equality of variances (Levene's test, $\alpha = 0.05$). As normality and homogeneity could be assumed, the numbers of F1 progeny produced in the test-item treatment was compared to numbers in the control, using t-test for independent samples ($\alpha = 0.05$). A NOEC with respect to springtail reproduction was determined.
Three separate samples of approximately 25-30 g of soil were preserved from both the test-item treatment and the control treatment for analytical verification of the concentration of test item present in the treated soil. The 0 DAT samples were frozen immediately after preparation and the remaining samples were kept alongside the test arenas until 14 DAT when they were made up to 50% WHC before being frozen. The samples were shipped frozen to Biochem Agrar for analytical verification of the test item concentrations in the soil with a method fully validated according to SANCO/3029/99 rev. 4 under project number 18 35 CRX 0025.

Results and discussion

Environmental conditions stayed within the recommended ranges.
The nominal initial test item concentration in the freshly prepared soil specimen was analytically confirmed (102% recovery for the active substance). The zoxamide concentrations stayed within the range of 102 – 109% of the nominal initial concentration throughout the whole study period.
At 14 days, there was 6% mortality in the control treatment, compared with 9% mortality (3% corrected mortality) in the 1000 mg test item/kg soil dry weight treatment concentration of Zoxium 240 SC. The result from the test item treatment was not statistically significantly different from the control (Fisher's Exact Test, $\alpha = 0.05$). The NOEC value was therefore 1000 mg test item/kg soil dry weight.

Table 10.4.2-9: Mortality of adult *H. aculeifer* after 14 days

Treatment (mg f.p./kg soil dry weight)	Initial number of introduced *Hypoaspis*	Mean mortality per treatment (%)	Effect in comparison to the control (%)
Control	80	6	-
1000	80	9	3

The mean number of progeny-produced per replicate was 235 in the control and 231 in the 1000 mg test item/kg soil dry weight treatment concentration of Zoxium 240 SC.

Table 10.4.2-10: Mean number of juveniles produced after 14 days

Treatment (mg f.p./kg soil dry weight)	Mean number of juveniles	Effect in comparison to the control (%)
Control	235	-
1000	231	1

Based on this data the EC_{20} and EC_{10} values for Zoxium 240 SC were > 1000 mg test item/kg soil dry weight. The result from the test item treatment was not statistically significantly different from the control (t-test for independent samples, $\alpha = 0.05$). The overall NOEC with respect to both mite survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item).
The reproduction data was not appropriate for regression analysis since only a single concentration of the test item was evaluated (limit test). Key effect concentrations (EC_{20} and EC_{10}) were therefore extrapolated with respect to this single test item treatment.

The following validity criteria were met for the study; therefore, these data are considered to be valid.
- The mean mortality of the original parental mites in the control treatment did not exceed 20% over 14 days (actual value of control in test = 6%).
- The mean number of juveniles recorded in the control treatment should be at least 50 per replicate at the end of the test (actual value of control in test = 235).
- The coefficient of variation of reproduction in the control should not exceed 30% (actual value of control in test = 5.7%).

The efficiency of the method used to extract the mites should be ≥ 90%. In a separate test carried out by the test facility this was determined to be 98.0% (98.0% for the adult female mites and for the juvenile mites).
The sensitivity of the test system has been determined in a separate bioassay using a formulated sample of dimethoate. As a result, an EC_{50} of 6.3 mg a.s./kg soil dry weight (95% confidence limits = 5.6 and 7.0 mg a.s./kg soil dry weight) was calculated.

Conclusion

In a laboratory bioassay with Zoxium 240 SC and the predatory soil mite *Hypoaspis aculeifer*, assessments of survival and reproductive performance were made over 14 days.
The lowest observed effect concentration (LOEC), the EC_{50}, EC_{20} and EC_{10} values were >1000 mg test item/kg soil dry weight. Statistical comparisons with the control indicated that the overall NOEC with respect to both soil mite survival and reproduction was 1000 mg test item/kg soil dry weight (equivalent to 217 mg a.s./kg soil dry weight, based on analysed a.s. content of 21.7% w/w in the test item) – the highest test item rate investigated (limit test).

According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints for *H. aculeifer* determined (5% peat content)**:**

The 14-day NOEC value based on reproduction = 27.78 mg a.s./kg dw

The 14-day EC10 value based on reproduction = 32.19 mg a.s./kg dw

The 14-day EC20 value based on reproduction = 38.46 mg a.s./kg dw

The 14-day EC50 value based on reproduction ≥ 50 mg a.s./kg dw

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Reference:	KCA 8.4.2
Report	Gray, J. 2021: RH-163353: Effect on reproduction of *Hypoaspis* (Geolaelaps) *aculeifer* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202391, GLP, Not published
Guideline(s):	OECD 226 (2016)
Deviations:	At day 14 the mean % MWHC was below the minimum of 40 % stated in the protocol for the water control and in the 0.82, 1.47, 2.65 and 4.76 mg a.s./kg dry substrate treatments. Moisture content over the exposure period also varied by >10% in both controls and at 0.82, 1.47, 2.65 and 4.75 mg a.s./kg dry substrate. This deviation was not considered to have had an adverse impact on the conduct or outcome of the study as the validity criteria were met in both water an solvent controls. In addition, there was no effect on the parental generation (NOEC > 50 mg a.s./kg dry substrate and all LCx values > 50 mg a.s./kg dry substrate). An adverse effect on reproduction was only recorded at 50 mg a.s./kg dry substrate where the moisture content was maintained at > 40 % MWHC throughout the exposure period.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-163353 (HHGCP001-00-2)
Purity	99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)
Species:	*Hypoaspis aculeifer*
Age:	Adult mites
Source:	Bias Labs Ltd., UK
Acclimation period:	--

Food:	juvenile collembola *ad-libitum*
Test system	50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum, aerated two to three times weekly. Each replicate contained ten female *H. aculeifer* in 20 g dry weight of artificial soil.
Soil:	artificial soil, 5% peat
Environmental conditions	
Temperature:	19.4 – 41.2 °C
Photoperiod:	16-hour photoperiod (432 to 609 lux)
Soil moisture:	32 - 48 % max. WHC
pH:	5.72 - 6.14
Application rate(s)	0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry soil
Negative control:	reverse osmosis (RO) water solvent control (acetone)
Positive control:	Dimethoat
Post exposure observation period	14 days
Remarks	None

