Renewal Assessment Report

under Regulation (EC) 1107/2009

Zoxamide

Zoxium 240 SC

Volume 3

Plant protection product
B.9 Ecotoxicology data

Rapporteur Member State: Latvia
Co-Rapporteur Member State: France

Version history

Date	Subject
July 2016	**Initial RAR**

B.9. ECOTOXICOLOGY DATA

SUMMARY OF THE DATA AND INFORMATION

Introduction

Zoxamide is a non-systemic fungicide belonging to the benzamide group of compounds. It is intended to protect against oomycete diseases such as Phytophthora infestans (late blight of potato) and Plasmopara viticola (downy mildew of grapevines). Zoxamide inhibits germ tube development and mycelium growth by inhibiting cell division. As a result, the fungal organism dies.

Zoxamide has previously been evaluated and was included in the Annex I of the Council Directive 2003/119/EC concerning placing of plant protection products on the market (91/414/EEC) in 2003. This document presents data and information on the metabolism and toxicology of zoxamide submitted in support of the renewal of approval of zoxamide under Regulation (EC) 1107/2009. Most of the data presented were also submitted to secure the first inclusion of zoxamide in Annex I to Directive 91/414/EEC. The evaluation of these data was presented in the Draft Assessment Report (DAR) for zoxamide (United Kingdom, 2001) and in 3 addenda. The critical endpoints for use in risk assessment were published in the Review Report for the active substance zoxamide (SANCO/10297/2003-Final).

In this report new data for the renewal of the approval of Zoxamide has been evaluated only. Studies and investigations already assessed within the EU DAR (2001) have been re-evaluated in this report. The conclusions have been updated to meet current scientific standards.

This document covers hazard and risk assessments which were not part of the original dossier and which are necessary to reflect changes

· in requirements of Regulations EU 283/2013 and 284/2013;

· in scientific and technical knowledge since the first inclusion;

· to representative uses (see Table B.9-1).

Table B.9-1: Summary of the representative uses of Zoxium 240 SC

Crop Zone	Pests or Group of pests controlled	Application				Application rate per treatment		PHI days
		method kind	growth stage & season	number min max	interval between applications (min)	water l/ha min - max	kg as/ha min - max	
Potato All zones	Foliar fungi Late blight	Foliar spraying	BBCH 20-80	Max. 5	8 days	1000	0.15 – 0.18	7
Table and wine grapes Central and Southern EU	Foliar fungi Downy mildew	Foliar spraying	BBCH 15-79	Max.5	8 days	1000	0.15 – 0.18	28

B.9.1 EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1 Effects on Birds

Avian toxicity tests with the formulation were not performed, since it is possible to extrapolate from data obtained with the active substance.

Additional studies to assess the acute oral toxicity of Zoxium 240 SC to birds are not required since the TER_A values for the active substance are about 100 or greater and the results from testing with mammals do not provide any evidence of significantly greater toxicity of the preparation compared to the active substance. This is consistent with the presumption against unnecessary testing, especially with vertebrates, presented in the EFSA guidance document.

B.9.1.2 Effects on terrestrial vertebrates other than birds

An acute toxicity of Zoxium 240 SC to mammals has been performed. Details are reported under Volume 3 CP-B.6.1

Table B.9.1.2.-1: Summary of effects of Zoxium 240 SC to mammals

Test species	Test System	Results[1]	Reference
Rat	Acute	LD_{50}: >5000 mg/kg bw	KCP 7.1.1/01/01 [redacted] (1999)

[1] As listed in the EC Review Report (2004)

B.9.2 RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.2.1 Risk assessment for birds

B.9.2.1.1 Toxicity

Table B.9.2.1-1: Summary of effects of zoxamide to birds

Test species	Test System	Duration of exposure	Results	Reference
Bobwhite quail *Colinus virginianus*	Acute	single appl.	LD_{50}: >2000 mg a.s./kg bw	IIA 8.1.1/01 [redacted] 1997a *
Bobwhite quail *Colinus virginianus*	short-term	8 days	LC_{50}: >5250 ppm LD_{50}: >1415.9 mg/kg bw/day**	IIA 8.1.2/01 [redacted] 1997b *
Mallard duck *Anas plathyrhynchos*	short-term	8 days	LC_{50}: >5250 ppm LD_{50}: > 1167 mg/kg bw/day**	IIA 8.1.2/02 [redacted] 1997c *
Mallard duck *Anas plathyrhynchos*	reproduction	20 weeks	NOEC: 1000 mg a.s./kg food NOEL: 114.3 mg/kg bw/day**	IIA 8.1.3/02 [redacted] 1998 *
Bobwhite quail *Colinus virginianus*	reproduction	20 weeks	NOEC: 1000 mg a.s./kg food NOEL: 158.2	IIA 8.1.3/01 [redacted] 1998 *

			mg/kg bw/day**	

* Already assessed in the DAR

** Conversion as calculated by the RMS based on study data

Endpoints used for risk assessment

In accordance with the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)[1] in cases where the short-term dietary LD_{50} is lower than the acute $LD_{50,}$ the dietary value should be used in the acute risk assessment. For both short-term dietary studies the LD_{50} values of >1415.9 and >1167 mg/kg bw/day appear to be lower than the acute LD_{50} values of >2000 mg a.s/kg bw day. However in each of the studies no significant sublethal effects were reported at the highest dose tested. The 'lower' values reported in the dietary studies reflect the highest dose tested which is also the NOEC. The LD_{50} values of >2000 mg a.s/kg bw day obtained for the acute studies are therefore considered to be more representative and relevant for assessing acute toxicity and are used in the risk assessment.

The endpoints retained for assessing the risk for birds exposed to zoxamide are reported in the table below.

B.9.2.1.1-2 Endpoints used for risk assessment of zoxamide for birds

Study type	Test substance	Species	Endpoint	Value
Acute oral toxicity	zoxamide	Bobwhite quail *Colinus virginianus*	LD_{50}	**> 2000 mg a.s./kg bw**
Long-term toxicity and reproduction	zoxamide	Mallard duck *Anas plathyrhynchos*	NOEC	=1000 ppm a.s. **=114.3 mg/kg bw/d**

Zoxamide metabolites

In the metabolism studies (Volume 3-CA B.7.2) conducted in grapes, tomato, cucumber and peas all metabolites found generally occurred at levels <10% of the TRR and the major residue observed in all studies was parent compound. In the potato metabolism study (Volume 3-CA B.7.2.1.2), no parent zoxamide was found in potato tubers. The main components of the residue in potato tubers were the metabolites RH-141452 and RH-141455, both have been found at significant levels >10% of the TRR.

RMS comments:
No information about endpoints for birds for metabolites RH-141452 and RH-141455, according to ecology of omnivorous birds it is unlikely that birds could feed on potato tubers, in conclusion the risk assessment for metabolites RH-141452 and RH-141455 for birds is considered not necessary.

B.9.2.1.2 Exposure

Exposure is calculated according to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** [2] based on the GAP detailed in table B.9-1.

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

B.9.2.1.3 Dietary risk assessment

The intended uses of zoxamide belongs to the "Bulbs and onion like crops, (…)" and "Vineyard" crop group. At first tier a risk envelop approach considering max. 5 applications of 180 g a.s./ha with an interval between applications of 8 days.

Table B.9.2.1.3-1: Screening step – estimates of acute exposure to zoxamide for application in potatoes at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
	Small omnivorous bird	158,8	28,58	1,7	48,59	41,2

Table B.9.2.1.3-2: Screening step – estimates of long-term exposure to zoxamide for application in potatoes at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
	Small omnivorous bird	64,8	11,66	2,2	13,60	8,4

Table B.9.2.1.3-3: Screening step – estimates of acute exposure to zoxamide for application in grapevines at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
	Small omnivorous bird	95,3	17,15	1,7	29,16	68,6

Table B.9.2.1.3-4: Screening step – estimates of long-term exposure to zoxamide for application in grapevines at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
	Small omnivorous bird	38,9	7,00	2,2	8,16	14,0

Table B.9.2.1.3-5: Acute (TERA) and long-term (TER_{LT}) risk to birds from zoxamide for applications in potatoes and grapevines at 180 g a.s./ha [5 applications]

Crop	Scenario (Annex I)	Clarified Scenario start	Clarified Scenario end	Generic focal species	Representative species	Shortcut value for mean RUDs	Shortcut value for 90th percentile RUDs	DDDacute	TERacute	DDD repro	TERrepro
Potatoes	BBCH 10 - 39	10	39	Small omnivorous bird "lark"	Woodlark (*Lullula arborea*)	10,9	24	7,5	265,84	2,3	43,93
Potatoes	BBCH ≥ 40	40	99	Small omnivorous bird "lark"	Woodlark (*Lullula arborea)*	3,3	7,2	2,3	886,14	0,7	145,11
Potatoes	BBCH ≥ 40	40	99	Small insectivorous bird "wagtail"	Yellow wagtail (*Motacilla flava*)	9,7	25,2	7,9	253,18	2,0	49,37
Vineyard	BBCH 10 - 19	10	19	Small insectivorous species "Redstart"	Black Redstart (*Phoenicurus ochruros*)	11,5	27,4	8,6	232,86	2,4	41,64
Vineyard	BBCH ≥ 20	20	99	Small insectivorous species "Redstart"	Black Redstart (*Phoenicurus ochruros*)	9,9	25,7	8,1	248,26	2,1	48,37
Vineyard	BBCH 10 - 19	10	19	Small granivorous bird "Finch"	Linnet (*Carduelis cannabina*)	6,9	14,8	4,6	431,10	1,4	69,40
Vineyard	BBCH 20 - 39	20	39	Small granivorous bird "Finch"	Linnet (*Carduelis cannabina)*	5,7	12,4	3,9	514,53	1,2	84,01
Vineyard	BBCH ≥ 40	40	99	Small granivorous bird "Finch"	Linnet (*Carduelis cannabina*)	3,4	7,4	2,3	862,19	0,7	140,84
Vineyard	Ripening	??		Frugivorous bird "Trush/ starling"	Song Thrush (*Turdus philomelos*)	14,4	28,9	9,1	220,77	3,0	33,25
Vineyard	BBCH 10 - 19	10	19	Small omnivorous bird "lark"	Wood Lark *(Lullula arborea*)	6,5	14,4	4,5	443,07	1,4	73,67
Vineyard	BBCH 20 - 39	20	39	Small omnivorous bird "lark"	Wood Lark (*Lullula arborea*)	5,4	12	3,8	531,69	1,1	88,68
Vineyard	BBCH ≥ 40	40	99	Small omnivorous bird "lark"	Wood Lark (*Lullula arborea*)	3,3	7,2	2,3	886,14	0,7	145,11

Conclusion: The TER_A and TER_{LT} value are greater than the trigger value of 10 for acute risk and the trigger value of 5 for long-term risk indicating an acceptable risk to birds following use of RH-117,281 2F (240SC).

B.9.2.1.4 Risk for birds and mammals through drinking water

Exposure to birds *via* drinking water is not explicitly included in the above daily dietary dose (DDD) calculation. Therefore, in line with EFSA Guidance on the risk assessment for birds and mammals (EFSA, 2009), the risk to birds through drinking contaminated water has been assessed. The 'puddle scenario' is considered relevant for the proposed uses of Zoxium 240 SC. This relates to birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. 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).