The zoxamide metabolite RH-163353 has been studied under laboratory test conditions whether it has harmful effects on the predatory soil mite *Hypoaspis* (Geolaelaps) *aculeifer* (Acari, Laelapidae).
Based on the results of a range-finding study, RH-163353 was evaluated in a bioassay at application rates of 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry soil. These were compared to an untreated (water only) control. A toxic reference item (dimethoate) was included in a separate validation study.
The test substrate was pre-moistened to 25.0% MWHC three days prior to application of the test substance. The test item was applied in acetone to a sand carrier. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water. A solvent control was included in the test.
Ten adult female *H. aculeifer* were introduced in seven replicate vessels per test item treatment and 11 for control. The vessels contained 20 g dry weight of an artificial soil (5% peat). The test organisms were fed with juvenile collembola *ad-libitum.* The moisture content was adjusted to 45% max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 14 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light:dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.
After 14 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. A Tullgren extraction funnel was used; the funnels are designed to extract organisms from substrate using specialist heat lamps with an increasing temperature gradient, prompting the adult and juvenile *H. aculeifer* to burrow downwards into collection pots containing fixation fluid. The fixation fluid from each replicate was emptied into a petri-dish. The collection pots were rinsed out using ca 1–2 mL RO water to ensure all organisms were removed from the collection pots. The number of the originally introduced adult mites, and the juvenile mites produced during the 14-day test, were then counted using a microscope, and recorded per replicate. Furthermore, any observed differences between the morphology of the mites were also recorded, where necessary.
Percentage adult mortality of the *H. aculeifer* in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and LC_{50} were determined. Corrected mortality, in comparison to both the water and solvent controls, was calculated using the Abbott's formula. Statistical analysis of the reproduction data, in comparison to both the water and pooled controls, was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. The NOEC and LOEC for the effects on survival were determined using a Bonferroni Adj t Test for comparison with the water control

and a Wilcoxon/Bonferroni Adj Test (pooled controls) and the ECx values were determined using Linear Interpolation (ICPIN). The NOEC and LOEC for the number of juveniles were determined using a Bonferroni Adj t Test and the EC_x values were determined using Linear Interpolation (ICPIN).
On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of the test item soil concentrations. The methods were validated according to SANCO 3029/99 rev. 4 with an LOQ of 0.016 mg/kg. Concentrations of RH-163353 were determined by extracting soil samples with an extraction solvent (acetonitrile/acetone 3:1 v/v containing 1% formic acid), then diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography time of flight mass spectrometry (LC-TOF/MS). The analytical procedure (SMV 3202390-01V, and its revisions) was used to determine RH-163353.
The analytical method validation for the enantiomeric ratio analysis for RH-163353 was conducted under Smithers ERS Study Number 3202586 (established analytical procedure, SMV 3202586-01V). A combination of the analytical procedures, SMV 3202390-01V and SMV 3202586-01V were used to assess the enantiomeric ratio of the test substance.

Results and discussion
Environmental conditions stayed within the recommended ranges (with the above deviations).
Post application the mean substrate concentrations were 0.8656, 1.4456, 2.9364, 4.4112, 8.2104, 15.5855, 24.2260 and 47.2443 mg a.s./kg dry substrate at application rates of 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. This is equivalent to 105.56, 98.34, 110.81, 92.67, 95.80, 101.01, 87.21 and 94.49% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. The recoveries of 87.21-110.81 % of nominal confirm the correct dosing of the test item. On day 14 the mean substrate concentrations were 0.6869, 1.5427, 2.4617, 3.9928, 7.8009, 12.9666, 25.2966 and 50.8642 mg a.s./kg dry substrate at application rates 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. This is equivalent to 83.77, 104.95, 92.90, 83.88, 91.03, 84.04, 91.06 and 101.73% of nominal at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate respectively. The moisture content was adjusted for each individual sample. Recoveries of 83.77-101.73 % of nominal at the end of the test confirm the stability of the test item over 14 days in soil under the test conditions.
At day 14 there was 45.43, 46.36, 47.01, 46.79, 46.84, 48.66, 48.56 and 48.82% of Isomer A present in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate groups respectively in comparison to a mean of 47.66% for the calibration samples. This was equivalent to 95.32, 97.27, 98.63, 98.18, 98.29, 102.10, 101.89 and 102.44% of the calibration mean. There was 54.57, 53.64, 52.99, 53.21, 53.16, 51.34, 51.44 and 51.15% of Isomer B present in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate groups respectively in comparison to a mean of 52.34% for the calibration samples. This was equivalent to 104.26, 102.49, 101.24, 101.66, 101.56, 98.09, 98.28 and 97.78% of the calibration mean. The mean ratio for the day 14 samples was 47.31% isomer A and 52.69% of isomer B of RH-163353. Compared to the enantiomeric ratio in the certificate of analysis of 48.6:51.4, this confirms the stability of the isomer ratio of RH-163353.
After 14 days of exposure, there was 12.5, 5.0, 5.0, 15.0, 5.0, 7.5, 12.5 and 5.0% mortality observed in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate groups respectively, in comparison to 4.0 % mortality in both the water and solvent controls. Corrected mortality was 8.9, 1.0, 1.0, 11.5, 1.0, 3.6, 8.9 and 1.0% observed in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate groups respectively. Based on these results, the NOEC and LOEC for 14-day survival were statistically determined to be 50 mg a.s./kg dry substrate and >50 mg a.s./kg dry substrate, respectively in comparison to both the water and pooled controls. The LC_{10}, LC_{20} and LC_{50} values for 14-day survival were all statistically determined to be >50 mg a.s./kg dry substrate in comparison to both the water and pooled controls.

Table 10.4.2-11: Mortality of adult *H. aculeifer* after 14 days

Treatment (mg a.s./kg dry substrate)	Initial number of introduced *Hypoaspis*	Mean mortality per treatment (%)	Effect in comparison to the water control (%)	Effect in comparison to the solvent control (%)
Water control	50	4.0	N/A	0.0
Solvent control	50	4.0	0.0	N/A
0.82	40	12.5	8.9	8.9
1.47	40	5.0	1.0	1.0
2.65	40	5.0	1.0	1.0
4.76	40	15.0	11.5	11.5
8.57	40	5.0	1.0	1.0
15.43	40	7.5	3.6	3.6
27.78	40	12.5	8.9	8.9
50	40	5.0	1.0	1.0

N/A = not applicable

The mean number of juveniles per vessel was 118.0, 126.5, 122.5, 137.0, 162.5, 143.3, 130.0 and 86.3 in the 0.82, 1.47, 2.65, 4.76, 8.57, 15.43, 27.78 and 50 mg a.s./kg dry substrate groups respectively, in comparison to 118.4 and 114.8 in the water and solvent controls respectively. At 50 mg a.s./kg dry substrate this corresponded to a reduction of 27.2%, when compared to the water control and 24.9% when compared to the solvent control. Increases of 0.3, 6.8, 3.5, 15.7, 37.2, 21.0 and 9.8% were recorded at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43 and 27.78 mg a.s./kg dry substrate groups respectively, in comparison to the water control. Increases of 2.8, 10.2, 6.7, 19.3, 41.6, 24.8 and 13.2% were recorded at 0.82, 1.47, 2.65, 4.76, 8.57, 15.43 and 27.78 mg a.s./kg dry substrate groups respectively, in comparison to the solvent control.

Table 10.4.2-12: Mean number of juveniles at day 14

Treatment (mg a.s./kg dry substrate)	Mean number of juveniles[a]	Effect in comparison to the water control (%)	Effect in comparison to the solvent control (%)	Effect in comparison to pooled controls (%)
Water control	118.4	N/A	+3.1	+1.5
Solvent control	114.8	-3.0	N/A	-1.5
0.82	118.0	-0.3	+2.8	+1.2
1.47	126.5	+6.8	+10.2	+8.5
2.65	122.5	+3.5	+6.7	+5.1
4.76	137.0	+15.7	+19.3	+17.5
8.57	162.5	+37.2	+41.6	+39.4
15.43	143.3	+21.0	+24.8	+22.9
27.78	130.0	+9.8	+13.2	+11.5
50	86.3	-27.2	-24.9	-26.0

N/A = not applicable
[a] pooled control mean = 116.6 juveniles

There were no other observations noted.