Zoxamide has a Koc of 1224 L/kg (mean). The maximum effective rate of use of Zoxium 240 SC is calculated for the proposed application rate of 0.18 kg a.s./ha (5 applications with 8 day spray interval) and represents the most critical GAP for drinking water. The effective application rate is calculated by multiplying the proposed application rates by MAF values based on the DT_{50} in soil (EFSA, 2009) for the active substance; laboratory DT_{50}soil for zoxamide were 2.03 to 13.75 days at 10 to 25°C. The ratios of effective application rate to relevant endpoints are presented in Table B.9.2.1.4-1.

Table B.9.2.1.4-1: Risk for birds and mammals through drinking water

Data from Data_Entry worksheet	**Concentration of the spray solution - C Spray (g/L)**	**Organic adsorption coefficient - KOC (mL/g)**	**Application rate (mg/m^2)**	**Birds LD50**	**Birds Reproductive End Point (mg/kg bw/d)**	**Mammals LD50**	**Mammals Reproductive End Point (mg/kg bw/d)**
	0,18	1224,00	18	2000,0	114,3	5000,0	360,0
Leaf senario							
Acute risk assessment - Leaf senario	**PEC pool (mg/L)**	**DWR for small granivorous bird (15.3g bw) in L/kg bw/d**	**TER birds**	**DWR for small granivorous mammal (21.7g bw) in L/kg bw/d**	**TER mammals**	**No refinement step required**	
	36	0,46	120,8	0,24	578,7		
Puddle senario							
Calculate predicted enviromental	**pore water term**	**soil term**	**MAF mean**	**PEC puddle (mg/L)**			
	0,02	0,0015	2,2	0,02134			
Acute risk assessment - Puddle senario	**DWR for small granivorous bird (15.3g bw) in L/kg bw/d**	**TER birds**	**DWR for small granivorous mammal (21.7g bw) in L/kg bw/d**	**TER mammals**	**No refinement step required**		
	0,46	203776,9	0,24	976431,0			
Reproductive risk assessment - Puddle senario	**DWR for small granivorous bird (15.3g bw) in L/kg bw/d**	**TER birds**	**DWR for small granivorous mammal (21.7g bw) in L/kg bw/d**	**TER mammals**	**No refinement step required**		
	0,46	11645,8	0,24	70303,0			

B.9.2.1.5 Effects on secondary poisoning

As the log Kow of zoxamide is greater than three (3.76) an assessment of secondary poisoning to earthworm-eating and fish-eating birds is required.

The risk assessment for earthworm-eating birds assumes a 100 g bird eating 104.6 g worms (fresh) per day (Table 9.2.1.5-1). The risk assessment for fish-eating birds assumes a 1000 g bird eating 159 g fresh fish per day (Table 9.2.1.5-2).

Table B.9.2.1.5-1: Food chain from earthworm to earthworm-eating birds and mammals

Food chain from earthworm to earthworm-eating birds and mammals								
Dry soil approach	**Foc (organic carbon content of soil)**	**BCF earthworm**	**PEC earthworm**	**DDD birds (based on 100g bird eating 104.6g worms per day)**	**TER Birds**	**DDD mammals (based on 10g mammal eating 12.8g worms per day)**	**TER Mammals**	**No refinement step required**
	0,02	0,04	0,02	0,02	6446,9	0,02	16656,54	

Table B.9.2.1.5-2: Food chain from fish to fish-eating birds and mammals

Food chain from fish to fish-eating birds and mammals						
Calculate predicted environmental concentration of active substance in fish	**PEC fish**	**DDD birds (based on 1000g bird eating 159g fish per day)**	**TER Birds**	**DDD mammals (based on 3000g mammal eating 425g fish per day)**	**TER Mammals**	**No refinement step required**
	1,72	0,15	787,44	0,13	2777,05	

RMS comments:

Effects on birds

Acute oral, dietary and reproduction toxicity tests have been conducted with zoxamide on the bobwhite quail and the mallard ducks.

The risk from dietary exposure of birds is considered acceptable.

The risk from consumption of contaminated water from puddles is not required in view of the active substance properties.

Zoxamide has a potential for bio-accumulation (log P = 3.76), risk assessment for secondary poisoning was performed. The risks for earthworms- and fish-eating birds are acceptable.

B.9.2.2 Risk assessment for terrestrial vertebrates other than birds

B.9.2.2.1 Toxicity

Table B.9.2.2.1-1: Summary of effects of Zoxium 240 SC to mammals

Test species	Test System	Results[1]	Reference
Rat	Acute	LD_{50}: >5000 mg/kg bw	KCP 7.1.1/01/01 [redacted] (1999)

[1] As listed in the EC Review Report (2004)

Table B.9.2.2.1-2: Summary of effects of zoxamide to mammals

Test species	Test System	Endpoints[1]	Reference
Rat	Parental	**NOAEL: 5000 ppm (360 mg/kg bw/day)**	CA 5.6.1/01, [redacted] (1998)
Rat	Reproductive	NOAEL: > 20 000ppm (1474 mg/kg bw/day)	CA 5.6.1/01, [redacted] (1998)
Rat	Offspring	**NOAEL: 5000 ppm (360 mg/kg bw/day)**	CA 5.6.1/01, [redacted] (1998)
Rabbit	Development	NOAEL: 1000 mg/kg bw/day)	CA 5.6.2/02, [redacted], (1997)
Rat	Development	NOAEL: 1000mg/kg bw/day)	CA 5.6.2/01, [redacted] (1995b)

[1] Updates from toxicology section Vol. 3 A.S. B6
Values in **bold** are used in risk assessment.

Zoxamide metabolites

In the metabolism studies (Volume 3-CA B.7.2) conducted in grapes, tomato, cucumber and peas all metabolites found generally occurred at levels <10% of the TRR and the major residue observed in all studies was parent compound. In the potato metabolism study (Volume 3-CA B.7.2.1.2), no parent zoxamide was found in potato tubers. The main components of the residue in potato tubers were the metabolites RH-141452 and RH-141455, both have been found at significant levels >10% of the TRR.

RMS comments:
No information about endpoints for mammals for metabolites RH-141452 and RH-141455. In this case the justification on safe use or assessment of metabolites RH-141452 and RH-141455 is considered necessary.

B.9.2.2.2 Exposure

Exposure is calculated according to the **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)** [3] based on the GAP detailed in table B.9-1.

B.9.2.2.3 Dietary risk assessment

[3] European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA journal 2009; 7(12):1438. [139 pp.]

Table B.9.2.2.3-1: Screening step – estimates of acute exposure to zoxamide for application in potatoes at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
	Small herbivorous mammal	118,4	21,31	1,7	36,23	138,0

Table B.9.2.2.3-2: Screening step – estimates of long-term exposure to zoxamide for application in potatoes at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
	Small herbivorous mammal	48,3	8,69	2,2	10,14	35,51

Table B.9.2.2.3-3: Screening step – estimates of acute exposure to zoxamide for application in grapevines at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF (90)	Daily Dietary Dose (Multiple)	TER
	Small herbivorous mammal	136,4	24,55	1,7	41,74	119,8

Table B.9.2.2.3-4: Screening step – estimates of long-term exposure to zoxamide for application in grapevines at 180 g a.s./ha [5 applications]

Acute risk assessment screening step	Indicator species	Short cut value	Daily Dietary Dose (single)	MAF mean	Daily Dietary Dose (Multiple)	TER
	Small herbivorous mammal	72,3	13,01	2,2	15,17	23,72

Table B.9.2.2.3-5: Acute (TERA) and long-term (TER_{LT}) risk to birds from zoxamide for applications in potatoes and grapevines at 180 g a.s./ha [5 applications]

Crop	Scenario (Annex I)	Clarified Scenario start	Clarified Scenario end	Generic focal species	Representative species	Shortcut value for mean RUDs	Shortcut value for 90th percentile RUDs	Relevant	DDD acute	TER acute	DDD repro	TER repro
Potatoes	BBCH ≥ 20	20	99	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	1,9	5,4	Relevant	1,7	2953,8	0,4	907,3
Potatoes	BBCH ≥ 40	40	99	Small herbivorous mammal "vole	Common vole (*Microtus arvalis*)	21,7	40,9	Relevant	12,8	390,0	4,5	79,4
Potatoes	BBCH 10 - 40	10	40	Large herbivorous mammal "lagomorph"	Rabbit (*Oryctolagus cuniculus*)	14,3	35,1	Relevant	11,0	454,4	3,0	120,6
Potatoes	BBCH ≥ 40	40	99	Large herbivorous mammal "lagomorph"	Rabbit (*Oryctolagus cuniculus*)	4,3	10,5	Relevant	3,3	1519,1	0,9	400,9
Potatoes	BBCH 10 - 39	10	39	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus*)	7,8	17,2	Relevant	5,4	927,4	1,6	221,0
Potatoes	BBCH ≥ 40	40	99	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus*)	2,3	5,2	Relevant	1,6	3067,4	0,5	749,5
Vineyard	BBCH 10-19	10	19	Large herbivorous mammal "lagomorph"	Brown Hare (*Lepus europaeus*)	6,7	16,3	Relevant	5,1	978,6	1,4	257,3
Vineyard	BBCH 20 - 39	20	39	Large herbivorous mammal "lagomorph"	Brown Hare (*Lepus europaeus*)	5,5	13,6	Relevant	4,3	1172,8	1,1	313,4
Vineyard	BBCH ≥ 40	40	99	Large herbivorous mammal "lagomorph"	Brown Hare (*Lepus europaeus*)	3,3	8,1	Relevant	2,5	1969,2	0,7	522,4
Vineyard	BBCH 10 - 19	10	19	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	4,2	7,6	Relevant	2,4	2098,8	0,9	410,4
Vineyard	BBCH ≥ 20	20	99	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus)*	1,9	5,4	Relevant	1,7	2953,8	0,4	907,3
Vineyard	BBCH ≥ 40	40	99	Small herbivorous mammal "vole	Common vole (*Microtus arvalis*)	21,7	40,9	Relevant	12,8	390,0	4,5	79,4
Vineyard	BBCH ≥ 40	40	99	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus)*	2,3	5,2	Relevant	1,6	3067,4	0,5	749,5

Conclusion: The TER_A and TER_{LT} value are greater than the trigger value of 10 for acute risk and the trigger value of 5 for long-term risk indicating an acceptable risk to mammals following use of RH-117,281 2F (240SC).

B.9.2.2.4 Risk for mammals through drinking water

Exposure to mammals *via* drinking water is not explicitly included in the above daily dietary dose calculation. Therefore, in line with EFSA Guidance on the risk assessment for birds and mammals (EFSA, 2009), the risk to mammals through drinking contaminated water has been assessed. The ‘puddle scenario’ is considered relevant for the proposed uses of Zoxium 240 SC. This relates to mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. 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).

Zoxamide has a Koc of 1224 L/kg (mean). The maximum effective rate of use of Zoxium 240 SC is calculated for the proposed application rate of 0.18 kg a.s./ha (5 applications with 8 days interval) and represents the most critical GAP for drinking water. The effective application rate is calculated by multiplying the proposed application rates by MAF values based on the DT_{50} in soil (EFSA, 2009) for the active substance; laboratory DT_{50}soil for zoxamide were 2.03 to 13.75 days at 10 to 25°C. The ratios of effective application rate to relevant endpoints are presented in the Table 9.2.1.4-1.

B.9.2.2.5 Effects on secondary poisoning

As the log Kow of zoxamide is greater than three (3.76) an assessment of secondary poisoning of earthworm-eating and fish-eating mammals is required.