Validity criteria:
The following validity criteria were met for the study; therefore, these data are considered to be valid.
- The mean mortality of the original parental mites in the control treatment did not exceed 20% over 14 days (actual mean mortality = 4.0% in both water and solvent controls)
- The mean number of juveniles was at least 50 in the controls over 14 days (actual mean number of juveniles = 118.4 and 114.8 in the water and solvent controls respectively)
- The coefficient of variance of the calculated reproduction in the control was 30% or less over 14 days (actual coefficient of variance = 6.56 and 5.86% in the water and solvent controls respectively)

H. aculeifer from the same source culture were used in a reference toxicity test with Dimethoate 400 g/L EC, a known toxic substance (performed under in-house GLP Smithers ERS. Study Number 3202598, December 2019). The EC_{50} value was estimated to be 2.043 mg a.i/kg dry substrate.

Table 10.4.2-13: NOEC, EC_{10}, EC_{20} and EC_{50} values

Parameter	Parent mortality	Value on reproduction compared to water control	Value on reproduction compared to pooled controls
	mg a.s./kg dry substrate (95 % confidence limits)		
NOEC	50	27.78	27.78
LOEC	>50	>50	>50
LC_{10}/EC_{10}	>50 (N/A)	32.19 (20.29 - 39.03)	32.19 (24.61 - 38.67)
LC_{20}/EC_{20}	>50 (N/A)	38.46 (29.08 – N/A)	38.46 (30.19 – 53.4)
LC_{50}/EC_{50}	>50 (N/A)	>50 (N/A)	>50 (N/A)

N/A = not available

Conclusion
In a laboratory bioassay with RH-163353 and the predatory soil mite *Hypoaspis aculeifer,* assessments of survival and reproductive performance were made over 14 days. After 14 days, based on adult mortality and the numbers of offspring produced, the following endpoints were noted in comparison to pooled controls:
14-day NOEC value for adult *H. aculeifer* survival = 50 mg a.s./kg dry substrate
14-day LC_{50} value for adult *H. aculeifer* survival ≥ 50 mg a.s./kg dry substrate
NOEC value based on reproduction = 27.78 mg a.s./kg dry substrate
EC_{10} value based on reproduction = 32.19 mg a.s./kg dry substrate
EC_{20} value based on reproduction = 38.46 mg a.s./kg dry substrate
EC_{50} value based on reproduction ≥ 50 mg a.s./kg dry substrate
The study is valid.

According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* According to EFSA (20017): *"Further data are needed to address the risk to soil macro-organisms other than earthworms for the metabolites RH-163353 and RH-141455 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints for *Hypoaspis aculeifer* (5% peat content):

The 14-day NOEC value based on reproduction = 50 mg a.s./kg dw

The 14-day EC50 value based on reproduction ≥50 mg a.s./kg dw

It was not possible to calculate EC10 and EC20 values since there was no significant effect at the concentration tested (limit test).

For risk assessment purposes endpoints are used as corrected values derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Reference:	KCA 8.4.2
Report	Gray, J., 2021: RH-141455: Effect on reproduction of *Hypoaspis* (Geolaelaps) *aculeifer* – Amended final report 1 Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202383, GLP, Not published
Guideline(s):	OECD 226 (2016)
Deviations:	The light period was less than the 16 hours specified. The analytical data confirmed that the applied dose was in excess of the required 50 mg a.s./kg but showed it was also outside the accepted range of 80-100% of nominal (+29.28% at Day 0 and + 50.01% at Day 14). However, as the dose was in excess of that required it is considered that this confirms no adverse effect on survival or reproduction at the intended dose. As all the validity criteria were met. Therefore, these deviations were not considered to have had any impact on the integrity or outcome of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-141455 (A19X08291)
Purity	92.77% (w/w)
Species:	*Hypoaspis aculeifer*
Age:	Adult mites
Source:	Bias Labs Ltd., UK
Acclimation period:	--
Food:	juvenile collembola *ad-libitum*
Test system	50 mm diameter glass vessel with a volume of approximately 120 mL, covered with a screw lid as a minimum, aerated two to three

	times weekly. Each replicate contained ten female *H. aculeifer* in 20 g dry weight of artificial soil.
Soil:	artificial soil, 5% peat
Environmental conditions	
Temperature:	20.1 – 20.4°C
Photoperiod:	16-hour photoperiod (642-763 lux)
Soil moisture:	40-51 % max. WHC
pH:	7.36 – 7.59
Application rate(s)	50 mg a.s./kg dry soil (limit test)
Negative control:	reverse osmosis (RO) water solvent control (acetone)
Positive control:	Dimethoat
Post exposure observation period	14 days
Remarks	None

The zoxamide metabolite RH-141455 has been studied under laboratory test conditions whether it has harmful effects on the predatory soil mite *Hypoaspis* (Geolaelaps) *aculeifer* (Acari, Laelapidae).
Based on the results of a range-finding study with no test item related effects up to and inclusive a test item concentration of 50 mg a.s./kg dry soil, RH-141455 was evaluated in a bioassay at an application rate of 50 mg a.s./kg dry soil (limit test) compared to an untreated (water only) control. A toxic reference item (dimethoate) was included in a separate validation study.
The test substrate was pre-moistened to 27.5% MWHC three days prior to application of the test substance. The test item was applied in acetone to a sand carrier. The acetone was allowed to evaporate before the treated sand was mixed with the soil and a required amount of reverse osmosis (RO) water. A solvent control was included in the test.
Ten adult female *H. aculeifer* were introduced in eight replicate vessels per test item treatment and control. The vessels contained 20 g dry weight of an artificial soil (5% peat). The test organisms were fed with juvenile collembola *ad-libitum.* The moisture content was adjusted to 55% max. WHC. The test vessels were aerated at least twice a week by removing the lid for several seconds. The pH (in 1 M KCl) of the treated substrate, sampled on days 0 and 14 (taken from abiotic vessels) was determined. The test was carried out in a temperature-controlled incubator at 20 ± 2°C and a 16:8 hour light:dark cycle (light intensity of 400 – 800 Lux), measured at least weekly throughout the test.
After 14 days the numbers of the original springtails still surviving and the numbers of any offspring they had produced were recorded. A Tullgren extraction funnel was used; the funnels are designed to extract organisms from substrate using specialist heat lamps with an increasing temperature gradient, prompting the adult and juvenile *H. aculeifer* to burrow downwards into collection pots containing fixation fluid. The fixation fluid from each replicate was emptied into a petri-dish. The collection pots were rinsed out using ca 1–2 mL RO water to ensure all organisms were removed from the collection pots. The number of the originally introduced adult mites, and the juvenile mites produced during the 14-day test, were then counted using a microscope, and recorded per replicate. Furthermore, any observed differences between the morphology of the mites were also recorded, where necessary.
Percentage adult mortality of the *H. aculeifer* in each vessel was calculated and a treatment mean presented. A no observed effect concentration (NOEC) and LC_{50} were determined. Corrected mortality, in comparison to both the water and solvent controls, was calculated using the Abbott's formula. Statistical analysis of the reproduction data, in comparison to both the water and pooled controls, was undertaken using CETIS version 1.8.6.8, based on the nominal test concentrations. Further statistical analysis was not undertaken as a limit test was conducted.
On the day of test item application and at the end of the test four 2 g substrate samples were taken from each treatment for chemical analysis (two to analyse and two to retain). The samples were stored frozen until analysis of RH-141455 soil concentrations. The methods were validated according to SANCO

3029/99 rev. 4 with an LOQ of 0.2 mg/kg. Concentrations of RH-141455 were determined by extracting soil samples with an extraction solvent (acetonitrile/acetone 2:1 v/v containing 1% formic acid), then diluting further with unfortified control sample extract to bring the response within the calibration range. Samples were analysed by liquid chromatography Fourier-transform mass spectrometry (LC-FT/MS). The analytical procedure (SMV 3202383-01V, and its revisions) was used to determine RH-141455.