The risk assessment for earthworm-eating mammals assumes a 10 g mammal eating 12.8 g worms (fresh) per day (see Table B.9.2.1.5-1). The risk assessment for fish-eating mammals assumes a 3000 g mammal eating 425 g fresh fish per day (see Table B.9.2.1.5-2).

RMS comments:

Effects on other terrestrial vertebrates
Acute oral and reproduction toxicity tests have been conducted with zoxamide. An acute toxicity is also available with the representative formulation. The product is not considered more toxic than expected and the risk assessment is focused on the active substance.

The risk from dietary exposure of mammals is considered acceptable.

The risk from consumption of contaminated water from puddles is not required in view of the active substance properties.

Zoxamide has a potential for bio-accumulation (log P = 3.76), risk assessment for secondary poisoning was performed. The risks for earthworms- and fish-eating mammals are acceptable.

B.9.3 EFFECTS ON AQUATIC ORGANISMS

B.9.3.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

The representative formulation of current RAR has changed since the first inclusion. For first inclusion representative formulation was blend containing zoxamide and mancozeb (Dithane/RH-117,281 DG Blend, (Lot No. WHC-3477; 69% mancozeb and 8.26% RH-117,281; TD No. 97-016), now it is Zoxium 240 SC formulation containing zoxamide (RH-117,281 2F (240 SC)).

B.9.3.1.1 Acute toxicity to fish

Report:	**KCP 10.2.1.1/01 [redacted] (2010)** **GOW 008: Acute toxicity to zebra fish (*Danio rerio*) in a 96-hour study under static exposure. Gowan Italia unpublished report**

Project no.:	CH-E-081/2010; BT104/10
Guidelines:	OECD 203; OPPTS 850.1075
Deviations:	None
GLP:	Yes
Previous evaluation:	No, data to support new representative formulation

Executive Summary

Seven juvenile fish of the species *Danio rerio* were exposed for 96 hours under static conditions to an aqueous test medium containing the test item at the five different concentrations of product, namely 10.0, 17.8, 31.6, 56.2 and 100.0 mg GOW 008/L, corresponding to 2.180, 3.880, 6.889, 12.252 and 21.800 mg a.s./L as active substance zoxamide. Besides these test concentrations, one tank without the test item was prepared as the negative control.

The test organisms were checked for mortality after 2, 24, 48, 72 and 96 hours from test initiation.

The actual test concentration of active ingredient zoxamide was analytically measured at the beginning and at the end of the test (96 hours). For the highest tested concentration (100.0 mg/L as product) the test was stopped after 48 hours of exposure and the analytical concentration check was carried out at that because all of the fish had died.

The following results for the test item GOW 008, based on the geometric mean of measured content of the active ingredient, after 96 hours are determined to be: the LC_{50} is 0.184 mg a.s./L (95% confidence intervals: 0.133 – 0.255 mg a.s./L) equivalent to 0.845 mg product/L (95% confidence intervals: 0.609 – 1.172 mg product/L), the NOEC is 0.215 mg a.s./L (corresponding to 0.987 mg product/L) and the LOEC is >0.215 mg a.s./L (corresponding to >0.987 mg product/L).

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	GOW 008
	Batch no.:	19052010
	Active ingredient/ content:	Zoxamide 246.1 g/L (21.8% w/w)

	Description:	Suspension concentrate
2.	**Test organism:**	Zebra fish (*Danio rerio*)
	Weight:	0.27 g (mean)
	Length:	2.0 cm (mean)
	Source:	Commercial supplier ([redacted]) on September 22, 2010
	Acclimation period:	47 days
	Diet:	During the holding period the fish were fed with commercial fish food provided by farmer (TETRAMIN Granules) until 48 hours before the test initiation
3.	**Treatment:**	0 10.0, 17.8, 31.6, 56.2 and 100.0 mg product/L, corresponding to 2.180, 3.880, 6.889, 12.252 and 21.800 mg a.s./L
4.	**Test vessels:**	60 L capacity glass aquaria
	Test water:	Reconstituted water
	Max. loading rate:	< 0.8 g fish/L test water
5.	**Environmental conditions:**	
	Temperature:	21.8 - 22.6 °C
	pH:	6.97 – 7.75
	Dissolved oxygen:	61.0 – 80.8 (%)
	Photoperiod:	16 hours light and 8 hours darkness daily photoperiod; light intensity was 50 - 500 lux, with 2 daily 30-minutes transition periods. The measured values were in the range 165 – 249 lux.

B. STUDY DESIGN AND METHODS

1. In-life phase: Nov 05 to 17, 2010

2. Test organism assignment and treatment

The main study was carried out for 96 hours under static conditions at five different concentrations, namely 10.0, 17.8, 31.6, 56.2 and 100.0 mg product/L, corresponding to 2.180, 3.880, 6.889, 12.252 and 21.800 mg a.s./L as active substance zoxamide. Besides this test concentration, one tank without the test item was prepared as the negative control. At the start of the test, 7 fish were randomly distributed to each aquarium; one replicate per concentration. The test was performed in single temperature-controlled aquarium and incubated in a temperature-controlled room. The fish were not fed during the 96-hour test.

3. Dose preparation

Prior to the start of the test, 45 litres of a solution of 100.0 mg/L were prepared by direct weighting into reconstituted water. The obtained solution appeared opalescent and showed whitish insoluble grains, therefore it was mixed by the mean of a magnetic stirrer for about 65 hours in dark conditions to achieve the maximum solubilisation of test item and then it was filtered by a cartridge filter (pore diameter 0.45 µm) to obtain a clear solution. The different test solutions were prepared by taking adequate aliquots of filtered solution and diluting them in the test aquaria with reconstituted water. The test solutions and the control were let to reach the desired temperature before adding the fish.

4. Measurements and observations

The test fish were observed at 2, 24, 48, 72 and 96 hours after test initiation for mortality and visible abnormalities.

100 mL samples were taken in duplicate from the test media and the control at the start and end of the test from each tank in order to determine the stability of the test item under the test conditions. For the highest concentration the test was stopped after 48 hours (at which point all fish were observed dead) and the analytical check was carried out at that time. The analyses were performed by an Agilent Liquid Chromatograph with Mass Spectrometer.

pH values and dissolved oxygen concentrations in the test item concentrations and in the control were measured daily.

5. Statistics

The LC_{50} was calculated at 24, 48, 72 and 96 hours of exposure time by a Trimmed Spearman-Karber method. The NOEC, LOEC, LC_0, LC_{50} and LC_{100} values were determined directly from the raw data at each time of observation. The CETIS v.1.026D software was used to carry out the statistical analyses.

II. RESULTS AND DISCUSSION

A. Mortality and signs of toxicity

The cumulative percent mortalities and the daily observations made during the test are presented in Table B.9.2.11-1.

Table B.9.2.11-1: Summary of cumulative mortality and clinical observations

Nominal concentration (mg test item/L)	Mortality			
	24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Control	0/7	0/7	0/7	0/7
10.0	0/7	0/7	0/7	0/7
17.8	0/7	0/7	0/7	0/7
31.6	2/7	3/7	3/7	3/7
56.2	2/7	2/7	3/7	3/7
100.0*	5/7	7/7	7/7	7/7

* The test was stopped after 48 hours since all the fish were found dead at that time

B. Toxicity endpoint

The following results for the test item GOW 008, based on the measured content of the active substance, were observed after 96 hours.

Table B.9.2.11-2: LC_{50}, NOEC and LOEC values determined on the basis of geometric mean of a.s. zoxamide measured concentrations

Time	NOEC (mg a.s./L)	LC_{50} – 95% Confidence range (mg a.s./L)	LOEC (mg a.s./L)
24 h	0.066	0.313 (0.205 – 0.477)	0.125
48 h	0.215	0.201 (0.146 – 0.276)	>0.215
72 h	0.215	0.184 (0.133 – 0.255)	>0.215
96 h	0.215	0.184 (0.133 – 0.255)	>0.215

C. Analytical verification

The concentration of active substance showed a large decrease in the freshly prepared solutions, being the average analytical recovery equal to 2.0% compared to the nominal values (range between 1.9% and

2.2%). At the end of the test (48 hours for the concentration of 10.0 mg/L and 96 hours for the other tested concentrations) the analytical recoveries ranged between 1.5% and 2.0% of the nominal values.

Since the analytical recovery was lower than the range 80% - 120%, the biological results referred to the geometric mean of the measured concentrations

Table B.9.2.11-3: Measured concentrations of zoxamide during the test

Nominal concentrations of zoxamide (mg a.s./L)	Measured concentrations (mg a.s./L)			Geometric mean (mg a.s./L)
	t = 0 h	t = 48 h	t = 96 h	
2.180	0.042	n.a.	0.042	0.042
3.880	0.073	n.a.	0.060	0.066
6.889	0.149	n.a.	0.104	0.125
12.252	0.238	n.a.	0.195	0.215
21.800*	0.472	0.439	n.a.	0.455

* For this concentration the test was stopped after 48 hours since all the fish were found dead at this time
n.a. not analysed

III.CONCLUSION

Under the experimental conditions, the 96-hour LC_{50} value of GOW 008, based on the geometric mean of the measured content of the active substance, was 0.184 mg a.s./L (95% confidence intervals: 0.133 – 0.255 mg a.s./L) equivalent to 0.845 mg product/L (95% confidence intervals: 0.609 – 1.172 mg product/L), the NOEC was 0.215 mg a.s./L (corresponding to 0.987 mg product/L) and the LOEC was >0.215 mg a.s./L (corresponding to >0.987 mg product/L).

RMS comments:
Endpoint is based on geometric mean measured concentrations and it is noted that stirring and filtration were needed for the preparation on the concentrations. The point C related to analytical verification indicates that only 2% of the active substance was recovered at all tested concentrations at t=0. It is due to the difficulties encountered when preparing the test concentrations with a cross-reference to point 3 of the study summary. Study is considered acceptable.

B.9.3.1.2 Acute toxicity to aquatic invertebrates

Report:	**KCP 10.2.1.2/01 Mantilacci S. (2010)** **Acute toxicity of product GOW008 on *Daphnia magna* in a 48-hour immobilization test under static exposure. Gowan Italia unpublished report**

Project no.: BT103/10
Guidelines: OECD 202
Deviations: None
GLP: Yes
Previous evaluation: No, data to support new representative formulation

Executive Summary

The acute immobilisation of *Daphnia magna* exposed to the test item GOW008 was determined in a laboratory limit study. The static limit immobilisation test was performed in order to demonstrate that the EC_{50} value after 48 hours of exposure to the test item GOW008 is greater than the concentration equivalent to the active substance solubility limit. The test was conducted with the following nominal test item concentrations: 0 and 3 mg test item/L (corresponding to 0.69 mg a.s./L). Forty test organisms (5 per replicate) were exposed to the test concentration and the control.

The zoxamide content in the test samples showed a mean recovery of 108.49% in the fresh solution and a mean recovery of 98.69 – 114.44% in the aged solutions. All study results were therefore based on the nominal value of the active substance.

No effects on daphnids were observed during the 24 and 48 hours of exposure at 3 mg/L of the test item (zoxamide measured concentration of 0.69 mg/L). The EC_{50} of GOW008 at 48 hours of exposure (concentration of the test item which results in a 50% immobilisation of the test organisms) was estimated to be greater than the concentration equivalent to the active substance solubility limit.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	GOW008
	Batch no.:	19052010
	Active ingredient/ content:	Zoxamide 21.8% w/w
	Description:	Suspension concentrate
2.	**Reference item:**	Potassium dichromate
3.	**Test organism:**	*Daphnia magna* Straus 1820
	Age at Test Start:	< 24 hours old
	Source:	Biology Department of the Milan University, Italy
	Diet:	Green algae *Pseudokirchneriella subcapitata* and yeast suspension (Daphnids were not fed during the test)
4.	**Treatment:**	0, 3 mg test item/L (corresponding to 0.69 mg a.s./L)
5.	**Test vessels:**	Glass beakers of 100 mL
	Test water:	Reconstituted water ISO 6341
6.	**Environmental conditions:**	
	Temperature:	20.33 - 21°C
	pH:	7.39 - 8.09
	Dissolved oxygen:	8.54 – 8.67 mg/L
	Photoperiod:	16 hours light (425-600 lux) : 8 hours darkness

B. STUDY DESIGN AND METHODS

1. In-life phase: Oct 27 to 29, 2010

2. Test organism assignment and treatment

Daphnids aged less than 24 hours at the start of the test, were exposed at a determinate concentration of the test item (GOW008) for a period of 48 hours. The dose of 3 mg/L of test item corresponds to an active substance concentration (0.69 mg a.s./L) around its limit of solubility in water. Forty daphnids were used for the treated and untreated groups, divided into eight groups of five animals each. The test solutions were not aerated and the daphnids were not fed during the test.

3. Dose preparation

The test solution was prepared in ISO water by dilution of a stock solution at 100 mg/L of the test item and 50 mL of the test and control (medium without test item) solutions were used for each treated and untreated replicate. The stock solution was sonicated for 30 minutes and the solutions were maintained on magnetic stirrer until the application.

4. Measurements and observations

The number of immobilised daphnids was assessed after 24 and 48 hours from the beginning of the test. The daphnids were considered in the immobile status if they were not able to swim after gentle agitation of the vessel and were counted using a stereomicroscope.

Samples to be analysed were taken at 0 hours (initial value) from fresh test solution and at 48 hours from the aged test solution in each replicate. The analyses were performed by an Agilent Liquid Chromatograph with Mass Spectrometer.

pH values and the dissolved oxygen concentrations were measured in the untreated and in the treated groups at test start and at the end of the test. Temperature and light intensity were continuously recorded by a data logger.

5. Statistics

No statistical analysis was performed as there were no mortalities recorded in the test item treated group.

II. RESULTS AND DISCUSSION

A. Immobilisation

The percentage of immobilisation of *Daphnia magna* observed after 24 and 48 hours of the exposure is given in Table B.9.2.11-4.

Table B.9.2.11-4: Effect concentration of GOW008 to *Daphnia magna*

Test item concentration (mg/L)	Number of exposed daphnids	Response at 24 h		Response at 48 h	
		Number of immobilised	%I	Number of immobilised	%I
0	40	0	0	0	0
3	40	0	0	0	0

After 24 hours of exposure, the EC_{50} value is 1.165 mg/L of potassium dichromate with evaluated confidence intervals (95%) of 1.052 mg/L and 1.370 mg/L.

B. Analytical verification

The zoxamide content in the test samples showed a mean recovery of 108.49% in the fresh solution and a mean recovery of 98.69 – 114.44% in the aged solutions. All study results were therefore based on the nominal value of the active substance.

Table B.9.2.11-5: Measured concentrations of zoxamide during the test

Nominal (mg test item)	Nominal (mg a.s./L)	Sampling (h)	Test item (mg a.s./L)	(%) of nominal
Control		0	Not detectable	-
		48	Not detectable	-
3	0.67	0	0.72	108.49
		48	0.70	104.32
		48	0.65	99.05
		48	0.70	105.44
		48	0.66	98.69
		48	0.70	104.76
		48	0.76	114.44
		48	0.69	102.70
		48	0.66	99.51

III. CONCLUSION

No effects on daphnids were observed during the 24 and 48 hours of exposure at 3 mg/L of the test item (zoxamide measured concentration of 0.69 mg a.s/L). The EC_{50} of GOW008 at 48 hours of exposure (concentration of the test item which results in a 50% immobilisation of the test organisms) was estimated to be greater than the concentration equivalent to the active substance solubility limit.

RMS comments:
The study is considered acceptable.

B.9.3.1.3 Effects on aquatic algae

Report:	**KCP 10.2.1.3/01 G. Scott Ward, C. W. Murdock (1998) Toxicity of RH-117,281 2F (240 SC) to *Selenastrum capricornutum* Printz. Rohm and Haas Company (now Dow AgroSiences).**

Report no.: 97RC-0094
Guidelines: Directive 92/69/EEC C.3 OECD 201; OPPTS 850.5400
Deviations: None
GLP: Yes
Previous evaluation: No, data to support new representative formulation

Executive Summary
The test was conducted by exposing *Selenastrum capricornutum* under static conditions for 96 hours to the following nominal exposure concentrations: 50, 100, 200, 400 and 800 µg formulated product/L RH-117,281 2F (240 SC), along with a control. There were three replicate flasks per test concentration and each contained initially approximately 1.0 x 10^4 cells/mL. An additional replicate was prepared only for the 72-hour water quality measurements and analytical samples.

Analytical determination of RH-117,281 2F (240 SC) concentrations in the exposure system was conducted at 0, 72, and 96 hours. The measured concentrations after 96 hours ranged from 68 to 83% of nominal concentrations indicating limited degradation under the conditions of the study. Due to losses of test substance in this static study, toxicity calculations were based on the measured concentrations at 0-hour which were 55.5, 94.9, 227, 426, and 923 µg/L for the nominal concentrations: 50, 100, 200, 400 and 800 µg formulated product/L RH-117,281 2F, respectively. These measured concentrations ranged from 95 to 115% of the nominal concentrations.

The E_bC_{50} and E_rC_{50} values for *Selenastrum capricornutum* exposed to RH-117,281 2F (240 SC) were 241 µg/L and 268 µg/L, respectively, for 0-72 hours. The 72-hour no-observed-effect-concentrations (NOEC) was 55.5 µg/L based both on biomass (area under the growth curve) and on growth rate.

The E_bC_{50} and E_rC_{50} values for *Selenastrum capricornutum* exposed to RH-117,281 2F (240 SC) were 242 µg/L and 274 µg/L, respectively, for 0-96 hours. The 96-hour no-observed-effect-concentrations (NOEC) was 55.5 µg/L based both on biomass (area under the growth curve) and on growth rate.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	RH-117,281 2F (240 SC)
	Batch no.:	YS-1239, TD No.97-018
	Active ingredient/ content:	Zoxamide: 22.35%
	Description:	Light brown liquid (suspension concentrate)
2.	**Test organism:**	*Selenastrum capricornutum* Printz
	Strain:	Not reported
	Source:	Department of Botany, University of Texas at Austin, USA
	Initial density:	10,000 cells/mL
3.	**Treatment:**	0, 50, 100, 200, 400 and 800 µg formulated product/L RH-117,281 2F
4.	**Test vessels:**	Glass test vessels (250 mL Erlenmeyer flasks containing 100 mL test medium)
	Test water:	Algal nutrient medium according to ASTM E1218-90 (1990)
	Shaking:	Continuously at 100 rpm
5.	**Environmental conditions:**	
	Temperature:	22.8 – 24.0°C
	pH:	7.43 – 7.48 (at test start); 7.68 – 8.45 (at test end)
	Photoperiod:	Continuous lighting (4311 - 4357 lux)

B. STUDY DESIGN AND METHODS

1. In-life phase: Jan 26 to 23, 1998

2. Test organism assignment and treatment

The test started (0 hours) by inoculation of a biomass of 10,000 algal cells per mL test medium. These cells were taken from an exponentially growing pre-culture which was set up 5 days prior to the test start under the same conditions as in the test. The test was performed with three replicates per test concentration and an additional replicate for analytical sampling and water chemistry.

3. Dose preparation

A 100 mg/L stock solution was prepared by dissolving 0.05 g of the test item into a 500 mL of freshwater culture medium. Appropriate volumes of this test concentration were diluted with culture medium to prepare the 800 µg/L test concentration and subsequently aliquots of the secondary stock solution were then diluted to prepare the rest of the nominal test concentrations.

4. Measurements and observations

The algal cell counts were accomplished utilising a hemacytometer and an Olympus Model BH-2 microscope.

100 mL samples were taken at 0 hour from the parent solutions. The 72-hour samples were obtained from the additional replicates prepared for exclusively for analytical sampling. At 96 hours, 100 mL samples were taken from all three replicates in each control and test substance treatment. Analysis of the test medium samples was performed by HPLC with UV detection.

For the assessment of any influence of the test item on the algal cells, the shape of the treated algal cells compared to the control was microscopically examined.

The pH and temperature in the test solutions was measured and recorded at 0, 3 and 4 days. The light intensity was measured daily throughout the test.

5. Statistics

A one-way ANOVA was conducted for each time with a Dunnett’s comparison to the control. Prior to the Dunnett's test, a Shapiro-Wilk's test and a Levene's test were conducted to test for normality and homogeneity of variance, respectively, over treatments at each time point. If the Shapiro-Wilk's and Levene's test did not indicate normality and significant heterogeneity, then the raw data for each replicate were transformed (square root transformation). If both the raw data and transformed data have shown non-normality or inequality of variance, then a nonparametric analysis of variance was performed using the ranks of the values. A logistic (sigmoid-shaped) model was used to for the calculation of the EC_{50} values. All statistical analyses were performed with the SAS program 6.10 (1994).

II. RESULTS AND DISCUSSION

A. Algal growth

The growth data (cell counts) from the definitive test are presented in Tables B.9.2.11-6. The growth data were subjected to an ANOVA and multiple means test (Dunnett's test). The multiple means test indicated a significant inhibition effect ($p \leq 0.05$) on the growth parameter of area under the growth curve for measured concentrations ≥ 94.9 µg/L, as compared to the control after 72 and 96 hours of exposure (Table B.9.2.11-7). For the growth parameter of growth rate, the multiple means test also indicated a significant inhibition effect *(*$p \leq 0.05$) for the measured concentrations ≥ 94.9 µg/L as compared to the control after 72 and 96 hours of exposure (Table B.9.2.11-8).

Table B.9.2.11-6: Mean number of cells (10^4 cells x mL^{-1}) at each observation time

0-hr measured concentration (µg/L)	Mean cell counts (x 10^4 cells/mL)				
	0 hour	24 hour	48 hour	72 hour	96 hour
0 (control)	1.1	4.0	12	47	170
55.5	--	3.8	13	47	160
94.9	--	3.6	11	39	130
227	--	2.8	8.8	31	110
426	--	1.5	0.82	0.85	0.63
923	--	1.3	0.33	0.037	0.15

Table B.9.2.11-7: Replicate biomass (area under the growth curve) values for *Selenastrum capricornutum* during exposure to RH-117,281 2F (240 SC)

0-Hour Measured Concentration (µg/L)	Replicate	0-24 hrs [a]	0-48 hrs [a]	0-72 hrs [a]	0-96 hrs [a]
control	A	36.00	204.0	801.0	3168
	B	38.64	206.6	930.4	3592
	C	27.96	201.6	946.0	3493
55.5	A	26.64	193.0	886.3	3385
	B	33.36	191.4	848.8	3234
	C	37.32	235.3	943.7	3425
94.9	A	32.04	191.8*	795.1*	2772*
	B	32.04	194.8*	795.1*	2945*
	C	24.00	152.0*	689.8*	2598*
227	A	27.96*	151.9*	615.6*	2239
	B	14.64*	108.0*	518.4*	2199
	C	18.72*	138.8*	604.9*	2282
426	A	3.96*	5.28*	-3.96*	-21.24
	B	1.32*	0.00*	-1.32*	-1.32
	C	8.04*	10.8*	1.56*	-7.68
923	A	3.96*	-2.76*	-26.76*	-52.08
	B	-1.32*	-12.0*	-33.36*	-56.04
	C	2.64*	-2.76*	-24.12*	-49.44

[a] Values calculated using cell count data and were obtained from SAS output. Values were rounded to two significant figures

* Multiple means test indicated a significant inhibition effect ($p \leq 0.05$) using growth rate data, as compared to the control.