Results and discussion
Environmental conditions stayed within the recommended ranges.
Post application the mean substrate concentration was 64.6394 mg a.s./kg dry substrate at 50 mg a.s./kg dry substrate. This is equivalent to 129.28% of nominal. The moisture content of the samples was corrected using the mean moisture content of the appropriate treatment rate. On Day 14 the mean substrate concentration was 66.80 mg a.s./kg dry substrate at 50 mg a.s./kg dry substrate. This is equivalent to 133.60% of nominal. The recoveries confirm an overdosing of the test item at study start and the stability of the test item over 14 days in soil under the test conditions.

Table 10.4.2-14: Mortality of adult *H. aculeifer* after 14 days

Treatment (mg a.s./kg dry substrate)	Initial number of introduced *Hypoaspis*	Mean mortality per treatment (%)	Effect in comparison to the water control (%)	Effect in comparison to the solvent control (%)
Water control	80	18.75	N/A	2.99
Solvent control	80	16.25	0	N/A
50	80	10.00	0	0

N/A = not applicable

After 14 days of exposure, there was 10.00% mortality observed in the 50 mg a.s./kg dry substrate group, in comparison to 18.75 and 16.25% mortality in the water and solvent controls respectively. Based on these results, the NOEC and LC_{50} for 14-day survival were empirically determined to be 50 mg a.s./kg dry substrate and >50 mg a.s./kg dry substrate, respectively.

Table 10.4.2-15: Mean number of juveniles at day 14

Treatment (mg a.s./kg dry substrate)	Mean number of juveniles[a]	Effect in comparison to the water control (%)	Effect in comparison to the solvent control (%)
Water control	123.4	N/A	0
Solvent control	120.6	2.27	N/A
50	125.9	0	0

N/A = not applicable
Coefficient of Variance for controls = 27.7 and 15.9% for the water and solvent controls respectively

The mean number of juveniles per vessel was 125.9 in the 50 mg a.s./kg dry substrate test groups, in comparison to 123.4 and 120.6 in the water and solvent controls respectively. There was no reduction when compared either the water or solvent control. Based on these results, the NOEC for reproduction was empirically determined to be 50 mg a.s./kg dry substrate and the EC_{10}, EC_{20} and EC_{50} values for reproduction were empirically determined to be >50 mg a.s./kg dry substrate, respectively.
There were no other observations noted.

Validity criteria:
The following validity criteria were met for the study; therefore, these data are considered to be valid.
- The mean mortality of the original parental mites in the control treatment did not exceed 20% over 14 days (actual mean mortality = 18.75 and 16.25% in the water and solvent controls respectively)
- The mean number of juveniles was at least 50 in the controls over 14 days (actual mean number of juveniles = 123.4 and 120.6 in the water and solvent controls respectively)

- The coefficient of variance of the calculated reproduction in the control was 30% or less over 14 days (actual coefficient of variance = 27.7 and 15.9% in the water and solvent controls respectively).

H. aculeifer from the same source culture were used in a reference toxicity test with Dimethoate 400 g/L EC, a known toxic substance (performed under in-house GLP Smithers ERS. Study Number 3202598, December 2019). The EC_{50} value was estimated to be 2.043 mg a.i/kg dry substrate.

Conclusion

In a laboratory bioassay with RH-141455 and the predatory soil mite *Hypoaspis aculeifer,* assessments of survival and reproductive performance were made over 14 days in a limit test. After 14 days, based on adult mortality and the numbers of offspring produced, the following endpoints were noted in comparison to pooled controls:

14-day NOEC value for adult *H. aculeifer* survival = 50 mg a.s./kg dry substrate
14-day LC_{50} value for adult *H. aculeifer* survival ≥50 mg a.s./kg dry substrate
NOEC value based on reproduction = 50 mg a.s./kg dry substrate
EC_{50} value based on reproduction ≥50 mg a.s./kg dry substrate
It was not possible to calculate EC_{10} and EC_{20} values since there was no significant effect at the concentration tested (limit test).
The study is valid.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No new data is submitted with this application.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Soil microorganisms nitrification

Comments of zRMS:	The study was conducted to OECD 216 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.5.1/01
Report:	ASSESSMENT OF THE EFFECTS OF THE PRODUCT GOW F716 (GWN-10616) ON SOIL MICROORGANISMS NITRIFICATION, Rossini, L., 2021, report No. BT138/17, Doc. No. 841-001
Guideline(s):	OECD No. 222 (2016), ISO 14240-1 (1997), OECD No. 216 (2000) - C21 (Dir 2004/73/EC (0.J. L152 2004)), ISO 14238 (2012)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Executive Summary

The potential effects of test item GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphonate (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, on the nitrogen (mineralisation) transformation by the soil microflora were examined in a laboratory test over a period of 28 days of exposure. The nominal test concentrations were 33.66 and 67.33 mg test item/kg soil dry weight (sdw). Differences in the rates of nitrogen transformation were ≤ 25 % at the end of the study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:

Test Material:	GOW F716 (GWN-10616)
Active substances	Potassium phosphonate and Zoxamide
Description:	Brownish liquid
Lot/Batch #:	L1704669001
Content of a.s.:	Potassium phosphonate measured as Phosphonic acid equivalent: 518 g/L, Zoxamide: 64 g/L

2. Vehicle and control:

Control:	Soil and sand
Reference item:	Dinoseb acetate

3. Test animals:

Species:	Soil microflora
Source:	Agricultural soil (sandy loam), taken from an agricultural field in Offenbach (Rhineland-Palatinate, Germany) 13th January 2017. No application of fertilisers and plant protection products were performed for at least 5 years prior to sampling. Soil details: sand: 59.6 %, C_{ORG} 6700 mg/kg sdw, pH: 5.9, carbon content of microbial biomass: 288.08 mg C/kg sdw (corresponding to 4.3 % of C_{ORG}), WHC: 35.6 %, CEC: 7.6 mEQ/100 g soil
Test unit:	Containers containing 1200 g moist soil

4. Environmental conditions

Temperature:	20 ± 2°C
Soil moisture:	Maximum water holding capacity 35.6 %
pH:	6.3 (soil pH)
Photoperiod:	Darkness

B. STUDY DESIGN AND METHODS

1. Experimental phase: 25.05.2017 – 23.06.2017

2. Experimental treatments

The effects of the test item GWN-10616 (containing active substances Potassium phosphonate and Zoxamide) on soil microbial nitrification (nitrogen transformation test) processes were studied according to OECD Guideline 216. The test item was mixed into a sandy loam agricultural soil, at concentrations of 33.66 and 67.33 mg test item/kg sdw. Based on the density value of the test item (1.425 g/mL), the applied quantity of 4.41 kg of test item/ha is corresponding to 3.09 L (soil density = 1310 kg/m^3).

The control consisted of soil treated with deionised water and was incubated at the same condition as the treated soil, in the dark at 20 ± 2°C. The reference item (Dinoseb acetate) was tested in a previous study to confirm the normal reaction of the soil against herbicides. The influence of the test item on the nitrification of lucerne meal was investigated and the results obtained in treated samples were compared to untreated sample data. The duration of the test was 28 days after treatment.

3. Statistics

The results of nitrogen transformation test in treated and control groups were compared using the software Tox Rat Pro version 3.2.1. The software Tox Rat Pro Version 3.2.1 was used to perform the

statistical analysis (Shapiro-Wilk's Test on Normal Distribution and Student – t test for Homogeneous Variances).

II. RESULTS AND DISCUSSION

A. Validity criteria

The study is considered valid, since the coefficients of variation between replicate control samples were less than 15 % (max CV = 10.46 %) for the nitrogen transformation test.

B. Biological results

The nitrate formation is an indicator of the nitrification activity of the soil microflora. Sandy soil spiked with lucerne meal, treated with the test item was analysed at 0, 7, 14 and 28 days after treatment in comparison with the control soils for the nitrate concentrations. After 28 days, 9.2 % deviation was observed at the concentration of 33.66 mg of test item/kg sdw (equivalent to 12.24 mg Phosphonic acid/kg sdw and 1.51 mg Zoxamide/kg sdw) and 6.65 % deviation was observed at the concentration of 67.33 mg of test item/kg sdw (equivalent to 24.48 mg Phosphonic acid/kg sdw and 3.02 mg Zoxamide/kg sdw) compared to control values, respectively.

Table 10.5-1: Effects of GWN-10616 on nitrogen transformation in soil amended with lucerne meal

Day	Control	33.66 mg test item/kg sdw		67.33 mg test item/kg sdw	
	NO_3 [mg/kg soil dw]	NO_3 [mg/kg soil dw]	Deviation to control [%]	NO_3 [mg/kg soil dw]	Deviation to control [%]
Nitrate content					
0	70.73	74.32	-5.07	72.41	-2.38
7	22.65	32.76	13.59	37.87	28.14
14	57.25	57.99	-21.11	61.66	20.07
28	118.39	117.57	9.20	116.98	6.65

III. CONCLUSIONS

Nitrogen transformation activities in soil treated in the laboratory with the test item GWN-10616 (containing active substances Potassium phosphonate and Zoxamide) at 33.66 and 67.33 mg test item/kg sdw were not substantially different compared to control soil by 28 days after treatment. The deviations in nitrogen transformation activity after 28 days were less than 25 % for all parameters examined.

EFSA (2017) has requested *"Further data to address the risk to soil microorganisms for metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints

On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.

The 28-day NOEC for nitrate concentrations = 0.195 mg a.s./kg dry soil (statistically determined).

The 28-day LOEC for nitrate concentrations >0.195 mg a.s./kg dry soil (statistically determined).

It was not possible to calculate EC10, EC25 and EC50 values since there was no significant effect at the highest concentration in the test.

Reference:	KCA 8.5
Report	Jarrom, R., 2019: RH-127450: Soil nitrogen transformation test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202377, GLP, Not published
Guideline(s):	OECD 216 (2000)
Deviations:	The protocol contained an error that stated the soil would be collected in accordance with ISO 10381-6. This is a previous revision of ISO 18400-102. This had no impact as the soil was collected in accordance with the most up to date revision.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-127450 (HHGCP002-00-1)
Purity:	99.22%
Test species	Micro-organisms naturally occurring in biologically active soil
Test soil	Lufa 2.3
Source:	LUFA Speyer, Germany
Soil batch no.:	F23 1219 (Lufa 19/002)
Date of sampling:	19th March 2019
Fertilisers:	no during ≥ 4 years
Pesticides:	no during ≥ 4 years
Nitrogen content (% N):	0.08 ± 0.02
Organic carbon content (% C):	0.66 ± 0.07
Microbial biomass (% of organic C):	1.5

Cation exchange capacity (meq/100g):	7.3 ± 1.1
pH:	5.6
WHCmax (%):	34.9 ± 1.8
Soil type (USDA)	sandy loam
Clay (%) (< 0.002 mm):	7.6 ± 0.5
Silt (%) (0.002-0.050 mm):	33.3 ± 0.6
Sand (%) (0.050-2.0 mm):	59.1 ± 0.4
Test vessels	500 mL amber glass jars
Filling:	100 g soil dry weight per vessel
Pre-incubation:	15 days under test environmental conditions
Environmental conditions	
Soil moisture:	approx. 50 % of max. WHC
Temperature:	20 ± 2°C
Air:	After treatment with the test substance, the test vessels were sealed and the lid perforated to allow air exchange.
Light conditions:	Dark
Application rate(s)	0.039 and 0.195 mg/kg dry soil
Post exposure observation period	28 days
Remarks	None

A fresh sample of LUFA 2.3 soil with a microbial biomass of 1.5 % of its organic carbon content was used for the study. The history of the soil batch demonstrated that no fertiliser and plant protection products were used over at least the last 4 years.
The effect of the test item RH-127450 on soil micro-organisms has been studied by investigation of the NO_3-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Results for a test item treated soil were compared to the results of a non-treated soil.
Soil equivalent to 400 g dry weight was weighed out. The test substance, incorporated in sand, was mixed by means of an electric hand mixer with the sand along with water to adjust the soil moisture to 50% maximum WHC. 2.0 g of powdered Lucerne (alfalfa) was also added (concentration in soil 0.5%). Application rates were 0.039 and 0.195 mg/kg dry soil. The test vessels were sealed and the lid perforated to allow air exchange. Triplicate samples of 100 g each per test rate and control were incubated for 28 days under environmentally controlled conditions.
After test item application on day 0, and after moisture adjustment on day 7, 14 and 28 of incubation, soil samples were taken on day 0 and at 7, 14 and 28 days after application from each test vessel. The samples were extracted with potassium chloride (0.1 M KCl, approximately 35 mL) and extract aliquots of 1.0 mL mixed with each 0.20 mL of Hach LCK 339 solution to analyse their NO_3-N content using a Hach Lange DR 3900 spectrophotometer. Two aliquots per sample were measured. The Limit of Quantification (LOQ) for this technique was 0.23 mg/L NO_3-N.
The data was subjected to statistical analysis (Dunnett Multiple Comparison Test) to determine the 28-day nitrate production NOEC and LOEC, using CETIS version 1.8.6.8. As there was no effect up to and including the highest test item concentration, EC_{10}, EC_{25} and EC_{50} values could not be calculated.

Results and discussion
Soil temperature remained within the required range of 20 ± 2 °C during incubation.
Over a 28-day period, the test substance had no significant effects on the amount of the nitrate produced in the soil type tested.

Table 10.5-2: Mean amount of nitrate measured over 28 days

Treatment (mg/kg dry soil)	Mean Nitrate Nitrogen NO_3-N (mg N/kg dry soil)				Mean Nitrate NO_3^- (mg N/kg dry soil)				Difference Compared to the Control (%)		
	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Control (0)	8.93	1.06	17.30	26.84	39.3	4.68	76.14	118.12	-	NA	-
0.039	9.35	1.72	21.03	26.53	41.12	7.56	92.52	116.75	61.5	21.5	-1.2
0.195	9.24	1.28	18.42	26.08	40.67	5.65	81.06	114.77	20.8	6.5	-2.8

NA = not applicable
1 mg/kg nitrate nitrogen (NO_3^-N) = 4.4 mg/kg nitrate (NO_3^-)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

Table 10.5-3: Mean amount of nitrate produced per day in the test soil

Treatment (mg/kg dry soil)	Mean Nitrate Nitrogen NO_3^-N (mg N/kg dry soil)			Mean Nitrate NO_3^- (mg N/kg dry soil)			Difference Compared to the Control (%)		
	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Control	-1.12	0.60	0.64	-4.95	2.63	2.81	-	NA	-
0.039	-1.09	0.83	0.61	-4.80	3.67	2.70	-3.0	39.5	-4.1
0.195	-1.14	0.66	0.60	-5.00	2.88	2.65	1.1	9.6	-6.0

NA = not applicable
1 mg/kg nitrate nitrogen (NO_3^-N) = 4.4 mg/kg nitrate (NO_3^-)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

The validity criterion was met:
The variation in NO_3-N concentrations between replicate control vessel samples was <15% on day 0, 7, 14 and 28. The coefficient of variation was calculated to be 2.92, 11.45, 12.57 and 9.24% for the day 0, 7, 14 and 28 extractions, respectively.