Table B.9.2.11-8: Replicate growth rate values for *Selenastrum capricornutum* during exposure to RH-117,281 2F (240 SC)

0-Hour Measured Concentration (µg/L)	Replicate	0-24 hrs [a]	0-48 hrs [a]	0-72 hrs [a]	0-96 hrs [a]
control	A	0.058	0.052	0.051	0.053
	B	0.054	0.048	0.052	0.052
	C	0.047	0.052	0.053	0.052

55.5	A	0.046	0.051	0.052	0.052
	B	0.052	0.049	0.052	0.051
	C	0.056	0.054	0.052	0.052
94.9	A	0.051	0.049	0.050*	0.049*
	B	0.051	0.050	0.050*	0.050*
	C	0.043	0.045	0.049*	00049*
227	A	0.047*	0.044*	0.047*	0.047*
	B	0.031*	0.040*	0.045*	0.048*
	C	0.037*	0.045*	0.046*	0.048*
426	A	0.011*	-0.0046*	-0.0095*	-0.017*
	B	0.0039*	-0.0046*	0.0013*	-0.0011*
	C	0.020*	-0.011*	-0.0049*	-0.0053*
923	A	0.011*	-0.034*	-0.041*	-0.024*
	B	-0.0043*	-0.025*	-0.032*	-0.017*
	C	0.0075*	-0.019*	-0.041*	-0.024*

[a] Values calculated using cell count data and were obtained from SAS output. Values were rounded to two significant figures

* Multiple means test indicated a significant inhibition effect ($p \leq 0.05$) using growth rate data, as compared to the control.

B. Toxicity endpoint

The toxicity endpoints of RH-117,281 2F (240 SC) for *Selenastrum capricornutum* are presented in Table 9.2.11-9.

Table B.9.2.11-9: Summary of endpoints for RH-117,281 2F (240 SC) on the growth of *Selenastrum capricornutum*

Hours	E_bC_{50} (95% confidence limits) (µg/L)[a]	NOEC (based on biomass) (µg/L)	E_rC_{50} (95% confidence limits) (µg/L)[b]	NOEC (based on biomass) (µg/L)
0-24	295 (215-289)	94.9	295 (235-355)	94.9
0-48	263 (236-262)	55.5	263(0-541)	94.9
0-72	268 (223-260)	55.5	268 (0-643)	55.5
0-96	274 (234-250)	55.5	274 (not calculated)	55.5

C. Analytical verification

At the 50, 100, 200 and 400 µg/L nominal concentration levels, measured concentrations of RH-1l7,281 2F (240 SC) ranged from 55.5 to 426 µg formulated product/L and 95 to 114% of nominal concentrations at test initiation (Table B.9.2.11-10). The 0-hour measurements were used as representative of the static test concentrations for calculation of the EC_{50} values due to expected losses observed at other sampling intervals. Measured concentrations after 96-hours ranged from 68 to 83% of nominal concentrations indicating limited degradation under the conditions of the study.

Table B.9.2.11-10: Measured concentrations during the test

Nominal concentration (µg/L)	0 hours		72 hours		96 hours	
	Measured concentration (µg/L)	% of nominal	Measured concentration (µg/L)	% of nominal	Measured concentration (µg/L)	% of nominal
0 (control)	<8.37	N/A	<8.46	N/A	<8.37	N/A
50	55.5	111	44.3	89	34.6	69
100	94.9	95	76.1	76	82.8	83
200	227	114	206	103	142	71
400	426	107	314	79	272	68
800	923*	115	756	95	631	79

Note: Measured concentrations of RH-117,281 2F (240 SC) were calculated by dividing the measured concentrations of the active substance zoxamide by 0.2235 (purity of the formulated product)
N/A not applicable
*The actual measurement was 331 µg/L. As there was a suspected sampling error the value was estimated based on mean percentages for the four lower test concentrations.

III. CONCLUSION

Under the experimental conditions, the E_bC_{50} and E_rC_{50} values for *Selenastrum capricornutum* Printz exposed to RH-117,281 2F (240 SC) were 241 µg/L and 268 µg/L, respectively, for 0-72 hours. The 72-hour no-observed-effect-concentrations (NOEC) was 55.5 µg/L based both on biomass (area under the growth curve) and on growth rate. The E_bC_{50} and E_rC_{50} values for *Selenastrum capricornutum* Printz exposed to RH-117,281 2F (240 SC) were 242 µg/L and 274 µg/L, respectively, for 0-96 hours. The 96-hour no-observed-effect-concentrations (NOEC) was 55.5 µg/L based both on biomass (area under the growth curve) and on growth rate.

0-72-hour E_bC_{50} = 241 µg/L (mean measured)
0-72-hour E_rC_{50} = 268 µg/L (mean measured)
72-hour NOEC = 55 µg/L (mean measured)
0-96-hour E_bC_{50} = 242 µg/L (mean measured)
0-96-hour E_rC_{50} = 274 µg/L (mean measured)

RMS comments:
EC_{10} value is not given in the study, no argumentation about absence of value are found, based on that results considered not sufficient. Validity criteria of the test are fulfilled and study is considered acceptable.

B.9.3.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms with the formulation were not performed, since it is possible to extrapolate from data obtained with the active substance.

B.9.3.3 Further testing on aquatic organisms

Further higher tier testing with RH-117,281 2F (240 SC) to assess the effects on aquatic organisms is not required since the TER_A values for the active substance are >100 and the TER_{LT} values are >10.

B.9.4 RISK ASSESSMENT FOR AQUATIC ORGANISMS

The toxicity of zoxamide and Zoxium 240 SC is summarised in Tables B.9.4-1 and B.9.4-2, respectively. The data on zoxamide were submitted for the first EU review and the endpoints reflect the values agreed in the Review Report (2004) or proposed in the DAR (2001) and addenda. Studies examining the acute toxicity of Zoxium 240 SC to fish, *Daphnia* and algae have been conducted to support this submission and the results are listed in Table B.9.4-2. Compared to zoxamide, the toxicity of Zoxium 240 SC is of no greater toxicity based on its a.s. content. Therefore, the toxicity data for the active substance can be used in the subsequent aquatic risk assessment. However, in the case of the *Daphnia* endpoint, the LC_{50} from the formulation study is used since the endpoint from the a.s. study is a "greater than" value and is also a slightly higher value. The endpoints for use in the following risk assessment are listed in Table B.9.4-1 and are in bold.

Table B.9.4-1: Summary of the toxicity of zoxamide to aquatic organisms

Test species	Test system	Endpoint (mg a.s./L)	Reference
Acute fish			
Oncorhynchus mykiss	96h – flow-through	LC_{50}: **0.16** (mean measured)	IIA 8.2.1/01 [redacted] 1995a
Lepomis macrochirus	96h – flow through	LC_{50}: >0.79 (mean measured)	IIA 8.2.1/02 [redacted] 1995b
Pimephales promelas	96h – flow-through	LC_{50}: >0.208 (mean measured)	IIA 8.2.1/03 (IIA 8.2.2.3/01) [redacted] 1998d
Brachydanio rerio	96h – flow through	LC_{50}: >0.73 (mean measured)	IIA 8.2.1/04 [redacted] 1998a
Cyprinodon variegatus	96h – flow-through	LC_{50}: >0.85 (mean measured)	IIA 8.2.1/05 [redacted] 1997
Long-term fish			
Oncorhynchus mykiss	95 d – flow-through, ELS	NOEC: **0.00348** (mean measured)	IIA 8.2.2.2/01 [redacted] 1996
Pimephales promelas	202 d – flow-through, FLC	NOEC: 0.06 (mean measured)	IIA 8.2.1/03 (IIA 8.2.2.3/01) [redacted] 1998d
Lepomis macrochirus	28 day – flow-through, bioaccumulation	BCF: 95-136	IIA 8.2.3/01 [redacted] 1998
Acute aquatic invertebrates			
Daphnia magna	48h – flow-through	EC_{50}: >0.78 (mean measured)	IIA 8.2.4/01 Sword, M.C., Gardner, C. 1995c
Mysidopsis bahia	96h - flow-through	LC_{50}: **0.076** (mean measured)	KCA 8.2.4.2/01 Roberts, C.A., Swigert, J.P. 1997

Test species	Test system	Endpoint (mg a.s./L)	Reference
Long-term aquatic invertebrates			
Daphnia magna	21d – flow through	NOEC: **0.039** (mean measured)	IIA 8.2.5/01 Murrell, H., Rhodes, J.E., Stewart, S. 1997
Chironomus riparius	28d– flow through	NOEC: **0.45** (nominal)	IIA 8.2.7/01 van der Kolk, J. 1998a
Mysidopsis bahia	27d- flow-through	NOEC: **0.0072** (mean measured)	KCA 8.2.5.2/01 Drottar, K.R., Krueger, H.O. 1998
Algae			
Selenastrum capricornutum	120h - static	E_bC_{50}: 0.023 E_rC_{50}: 0.048 (mean measured)	IIA 8.2.6/01 Ziegler, T.A., Stewart, S. 1996
Anabaena flos-aquae	96h - static	E_bC_{50}: >0.86 E_rC_{50}: >0.86 (mean measured)	IIA 8.2.6/02 Drottar, K.R., Sutherland, C.A., Krueger, H.O. 1998e
Scenedesmus subspicatus	96h - static	E_bC_{50}: **0.011** E_rC_{50}: 0.018 (mean measured)	IIA 8.2.6/03 Drottar, K.R., Sutherland, C.A., Krueger, H.O. 1998f
Navicula pelliculosa	96h - static	E_bC_{50}: >0.93 E_rC_{50}: >0.93 (mean measured)	IIA 8.2.6/04 Drottar, K.R., Sutherland, C.A., Krueger, H.O. 1998g
Skeletonema costatum	96h - static	E_bC_{50}: >0.91 E_rC_{50}: >0.91 (mean measured)	IIA 8.2.6/05 Drottar, K.R., Krueger, H.O. 1998c
Aquatic plants			
Lemna gibba	14d – static renewal	7d-EC_{50}: >0.018 14d-EC_{50}: **0.017** (mean measured)	IIA 8.2.6/01 Ziegler, T.A., Stewart, S. 1996

The endpoints for use in the risk assessment are highlighted in **bold**

The results of the studies conducted with aquatic organisms that address the acute toxicity of Zoxium 240 SC are summarised in Table B.9.2.15-2. These data have not been previously assessed at the EU level.