Conclusions
The effect of the test substance RH-127450 on soil micro-organisms has been studied by investigation of the NO_3-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Test results of the test item treated soil were compared to the results of a non-treated soil.
On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.
The NOEC for nitrate concentrations on day 28 was statistically determined to be 0.195 mg/kg dry soil, the LOEC for nitrate concentrations on day 28 was statistically determined to be >0.195 mg/kg dry soil.
It was not possible to calculate EC_{10}, EC_{25} and EC_{50} values since there was no significant effect at the highest concentration in the test.
The study is valid.

EFSA (2017) has requested *"Further data to address the risk to soil microorganisms for metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints

At Day 28 both treatment groups showed < 25% change in nitrate production when compared to the control.

The 28-day NOEC for nitrate concentrations = 0.350 mg a.s./kg dry soil (statistically determined).

The 28-day LOEC for nitrate concentrations >0.350 mg a.s./kg dry soil (statistically determined).

It was not possible to calculate EC10, EC25 and EC50 values since there was no significant effect at the highest concentration in the test.

Reference:	KCA 8.5
Report	Jarrom, R., 2019: RH-24549: Soil nitrogen transformation test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No. 3202396, GLP, Not published
Guideline(s):	OECD 216 (2000)
Deviations:	The protocol contained an error that stated the soil would be collected in accordance with ISO 10381-6. This is a previous revision of ISO 18400-102. This had no impact as the soil was collected in accordance with the most up to date revision.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-24549 (FCC25806)
Purity:	99.59 % (w/w)
Test species	Micro-organisms naturally occurring in biologically active soil
Test soil	Lufa 2.3
Source:	LUFA Speyer, Germany
Soil batch no.:	F23 1219 (Lufa 19/002)
Date of sampling:	19th March 2019
Fertilisers:	no during ≥ 4 years
Pesticides:	no during ≥ 4 years
Nitrogen content (% N):	0.08 ± 0.02
Organic carbon content (% C):	0.66 ± 0.07
Microbial biomass (% of organic C):	1.5

Cation exchange capacity (meq/100g):	7.3 ± 1.1
pH:	5.6
WHCmax (%):	34.9 ± 1.8
Soil type (USDA)	sandy loam
Clay (%) (< 0.002 mm):	7.6 ± 0.5
Silt (%) (0.002-0.050 mm):	33.3 ± 0.6
Sand (%) (0.050-2.0 mm):	59.1 ± 0.4
Test vessels	500 mL amber glass jars
Filling:	100 g soil dry weight per vessel
Pre-incubation:	8 days under test environmental conditions
Environmental conditions	
Soil moisture:	approx. 45 % of max. WHC
Temperature:	20 ± 2°C
Air:	After treatment with the test substance, the test vessels were sealed and the lid perforated to allow air exchange.
Light conditions:	Dark
Application rate(s)	0.070 and 0.350 mg/kg dry soil
Post exposure observation period	28 days
Remarks	None

A fresh sample of LUFA 2.3 soil with a microbial biomass of 1.5 % of its organic carbon content was used for the study. The history of the soil batch demonstrated that no fertiliser and plant protection products were used over at least the last 4 years.
The effect of the test item RH-24549 on soil micro-organisms has been studied by investigation of the NO_3-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Results for a test item treated soil were compared to the results of a non-treated soil.
Soil equivalent to 400 g dry weight was weighed out. The test substance, incorporated in sand, was mixed by means of an electric hand mixer with the sand along with water to adjust the soil moisture to 45% maximum WHC. 2.0 g of powdered Lucerne (alfalfa) was also added (concentration in soil 0.5%). Application rates were 0.070 and 0.350 mg/kg dry soil. The test vessels were sealed and the lid perforated to allow air exchange. Triplicate samples of 100 g each per test rate and control were incubated for 28 days under environmentally controlled conditions.
After test item application on day 0, and after moisture adjustment on day 7, 14 and 28 of incubation, soil samples were taken on day 0 and at 7, 14 and 28 days after application from each test vessel. The samples were extracted with potassium chloride (0.1 M KCl, approximately 35 mL) and extract aliquots of 1.0 mL mixed with each 0.20 mL of Hach LCK 339 solution to analyse their NO_3-N content using a Hach Lange DR 3900 spectrophotometer. Two aliquots per sample were measured. The Limit of Quantification (LOQ) for this technique was 0.23 mg/L NO_3-N.
The data was subjected to statistical analysis (Dunnett Multiple Comparison Test) to determine the 28-day nitrate production NOEC and LOEC, using CETIS version 1.8.6.8. As there was no effect up to and including the highest test item concentration, EC_{10}, EC_{25} and EC_{50} values could not be calculated.

Results and discussion
Soil temperature remained within the required range of 20 ± 2 °C during incubation.
Over a 28-day period, the test substance had no significant effects on the amount of the nitrate produced in the soil type tested.

Table 10.5-4: Mean amount of nitrate measured over 28 days

Treatment (mg/kg dry soil)	Mean Nitrate-Nitrogen NO_3^-N (mg N/kg dry soil)				Mean Nitrate NO_3^- (mg N/kg dry soil)				Difference compared to the Control (%)		
	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Control	10.09	7.10	28.87	34.70	44.39	31.26	127.02	152.68	-	NA	-
0.070	8.82	6.98	30.24	35.78	38.79	30.73	133.08	157.44	-1.7	4.8	3.1
0.350	10.78	7.12	27.75	36.20	47.42	31.33	122.10	159.26	0.2	-3.9	4.3

NA = not applicable
1 mg/kg nitrate nitrogen (NO_3^-N) = 4.4 mg/kg nitrate (NO_3^-)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

Table 10.5-5: Mean amount of nitrate produced per day in the test soil

Treatment (mg/kg dry soil)	Mean Nitrate-Nitrogen NO_3^-N (mg N/kg dry soil)			Mean Nitrate NO_3^- (mg N/kg dry soil)			Difference compared to the Control (%)		
	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Control	-0.43	1.34	0.88	-1.88	5.90	3.87	-	NA	-
0.070	-0.26	1.53	0.96	-1.15	6.73	4.24	-38.6	14.1	9.6
0.350	-0.52	1.21	0.91	-2.30	5.33	3.99	22.5	-9.6	3.3

NA = not applicable
1 mg/kg nitrate nitrogen (NO_3^-N) = 4.4 mg/kg nitrate (NO_3^-)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

The validity criterion was met:
The variation in NO_3-N concentrations between replicate control vessel samples was <15% on day 0, 7, 14 and 28. The coefficient of variation was calculated to be 7.64, 1.92, 4.23 and 7.73% for the day 0, 7, 14 and 28 extractions, respectively.

Conclusions
The effect of the test substance RH-24549 on soil micro-organisms has been studied by investigation of the NO_3-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Test results of the test item treated soil were compared to the results of a non-treated soil.
On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.
The NOEC for nitrate concentrations on day 28 was statistically determined to be at 0.350 mg/kg dry soil, the LOEC for nitrate concentrations on day 28 was statistically determined to be >0.350 mg/kg dry soil.
It was not possible to calculate EC_{10}, EC_{25} and EC_{50} values since there was no significant effect at the highest concentration in the test.
The study is valid.

EFSA (2017) has requested *"Further data to address the risk to soil microorganisms for metabolites RH-127450, RH-24549, RH-163353 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5)."* This active substance related study has already been provided to the RMS Latvia. Thus, the summary of the study is only presented for completeness sake. The study is only indicated in the list of data submitted or referred to by the applicant and relied on.

Review Comments:

The confirmatory-like studies were evaluated by the RMS-LV for zoxamide and its metabolites in an interzonal procedure. All details are to be found in the file: Zoxamide_ confirmatory_ like_data_Part_B5_B6_B8_B9_ XXXX _LV_2023, Part B – Section 9, available on CIRCABC.

RMS-LV conclusion:

The study is considered valid and acceptable.

Agreed endpoints

On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.

The 28-day NOEC for nitrate concentrations = 0.365 mg a.s./kg dry soil (statistically determined)

The 28-day LOEC for nitrate concentrations >0.365 mg a.s./kg dry soil (statistically determined).

It was not possible to calculate EC10, EC25 and EC50 values since there was no significant effect at the highest concentration in the test.

Reference:	KCA 8.5
Report	Jarrom, R., 2020: RH-163353: Soil nitrogen transformation test Gowan Crop Protection Ltd., UK Smithers ERS Ltd., UK, Report No.3202392, GLP, Not published
Guideline(s):	OECD 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test material (Lot/Batch No.)	RH-163353 (HHGCP001-00-2)
Purity:	99.48 % (w/w); enantiomeric ratio 48.6:51.4 (racemate)
Test species	Micro-organisms naturally occurring in biologically active soil
Test soil	Lufa 2.3
Source:	LUFA Speyer, Germany
Soil batch no.:	F23 4119 (Lufa 19/003)
Date of sampling:	7th October 2019
Fertilisers:	no during ≥ 4 years
Pesticides:	no during ≥ 4 years
Nitrogen content (% N):	0.07 ± 0.02
Organic carbon content (% C):	0.65 ± 0.08
Microbial biomass (% of organic C):	3.8
Cation exchange capacity (meq/100g):	6.8 ± 1.4
pH:	5.99
WHCmax (%):	35.2 ± 1.8

Soil type (USDA)	sandy loam
Clay (%) (< 0.002 mm):	7.3 ± 0.9
Silt (%) (0.002-0.050 mm):	33.3 ± 0.6
Sand (%) (0.050-2.0 mm):	59.4 ± 0.7
Test vessels	500 mL amber glass jars
Filling:	100 g soil dry weight per vessel
Pre-incubation:	8 days under test environmental conditions
Environmental conditions	
Soil moisture:	approx. 45 % of max. WHC
Temperature:	20 ± 2°C
Air:	After treatment with the test substance, the test vessels were sealed and the lid perforated to allow air exchange.
Light conditions:	Dark
Application rate(s)	0.073 and 0.365 mg/kg dry soil
Post exposure observation period	28 days
Remarks	None

A fresh sample of LUFA 2.3 soil with a microbial biomass of 3.8 % of its organic carbon content was used for the study. The history of the soil batch demonstrated that no fertiliser and plant protection products were used over at least the last 4 years.
The effect of the test item RH-163353 on soil micro-organisms has been studied by investigation of the NO_3-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Results for a test item treated soil were compared to the results of a non-treated soil.
Soil equivalent to 400 g dry weight was weighed out. The test substance, incorporated in RO water (2.92 mg/ stock solution), was mixed by means of an electric hand mixer along with further RO water to adjust the soil moisture to 45% maximum WHC. 2.0 g of powdered Lucerne (alfalfa) was also added (concentration in soil 0.5%). Application rates were 0.073 and 0.365 mg/kg dry soil. The test vessels were sealed and the lid perforated to allow air exchange. Triplicate samples of 100 g each per test rate and control were incubated for 28 days under environmentally controlled conditions.
After test item application on day 0, and after moisture adjustment on day 7, 14 and 28 of incubation, soil samples were taken on day 0 and at 7, 14 and 28 days after application from each test vessel. The samples were extracted with potassium chloride (0.1 M KCl, approximately 35 mL) and extract aliquots of 1.0 mL mixed with each 0.20 mL of Hach LCK 339 solution to analyse their NO_3-N content using a Hach Lange DR 3900 spectrophotometer. Two aliquots per sample were measured. The Limit of Quantification (LOQ) for this technique was 0.23 mg/L NO_3-N.
The data was subjected to statistical analysis (Dunnett Multiple Comparison Test) to determine the 28-day nitrate production NOEC and LOEC, using CETIS version 1.8.6.8. As there was no effect up to and including the highest test item concentration, EC_{10}, EC_{25} and EC_{50} values could not be calculated.

Results and discussion
Soil temperature remained within the required range of 20 ± 2 °C during incubation.
Over a 28-day period, the test substance had no significant effects on the amount of the nitrate produced in the soil type tested.

Table 10.5-6: Mean amount of nitrate measured over 28 days

Treatment (mg/kg dry soil)	Mean Nitrate-Nitrogen NO_3^-N (mg N/kg dry soil)				Mean Nitrate NO_3^- (mg N/kg dry soil)				Difference compared to the control (NO_3^-) (%)		
	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Control	10.95	7.69	21.28	29.22	48.18	33.85	93.65	128.57	N/A		
0.073	13.20	10.03	20.63	30.17	58.09	44.14	90.77	132.73	30.04	-3.1	3.2
0.365	13.36	7.99	22.09	30.61	58.77	35.14	97.20	134.70	3.8	3.8	4.8

NA = not applicable
1 mg/kg nitrate nitrogen (NO_3^-N) = 4.4 mg/kg nitrate (NO_3^-)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

Table 10.5-7: Mean amount of nitrate produced per day in the test soil

Treatment (mg/kg dry soil)	Mean Nitrate-Nitrogen NO_3^-N (mg N/kg dry soil)			Mean Nitrate NO_3^- (mg N/kg dry soil)			Difference compared to the Control (%)		
	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Control	-0.47	0.74	0.65	-2.05	3.25	2.87	-	NA	-
0.073	-0.45	0.53	0.61	-1.99	2.33	2.67	-2.64	-28.13	-7.15
0.365	-0.77	0.62	0.62	-3.38	2.75	2.71	64.98	-15.48	-5.55

NA = not applicable
1 mg/kg nitrate nitrogen (NO_3^-N) = 4.4 mg/kg nitrate (NO_3^-)
Negative percentage difference compared to the control indicates lower nitrate production than the control.

The validity criterion was met:
The variation in NO_3-N concentrations between replicate control vessel samples was <15% on day 0, 7, 14 and 28. The coefficient of variation was calculated to be 4.28, 12.24, 4.86 and 3.10% for the Day 0, 7, 14 and 28 extractions, respectively.