Table B.9.4-2: Summary of the toxicity of Zoxium 240 SC to aquatic organisms

Test species	Test system	Endpoint (mg/L)	Reference
Acute fish			
Danio rerio	96h – static	LC_{50}: 0.184 mg a.s./L (0.865 mg formulation/L)	KCP 10.2.1.1/01 [redacted] 2010
Acute aquatic invertebrates			
Daphnia magna	48h – static	EC_{50}: **>0.69 mg a.s./L** (>3.0 mg formulation/L)	KCP 10.2.1.2/01 Mantilacci, S. 2010
Algae			

*Selenastrum capricornutum***	96h– static	E_bC_{50}: 0.24 mg formulation/L (0.0514 mg a.s./L)* E_rC_{50}: 0.274 mg formulation/L (0.0582 mg a.s./L)*	KCP 10.2.1.3/01 Ward, G.S., Murdock, C. W. 1998

*endpoints expressed as mg formulation/L are converted to mg a.s./L considering the purity of the formulation (21.24%)
** Study was performed with RH-117,281 2F, a very similar formulation to Zoxium 240 SC. Refer to Vol. 4 Part C 1 Table 1.3.2.-3 for details of both formulations.
The endpoints for use in the risk assessment are highlighted in **bold**

Classification in accordance with regulation EC 1272/2008 (CLP):

Based on data in Vol.3 Part B.8 in study of Callow & Hilton (2013b), Table B.8.2.2.3-5 to 8, 60% of degradation at 10 ºC was achieved after 28 days, what means the active substance zoxamide can be considered as not readily degradable. Zoxamide has low bioaccumulation potential (BCF whole fish: 136 < 500) and on the above acute and chronic ecotoxicology data on the formulated product, the following classifications are proposed for Zoxium 240 SC:

- Acute (short-term) aquatic hazard: Acute Category 1
- Chronic (long-term) aquatic hazard: Chronic Category 1 (factor M for zoxamide is 10^4)

Metabolites

Testing was also conducted with the major surface water metabolites R-127450 and R-163353 and the results from the DAR are presented in Tables B.9.4-3, B.9.2.15-4 and B.9.4-5. There are also toxicity data available for metabolite RH-139432; however, this has not been identified as a major surface water metabolite (see RAR Part B8).

Table B.9.4-3: Summary of the toxicity of RH-127450 to aquatic organisms

Test species	Test system	Endpoint (mg/L)	Reference
Acute fish			
Oncorhynchus mykiss	96h – static	LC_{50}: >5 (nominal)	IIA 8.2.1/06 [redacted] 1998a
Acute aquatic invertebrates			
Daphnia magna	48h – semi-static	EC_{50}: >5 (nominal)	IIA 8.2.4/02 Rhodes, J.E., Williams, S. 1998b
Algae			
Selenastrum capricornutum	96h - static	EC_{50}: 3.2 **E_bC_{50}: 2.8** E_rC_{50}: 4.1 (mean measured)	IIA 8.2.6/07 Rhodes, J.E., Williams, S. 1998c

The endpoints for use in the risk assessment are highlighted in **bold**

[4] The M-factor is applied when classifying mixtures on the basis of their individual components according to Regulation (EC) No 1272/2008.

Table B.9.4-4: Summary of the toxicity of RH-139432 to aquatic organisms

Test species	Test system	Endpoint (mg/L)	Reference
Acute fish			
Oncorhynchus mykiss	96h – flow-through	LC_{50}: 2 (nominal)	CA 8.2.1/07 [redacted] 2002
Acute aquatic invertebrates			
Daphnia magna	48h – semi-static	EC_{50}: 17 (nominal)	CA 8.2.4.1/03 Caferella, M.A. 2002
Algae			
Scenedesmus subspicatus	96h - static	EC_{50}: **21** 72h-E_bC_{50}: 26 72h-E_rC_{50}: >30 (mean measured)	CA 8.2.6.1/09 Hoberg, J.R. 2002

The endpoints for use in the risk assessment are highlighted in **bold**

Table B.9.4-5: Summary of the toxicity of RH-163353 to aquatic organisms

Test species	Test system	Endpoint (mg/L)	Reference
Algae			
Selenastrum capricornutum	96h - static	E_bC_{50}: >23 E_rC_{50}: >23 (mean measured)	IIA 8.2.6/08 Rhodes, J.E., Williams, S. 1999

According to the environmental fate characterisation of the active substance, the metabolites identified as relevant for the aquatic risk assessment based on the criteria set out in EU Regulation 1107/2009 are RH-127450, RH-24549, RH-141455, RH-163353 and RH-141288. Aquatic ecotoxicity tests for metabolites RH-24549, RH-141455 and RH-141288 have not been conducted. Considering the structure of metabolites RH-24549 and RH-141455 it can be concluded that the active part (haloketone toxophore) is no longer intact, therefore, it is very unlikely that they could exhibit an increased toxicity compared to the active substance (more information on structure of metabolites and degradation scheme see Vol.3 part B.8 AS point B.8.2.2. and Figure B.8.2.2.3-3). Metabolite RH-141455 has been shown not to have fungicidal activity. This is also shown by the toxicity data of the structurally similar metabolite RH-139432 to aquatic organisms. Moreover, metabolite RH-24549 is a soil metabolite formed at the end of the metabolic pathway. To address potential concerns, the available toxicity data on the metabolite RH-139432 is used in the risk assessment to represent the metabolites RH-24549 and RH-141455.

Regarding RH-139432, as this not a relevant metabolite, PEC_{sw} values have not been calculated and consequently no aquatic risk assessment has been performed for this metabolite.

Metabolite RH-141288 is structurally very similar to the major metabolite R-127450 but more polar and hydrophilic and therefore it is considered that the potential risks posed by RH-141288 to aquatic organisms are covered by the risk assessment on RH-127450 for which data are available and the calculated PEC_{sw} values for RH-127450 are higher than for RH-141288 (see RAR Section B8).

For metabolite RH-163353 no toxicity data on fish and *Daphnia* are available; however, this metabolite is also structurally similar to RH-127450 but more polar and hydrophilic. It is also less toxic to algae than RH-127450. It is therefore considered acceptable to use the available acute fish and *Daphnia* toxicity data on metabolite RH-127450 in order to assess the risk from the metabolite RH-163353.

In line with SANCO/3268/2001, only if the metabolite is more acutely toxic than the parent is a chronic risk assessment required. As this is not the case, none of the metabolites have been considered in any of the chronic risk assessments.

B.9.4.1 Acute toxicity exposure ratios (TER_A) for fish

TER_A values for fish following applications of Zoxium 240 SC to potatoes and grapevines have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple applications (see RAR Document B8 point 8.5. for details) and the acute toxicity endpoint for the active substance. Results are listed in Table B.9.4.1-1.

Table B.9.4.1-1: Acute TER values for fish from the proposed worst-case uses of Zoxium 240 SC using the maximum initial FOCUS Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	160	170	100
D4 Pond	Drift	0.047[2]		3404	
D4 Stream	Drift	0.709[1]		226	
D6 Ditch (1st)	Drift	0.937[1]		171	
D6 Ditch (2nd)	Drift	1.370 [2]		116.8	
R1 Pond	Run-off	0.534[2]		300	
R1 Stream	Run-off	2.415[2]		**66.3**	
R2 Stream	Drift	0.877[1]		182	
R3 Stream	Run-off	0.961[2]		166	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	160	109	100
R1 Pond	Drift	0.126[2]		1270	
R1 Stream	Drift	1.666[2]		**96**	
R2 Stream	Drift	0.984[1]		163	
R3 Stream	Drift	1.043[1]		153	
R4 Stream	Run-off	3.145[2]		**50.9**	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	160	**45.1**	100
R1 Pond	Drift	0.308[2]		519	
R1 Stream	Drift	2.264[1]		**70.7**	
R2 Stream	Drift	3.034[1]		**52.7**	
R3 Stream	Drift	3.190[1]		**50.1**	
R4 Stream	Run-off	2.263[1]		**70.7**	

[1] Calculated for a single application

[2] Calculated for multiple applications

TER values below the trigger are highlighted **in bold**

Except for the R1 (stream) and D6 (ditch 2nd) for application to potatoes, the calculated TER_A values are greater than the trigger value indicating an acceptable acute risk to fish from the proposed use of Zoxium 240 SC in potatoes for most scenarios. Except for the R1 (stream) and R4 (stream) for application to grapevine, the calculated TER_A values are greater than the trigger value indicating an acceptable acute risk to fish from the proposed use of Zoxium 240 SC in grapevines for most scenarios. However, except for the R1 (pond) scenario the calculated TER_A values are lower than the trigger value for late application in grapevines, indicating a potential acute risk to fish from the proposed worst-case use of Zoxium 240 SC in grapevines.

The initial refinement is to apply a 10 m buffer zone or a 10 m buffer zone with a 10 m vegetative strip as a risk mitigation measure depending on the main route of entry into the water body for each scenario (spray drift or run-off). Calculation of the subsequent acute TER values from the use of the initial FOCUS Step 4 PEC_{sw} for the failing scenarios are summarised in Table B.9.4.1-2.

Table B.9.4.1-2: Acute TER values for fish from the proposed worst-case uses of Zoxium 240 SC using the maximum initial FOCUS Step 4 PEC_{sw} values

Scenario	Maximum initial Step 4 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
	10 m *			
Potatoes:				
R1 Stream	1.028[2]	160	156	100
Grapevines (early application):				
R1 Stream	0.460†[1]	160	347.8	100
R4 Stream	0.674†[1]		237.4	100
Grapevines (late application):				
D6 Ditch	0.776[2]	160	206	100
R1 Stream	0.482[2]		332	
R2 Stream	0.647[2]		247.3	
R3 Stream	1.579†[2]		101.3	
R4 Stream	0.950†[2]		168	

10 m *: Mitigation (spray drift): 10 m buffer zone
[1] Calculated for a single application
[2] Calculated for multiple applications
† Run-off is the main route of entry when this mitigation is considered.

The calculated TER_A values are greater than the trigger value indicating an acceptable acute risk from the proposed uses of Zoxium 240 SC in both potatoes and grapevines when risk mitigation measures of a 10 m buffer zone with a 10 m vegetative strip are implemented.

<u>Metabolites</u>

TER_A values for fish following applications of Zoxium 240 SC to potatoes and grapevines have been determined using the maximum initial FOCUS Step 1 PEC_{sw} (see Document M-CP Section 9, Point 9.2.5 for details) for the surface water metabolites of zoxamide: RH-127450, RH-24549, RH-141455 and RH-163353. Results are listed in Table B.9.4.1-3.

Table B.9.4.1-3: Acute TER values for fish from the proposed worst-case uses of Zoxium 240 SC using the maximum initial FOCUS Step 1 PEC_{sw} values for surface water metabolites of zoxamide

Crops	Maximum initial Step 1 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
RH-127450				
Potatoes	24.4	>5000	>205	100
Grapevines	30.0		>167	100
RH-24549				
Potatoes	50.8	2000[1]	**39.4**	100
Grapevines	53.0		**37.7**	100
RH-141455				
Potatoes	17.3	2000[1]	116	100
Grapevines	18.5		108	100
RH-163353				
Potatoes	44.6	>5000[2]	>112	100
Grapevines	52.1		**>96**	100

[1] Acute fish toxicity data for metabolite RH-139432
[2] Acute fish toxicity data for metabolite RH-127450
TER values below the trigger are highlighted **in bold**

The calculated TER_A values for RH-127450 and RH-141455 are greater than the trigger value indicating an acceptable acute risk from the proposed uses of Zoxium 240 SC in both potatoes and grapevines. However, TER_A values for RH-24549 (for both potatoes and grapevines) and RH-163353 (for grapevines) are lower than the trigger value indicating a potential concern. For these scenarios acute TER values for fish have been calculated using maximum FOCUS Step 2 PEC_{sw} values (see Table B.9.4.1-4).