Conclusions
The effect of the test substance RH-163353 on soil micro-organisms has been studied by investigation of the NO_3-N formation in a field fresh arable LUFA 2.3 soil amended with a nitrogen source, powdered Lucerne. Test results of the test item treated soil were compared to the results of a non-treated soil.
On day 28 after test start both treatment groups showed < 25% change in nitrate production compared to the control.
The NOEC for nitrate concentrations on day 28 was statistically determined to be at 0.365 mg/kg dry soil, the LOEC for nitrate concentrations on day 28 was statistically determined to be >0.365 mg/kg dry soil.
It was not possible to calculate EC_{10}, EC_{25} and EC_{50} values since there was no significant effect at the highest concentration in the test.
The study is valid.

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

A 2.6.1 KCP 10.6.1 Summary of screening data

No new data is submitted with this application.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study was conducted to OECD 227 the guideline and according to the principles of GLP. All validity criterions were met. The study is considered to be reliable and suitable for the risk assessment.

Reference: KCP 10.6.2/01
Report: EFFECTS OF GWN-10616 ON TERRESTRIAL PLANTS VEGETATIVE VIGOUR TEST, Colli, M., 2021, report No. BT214/21, Doc. No. 851-001
Guideline(s): OECD No. 227 (2006), ISO 11268 (2012)
Deviations: None
GLP: Yes
Acceptability: Yes

Executive Summary
The potential chronic effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, on the vegetative vigour of six different plant species (*Glycine max, Cucumis sativus, Brassica napus, Helianthus annuus, Allium cepa* and *Avena sativa*) at BBCH stage 12-14 was tested. The study was conducted over a period of 21 days. In parallel, untreated controls were tested.

Plants were treated with a single rate of 4275 g test item/ha. On all plant species tested, GWN-10616 did not cause any mortality, phytotoxic effects and adverse effects on biomass at the applied rate.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:
Test Material: GWN-10616
Active substances: Potassium phosphite and Zoxamide
Description: Beige homogeneous and viscous liquid
Lot/Batch #: P2102669001
Content of a.s.: Potassium phosphite measured as Phosphonic acid equivalent: 507 g/L, Zoxamide: 62 g/L

2. Control: Deionised water

3. Tested seeds:
Species: Glycine max (soybean), Cucumis sativus (cucumber), Brassica napus (oilseed rape), Helianthus annuus (sunflower), Allium cepa (onion) and Avena sativa (oat)
Source: Commercial supplier (Bavicchi, Italy)
Environmental conditions
Temperature: 16.9 – 21.6 °C
Relative humidity: 43.4 – 78.7 %
Soil: Standard soil type 2.3 (Lufa Speyer – Germany)
Photoperiod: 16 h light (intensity: 341.1 – 358.1 PAR (µE/m2/s))

B. STUDY DESIGN AND METHODS

1. Experimental phase: 10.11.2021 – 01.12.2021

2. Experimental treatments

The study was conducted in a limit test design to investigate potential chronic effects of GWN-10616, a suspension concentrate formulation containing nominal 500 g/L Potassium phosphite (measured as Phosphonic acid equivalent) and 60 g/L Zoxamide as active substances, applied at a rate of 4275 g test item/ha, on the vegetative vigour of six different plant species (*Glycine max, Cucumis sativus, Brassica napus, Helianthus annuus, Allium cepa* and *Avena sativa*) at BBCH stage 12 – 14 was tested.

Six different plant species were planted in pots containing standard soil type 2.3 (Lufa Speyer – Germany). The experimental design consisted of 1 test item treatment group at the application rate of 4275.0 g test item/ha and an untreated control group (deionised water), with minimum 24 plants per group (5 or 12 replicate pots with 5 or 2 plants each, depending on species). Plants were treated at BBCH 12 – 14.

The test item solution was prepared in deionised water immediately before application and was applied with spray equipment calibrated to deliver an output of 400 L/ha (± 10%). The pots were then placed on a bench top in randomised order in a climatic chamber under controlled test conditions for 21 days.

3. Observations

Effects on plants as mortality and visual phytotoxicity (deformations, modifications in colour, necrosis) were recorded at 7, 14 and 21 days after the treatment (DAT). At the end of the test, the biomass (fresh shoot weight for each pot) was measured in addition.

4. Analytics

A sample of the test item solution was analysed in order to verify the correct application of the test item. The analysis of the active substances content in the test item solution was carried out with an analytical method validated in a separate GLP study (BT233/21).

5. Statistics

Mortality and biomass data for each plant species were analysed using appropriate statistical methods to demonstrate that the rate that cause 50% mortality/effect was greater than the tested application rate. The software ToxRat Pro version 3.3.0 was used to perform the statistical analysis.

II. RESULTS AND DISCUSSION

A. Validity criteria

According to OECD 227 (2006), the test is considered valid because the seedling emergence (before test start) was at least 70 % in control and treated groups (actual: 94.3 to 98.6 %), the control plants do not exhibit visible phytotoxic effects (modification in colour, necrosis, leaf and stem deformations were 0 % in all tested species) and the control plants exhibit only normal variation in growth and morphology for that particular species, the mean control plant survival was 100 % at the end of the test and the environmental conditions for particular species were identical and growing media contained the same amount of soil matrix, support media or substrate from the same source.

B. Analytical test results

The concentrations of the active substances Zoxamide and Phosphonic acid in the application solution used to treat the plants were determined. All measured concentrations of the test item were inside ± 20 % of the nominal values. Accordingly, endpoints of the test were calculated with respect to the nominal concentration of the test item.

C. Biological test results

The results for mortality, phytotoxic and biomass effects in the vegetative vigour study are presented in Table 10.6.2-1.

As no mortalities and no statistically significant reduction in biomass were observed at the end of the test, the corresponding NOER is estimated to be ≥ 4275 g test item/ha (equivalent to 181.69 g Zoxamide/ha

and 1484.71 g Potassium phosphite/ha), and the LR_{50} and ER_{50} values are estimated to be > 4275 g test item/ha. No phytotoxic effects occurred in any of the test item treatment groups.

Table 10.6.2-1: Effects of GWN-10616 (applied at 4275 g test item/ha) on mortality, phototoxicity and biomass (fresh shoot weight) in a 21-day vegetative vigour test

Species	Mortality [%]	Deformation [%]	Modification in colour [%]	Necrosis [%]	Reduction in fresh shoot weight* [%]
Glycine max	0	0	0	0	-11.6
Helianthus annuus	0	0	0	0	-7.9
Cucumis sativus	0	0	0	0	9.7
Brassica napus	0	0	0	0	8.6
Allium cepa	0	0	0	0	2.7
Avena sativa	0	0	0	0	3.0

* negative values refer to an increase in biomass compared to the control.

III. CONCLUSION

The effects of the test item GWN-10616 on the vegetative vigour of six plant species were investigated in a limit test following the test guideline OECD 227 (2006). The test item was applied at a single application rate of 4275 g test item/ha corresponding to 181.69 g Zoxamide/ha and 1484.71 g Potassium phosphite/ha.

As no mortalities and no statistically significant reduction in biomass were observed at the end of the test, the corresponding NOER is estimated to be ≥ 4275 g test item/ha, and the LR_{50} and ER_{50} values are estimated to be > 4275 g test item/ha. No phytotoxic effects occurred in any of the test item treatment groups.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No new data is submitted with this application.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No new data is submitted with this application.

A 2.8 KCP 10.8 Monitoring data

No new data is submitted with this application.