Table B.9.4.1-4: Acute TER values for fish from the proposed worst-case uses of Zoxium 240 SC using the maximum FOCUS Step 2 PEC_{sw} values for surface water metabolites of zoxamide

Crops	Maximum initial Step 2 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
RH-24549				
Potatoes	2.55	2000[1]	784	100
Grapevines	3.92		510	100
RH-163353				
Grapevines	10.0	>5000[2]	>500	100

[1] Acute fish toxicity data for metabolite RH-139432
[2] Acute fish toxicity data for metabolite RH-127450

The calculated TER_A values for RH-24549 and RH-163353 greater than the trigger value indicating an acceptable acute risk from the proposed uses of Zoxium 240 SC in both potatoes and grapevines.

B.9.4.2 Long-term toxicity exposure ratios (TER_{LT}) for fish

TER_{LT} values following applications of Zoxium 240 SC to potatoes and grapevines for fish have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple applications (see RAR Document B8 point 8.5) and the long-term toxicity endpoint for the active substance. Results are listed in Table B.9.4.2-1.

Table B.9.4.2-1: Long-term TER values for fish from the proposed worst-case uses of Zoxium 240 SC using the maximum initial FOCUS Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Long-term endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	3.48	**3.69**	10
D4 Pond	Drift	0.047[2]		74.0	
D4 Stream	Drift	0.709[1]		**4.91**	
D6 Ditch (1st)	Drift	0.937[1]		**3.71**	
D6 Ditch (2nd)	Drift	1.370 [2]		**2.54**	
R1 Pond	Run-off	0.534[2]		**6.52**	
R1 Stream	Run-off	2.415[2]		**1.44**	
R2 Stream	Drift	0.877[1]		**3.97**	
R3 Stream	Run-off	0.961[2]		**3.62**	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	3.48	**2.37**	10
R1 Pond	Drift	0.126[2]		28	
R1 Stream	Drift	1.666[2]		**2.1**	
R2 Stream	Drift	0.984[1]		**3.54**	
R3 Stream	Drift	1.043[1]		**3.34**	
R4 Stream	Run-off	3.145[2]		**1.11**	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	3.48	**0.98**	10
R1 Pond	Drift	0.308[2]		11.3	
R1 Stream	Drift	2.264[1]		**1.54**	
R2 Stream	Drift	3.034[1]		**1.15**	
R3 Stream	Drift	3.190[1]		**1.09**	
R4 Stream	Run-off	2.263[1]		**1.54**	

[1] Calculated for a single application
[2] Calculated for multiple applications
TER values below the trigger are highlighted **in bold**

Apart from D4 (pond) for potatoes and R1 (pond) for grapevines, the calculated TER_{LT} values are lower than the trigger value indicating a potential long-term risk to fish from the proposed worst-case use of Zoxium 240 SC in potatoes and grapevines (for both early and late applications).

A step-wise approach has been conducted to take into account risk mitigation measures to refine the risk assessment for the failing scenarios. The refinement steps taken and the corresponding calculated TER_{LT} values are summarised in Table B.9.4.2-2.

Table B.9.4.2-2: Long-term TER values for fish the proposed worst-case uses of Zoxium 240 SC using the maximum FOCUS Step 4 PEC_{sw} values

Scenario	Maximum Step 4 PEC_{sw} (µg a.s./L)				Long-term endpoint (µg a.s./L)	TER	Trigger value
	10 m*	**10 m****	**20 m***	**20 m****			
Potatoes:							
D3 Ditch	0.164[1]	-	-	-		21.2	
D4 Stream	0.159[1]	-	-	-		21.9	
D6 Ditch (1st)	0.163[1]	-	-	-		21.3	
D6 Ditch (2nd) [1]	**-**	**1.370[2]**	-	-	3.48	**2.54**	10
R1 Pond	0.235[2]	-	-	-		14.81	
R1 Stream	1.028[2]	-	0.539[2]			6.46	
R2 Stream	0.312[1]	-	-	-		11.1	
R3 Stream	0.618†[1]	0.282[1]	-	-		12.3	
Grapevines (early application):							
D6 Ditch	0.303[2]	-	-	-		11.5	
R1 Stream	-	0.733†[2]	-	**0.397[2]**		**8.77**	
R2 Stream	0.403†[2]	0.199‡[2]	-	-	3.48	17.49	10
R3 Stream	0.265[1]	-	-	-		13.1	
R4 Stream	0.674†[1]	0.305[1]	-	-		11.4	
Grapevines (late application):							
D6 Ditch	0.776[2]	-	0.272[2]	-		12.8	
R1 Stream	0.597[1]	-	0.209[1]	-		16.6	
R2 Stream	0.801[1]	-	0.281[1]	-		12.4	
R3 Stream	1.579†[2] (10 m drift)	0.707†[2] (10 m*)	1.579†[2] (20 m drift)	**0.368‡[2]** (20 m *)	3.48	**9.46**	10
R4 Stream	0.950†[2]		**0.494†[2]**	-		**7.04**	

[1]Drainage: no mitigation measures can be applied
10 m *: Mitigation (spray drift): 10 m buffer zone
10 m **: Mitigation (spray drift and run-off): 10 m buffer zone and 10 m vegetative strip
20 m *: Mitigation (spray drift): 20 m buffer zone
20 m **: Mitigation (spray drift and run-off): 20 m buffer zone and 20 m vegetative strip
PECsw values highlighted **in bold** have been used in the calculation of TER values
† Run-off is the main route of entry when this mitigation is considered.
‡ No mitigation can be performed for drainage within the programme.
[1] Calculated for a single application
[2] Calculated for multiple applications
TER values below the trigger are highlighted **in bold**

Except for the D6 (ditch 2nd) for potatoes and R1 (stream) for grapevines (early application), R3 (stream) and R4 (stream) for grapevines (late application), the calculated TER_{LT} values are greater than the trigger value indicating an acceptable long-term risk to fish from the proposed worst-case use of Zoxium 240 SC for these scenarios using buffer zones and vegetated filter strip of 10 m except for scenario R1 (stream) for potatoes and D6 (ditch), R1 (stream), R2 (stream) and R3 (stream) for grapevines (late application) risk is acceptable using 20 m buffer zone with vegetated filter strip. For failing scenarios, further refinements have been performed to demonstrate an acceptable risk.

Risk assessment performed using the worst-case PEC_{sw} values calculated by the manual module have also been refined to demonstrate safe use.

Further refinement is based on the use of time weighted average (TWA) PEC_{sw} values which is deemed appropriate due to the following reasons.

The endpoint used in the long-term risk assessment for fish is derived from a flow-through 95 day study during which effects (survival of fry) were observed in fish after 69 days of exposure (35 days post-hatch) (Downing, et al., 1996). The study meets certain conditions which have been presented in the EFSA Guidance (2013)[5] and this allows the use of a TWA-PEC_{sw} exposure value:

- The exposure was maintained in the system and the endpoint was expressed in mean measured concentration (ranging from 106 to 118% of the nominal concentrations).
- The effect endpoint (survival of fry) is not based on a developmental process during a specific sensitive life stage that may last a short time only.
- The effect endpoint (survival of fry) is not based on mortality occurring early in the test, which would have indicated an acute effect. Reduced numbers of surviving fry were first observed at the highest concentration tested 16 days post-hatching of the eggs which is equivalent to a total duration of exposure of 52 days of eggs and fry to the test item.
- There is no indication from the toxic mode of action of the active substance or substances with a similar toxic mode of action that latency of effects may occur.

As suggested by the EFSA Guidance (2013) a default 7-day TWA time window of the TWA-PEC_{sw} should be used if no further information on the relation between exposure pattern and time-to-onset of the relevant effect is provided.

TER_{LT} values following applications of Zoxium 240 SC to potatoes and grapevines for fish have been re-calculated using the FOCUS Step 3 7-d TWA-PEC_{sw} values (see Vol. 3 Document B.8, Point 8.5 for details) and the long-term toxicity endpoint for the active substance. Results are listed in Table B.9.4.2-4.

Table B.9.4.2-4: Refined long-term TER values for fish from the proposed worst-case uses of Zoxium 240 SC using the maximum FOCUS Step 3 7-day TWA-PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum Step 3 7-d TWA-PEC_{sw} (µg a.s./L)	Long-term endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.158[1]	3.48	22.02	10
D4 Pond	Drift	0.078 [2]		46.6	
D4 Stream	Drift	0.011[2]		316.4	
D6 Ditch (1st)	Drift	0.083[1]		42	

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

D6 Ditch (2nd)	Drainage	0.126[2]		15	
R1 Pond	Run-off	**0.503[2]**		**6.92**	
R1 Stream	Run-off	0.344[2]		10.1	
R2 Stream	Drift	0.071[2]		49.0	
R3 Stream	Run-off	0.150[2]		23.2	
Grapevines (early application):					
D6 Ditch	Drift	**0.985[2]**	3.48	**3.53**	10
R1 Pond	Drift	0.119[2]		29.2	
R1 Stream	Drift	0.206[2]		16.9	
R2 Stream	Drift	0.061[2]		57	
R3 Stream	Drift	0.097[2]		35.9	
R4 Stream	Run-off	0.738[2]		**4.72**	
Grapevines (late application):					
D6 Ditch	Drift	**2.372[2]**	3.48	**1.47**	10
R1 Pond	Drift	0.290[2]		12	
R1 Stream	Drift	0.070 [1]		49.7	
R2 Stream	Drift	0.048[1]		72.5	
R3 Stream	Drift	**0.497[2]**		**7**	
R4 Stream	Drift	0.337[2]		10.3	

[1] Calculated for a single application;
[2] Calculated for multiple applications;
TER values below the trigger are highlighted **in bold**

Apart R1 (pond) for potatoes, D6 (ditch) and R4 (stream) for grapevines (early application) and from D6 (ditch) and R3 (stream) scenarios for grapevines (late application), all calculated TER_{LT} values are greater than the trigger value indicating an acceptable long-term risk to fish from the proposed worst-case use of Zoxium 240 SC in potatoes and grapevines, without the use of risk mitigation.

A step-wise approach has been taken in applying risk mitigation measures to refine the failing scenarios using the 7-d TWA Step 4 PEC_{sw} values. The refinement steps taken and the subsequently calculated TER_{LT} values are summarised in Table B.9.4.2-5.

Table B.9.4.2-5: Refined long-term TER values for fish the proposed worst-case uses of Zoxium 240 SC using the maximum Step 4 7-d TWA-PEC_{sw} FOCUS values

Scenario	Maximum Step 4 7-d TWA-PEC_{sw} (µg a.s./L)			Long-term endpoint (µg a.s./L)	TER	Trigger value
	10 m	10 m *	20 m *			
Potatoes:						
R1 Pond	**0.223[2]**	-	-	3.48	15.6	10
Grapevines (early application):						
D6 Ditch	**0.223[2]**	-	-	3.48	15.6	10
R4 Stream	-	**0.326[2]**	0.168		10.7	
Grapevines (late application):						
D6 Ditch	0.539[2]	-	**0.194[2]**	3.48	17.9	10
R3 Stream	0.394	**0.197**	-		17.7	

[1] Calculated for a single application;

[2] Calculated for multiple applications;
10 m : Mitigation (spray drift): 10 m buffer zone
10 m *: Mitigation (spray drift and run-off): 10 m buffer zone and 10 m vegetative strip
20 m *: Mitigation (spray drift): 20 m buffer zone
PECsw values highlighted **in bold** have been used in the calculation of TER values

The calculated TER_{LT} values using the worst case Step 4 7-day TWA-PEC_{sw} values are greater than the trigger indicating an acceptable long-term risk from the proposed uses of Zoxium 240 SC in grapevines and potatoes when risk mitigation measures of a 20 m buffer zone is implemented.

B.9.4.3 Acute toxicity exposure ratios (TER_A) for aquatic invertebrates

Daphnia magna

TER_A values following applications of Zoxium 240 SC to potatoes and grapevines for *Daphnia* have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple application (see RAR Document B8, Point 8.5 for details) and the acute toxicity endpoint from the formulated product. Results are listed in Table B.9.4.3-1.

Table B.9.4.3-1: Acute TER values for *Daphnia* from the proposed worst-case uses of Zoxium 240 SC using the maximum initial FOCUS Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	>690	>732	100
D4 Pond	Drift	0.047[2]		>14681	
D4 Stream	Drift	0.709[1]		>973	
D6 Ditch (1st)	Drift	0.937[1]		>736	
D6 Ditch (2nd)	Drainage	1.370 [2]		>504	
R1 Pond	Run-off	0.534[2]		>1292	
R1 Stream	Run-off	2.415[2]		>286	
R2 Stream	Drift	0.877[1]		>787	
R3 Stream	Run-off	0.961[2]		>719	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	>690	>469	100
R1 Pond	Drift	0.126[2]		>5476	
R1 Stream	Drift	1.666[2]		>414	
R2 Stream	Drift	0.984[1]		>701	
R3 Stream	Drift	1.043[1]		>662	
R4 Stream	Run-off	3.145[2]		>219	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	>690	>195	100
R1 Pond	Drift	0.308[2]		>2240	
R1 Stream	Drift	2.264[1]		>305	

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
R2 Stream	Drift	3.034[1]		>227	
R3 Stream	Drift	3.190[1]		>216	
R4 Stream	Run-off	2.263[1]		>305	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme.

The calculated TER_A values are greater than the trigger value indicating an acceptable acute risk to *Daphnia* from the proposed uses of Zoxium 240 SC in both potatoes and grapevines.

Mysidopsis bahia

Under EU regulation 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, as data on the saltwater crustacean *Mysidopsis bahia* are available for zoxamide and the endpoint is lower than the *Daphnia* endpoint a risk assessment has been conducted. TER_A values following applications of Zoxium 240 SC to potatoes and grapevines for *Mysidopsis bahia* have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple application (see RAR Document B8 Point 8.5 for details) and the acute toxicity endpoint for the active substance. Results are listed in Table B.9.4.3-2.

Table B.9.4.3-2: Acute TER values for *Mysidopsis bahia* from the proposed worst-case uses of Zoxium 240 SC using the maximum initial Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	76	**80.6**	100
D4 Pond	Drift	0.047[2]		1617	
D4 Stream	Drift	0.709[1]		107	
D6 Ditch (1st)	Drift	0.937[1]		**81.1**	
D6 Ditch (2nd)	Drainage	1.370‡ [2]		**55.5**	
R1 Pond	Run-off	0.534[2]		142	
R1 Stream	Run-off	2.415[2]		**31.5**	
R2 Stream	Drift	0.877[1]		**86.7**	
R3 Stream	Run-off	0.961[2]		**79.1**	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	76	**51.7**	100
R1 Pond	Drift	0.126[2]		603.2	
R1 Stream	Drift	1.666[2]		**45.6**	
R2 Stream	Drift	0.984[1]		**77.2**	
R3 Stream	Drift	1.043[1]		**72.9**	
R4 Stream	Run-off	3.145[2]		**24.2**	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	76	**21.4**	100

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
R1 Pond	Drift	0.308[2]		247	
R1 Stream	Drift	2.264[1]		**33.6**	
R2 Stream	Drift	3.034[1]		**25.0**	
R3 Stream	Drift	3.190[1]		**23.8**	
R4 Stream	Run-off	2.263[1]		**33.6**	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme
TER values below the trigger are highlighted **in bold**

Except for the D4 (pond), D4 (stream) and the R1 (pond) scenarios for application to potatoes, the calculated TER_A values are lower than the trigger value indicating a potential risk to *Mysidopsis bahia* from the proposed uses of Zoxium 240 SC in potatoes. For grapevines, except for the R1 (pond) scenario, the calculated TER_A values are lower than the trigger value, indicating a potential risk to *Mysidopsis bahia* from the proposed worst-case use of Zoxium 240 SC in grapevines (for both early and late applications).

A step-wise approach has been conducted to take into account risk mitigation measures to refine the failing scenarios. The refinement steps taken and the subsequently calculated TER_A values are summarised in Table B.9.4.3-3.

Table B.9.4.3-3: Acute TER values for *Mysidopsis bahia* the proposed worst-case uses of Zoxium 240 SC using the maximum FOCUS Step 4 PEC_{sw} values

Scenario	Maximum Step 4 PEC_{sw} (µg a.s./L)			Acute endpoint (µg a.s./L)	TER	Trigger value
	10 m *	10 m**	20 m *			
Potatoes:						
D3 Ditch	0.164	-	-	76	463	100
D6 Ditch (1st)	0.163	-	-		466	
D6 Ditch (2nd)	1.370[1]				**55.5**	
R1 Stream	-	1.027	**0.539**		141	
R2 Stream	0.312	-	-		243	
R3 Stream	0.618‡	-	-		123	
Grapevines (early application):						
D6 Ditch	0.213	-	-	76	357	100
R1 Stream	0.733‡	-	-		103.7	
R2 Stream	0.403	-	-		189	
R3 Stream	0.265	-	-		287	
R4 Stream	0.674	-	-		113	
Grapevines (late application):						
D6 Ditch	0.776	-	0.272	76	279	100
R1 Stream	0.597	-	-		127	
R2 Stream	0.801		0.281		270	

Scenario	Maximum Step 4 PEC_{sw} (µg a.s./L)			Acute endpoint (µg a.s./L)	TER	Trigger value
	10 m *	10 m**	20 m *			
R3 Stream	1.579†[2] (10 m drift)	0.707†[2] (10 m*)	-		108	
R4 Stream	-	0.950	0.494		154	

[1]Drainage: no mitigation measures can be applied; value remains as calculated at Step 3
10 m *: Mitigation (spray drift): 10 m buffer zone
10 m **: Mitigation (spray drift and run-off): 10 m buffer zone and 10 m vegetative strip
‡ No mitigation can be performed for drainage within the programme
PECsw values highlighted **in bold** have been used in the calculation of TER values
TER values below the trigger are highlighted **in bold**

The TER_A values for all scenarios except D6 Ditch (2[nd]) scenario for application to potatoes are higher than the trigger demonstrating an acceptable risk to the marine crustacean *Mysidopsis bahia* when risk mitigation measures of a 20 m buffer with a 20 m vegetative strip are implemented.

A refined risk assessment for the D6 Ditch (2[nd]) scenario is performed using the geomean approach as described in EFSA Guidance (2013). In this approach, the geomean LC_{50} value for species belonging to the same taxonomic group is calculated. In this case, a geomean LC_{50} value is calculated for crustaceans using the LC_{50} values of zoxamide for *Daphnia magna* and *Mysidopsis bahia*. This 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 Guidance, 2013). In addition, the resulting geomean LC_{50} (229 µg a.s./L) is less than an order of magnitude greater than the LC_{50} of the most sensitive species LC_{50}; therefore, it is considered that the geomean approach in this case is not biased by using data on insensitive species.

Using this refined LC_{50} value the resulting TER_A for the D6 Ditch (2[nd]) scenario is 167.5 (trigger = 100), therefore indicating that the acute risks to aquatic invertebrates from the uses of Zoxium 240 SC are acceptable even for this exposure scenario for which risk mitigation measures such as buffer zones or vegetative strip zones have no effect.

Metabolites

TER_A values for *Daphnia* following applications of Zoxium 240 SC to potatoes and grapevines have been determined using the maximum initial FOCUS Step 1 PEC_{sw} (see Document M-CP Section 9, Point 9.2.5 for details) for the surface water metabolites of zoxamide: RH-127450, RH-24549, RH-141455 and RH-163353. Results are listed in Table B.9.4.3-4.

Table B.9.4.3-4: Acute TER values for *Daphnia* from the proposed worst-case uses of Zoxium 240 SC using the maximum initial FOCUS Step 1 PEC_{sw} values for surface water metabolites of zoxamide

Crops	Maximum initial Step 1 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
RH-127450				
Potatoes	24.4	>5000	>205	100
Grapevines	30.0		>167	100
RH-24549				
Potatoes	50.8	17000[1]	335	100
Grapevines	53.0		321	100

RH-141455				
Potatoes	17.3	17000[1]	983	100
Grapevines	18.5		919	100
RH-163353				
Potatoes	44.6	>5000[2]	>112	100
Grapevines	52.1		**>96**	100

[1] Acute *Daphnia* toxicity data for metabolite RH-139432
[2] Acute *Daphnia* toxicity data for metabolite RH-127450
TER values below the trigger are highlighted **in bold**

The calculated TER_A values for RH-127450, RH-24549 and RH-141455 are greater than the trigger value indicating an acceptable acute risk from the proposed uses of Zoxium 240 SC in both potatoes and grapevines. However, the TER_A value for RH-163353 (for grapevines) is lower than the trigger value indicating a potential concern. For this scenario the acute TER value for *Daphnia* has been calculated using the maximum FOCUS Step 2 PEC_{sw} value (see Table B.9.4.3-5).

Table B.9.4.3-5: Acute TER value for *Daphnia* from the proposed worst-case uses of Zoxium 240 SC using the maximum initial FOCUS Step 2 PEC_{sw} values for surface water metabolites of zoxamide

Crops	Maximum initial Step 2 PEC_{sw} (µg a.s./L)	Acute endpoint (µg a.s./L)	TER	Trigger value
RH-163353				
Grapevines	10.0	>5000[1]	>500	100

[1] Acute *Daphnia* toxicity data for metabolite RH-127450

The calculated TER_A value for RH-163353 is greater than the trigger value indicating an acceptable acute risk from the proposed uses of Zoxium 240 SC in grapevines.

B.9.4.4 Long-term toxicity exposure ratios (TER_{LT}) for aquatic invertebrates

Daphnia magna

TER_{LT} values following applications of Zoxium 240 SC to potatoes and grapevines for *Daphnia* have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple applications (see RAR Document B 8, Point 8.5 for details) and the acute toxicity endpoint for the active substance. Results are listed in Table B.9.4.4-1.

Table B.9.4.4-1: Long-term TER values for *Daphnia* from the proposed worst-case uses of Zoxium 240 SC using the maximum initial Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Long-term endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	39	41.4	10
D4 Pond	Drift	0.047[2]		830	
D4 Stream	Drift	0.709[1]		55.0	

D6 Ditch (1st)	Drift	0.937[1]		41.6	
D6 Ditch (2nd)	Drainage	1.370 [2]		28.5	
R1 Pond	Run-off	0.534[2]		73	
R1 Stream	Run-off	2.415[2]		16.1	
R2 Stream	Drift	0.877[1]		44.5	
R3 Stream	Run-off	0.961[2]		40.6	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	39	26.5	10
R1 Pond	Drift	0.126[2]		312	
R1 Stream	Drift	1.666[2]		23.4	
R2 Stream	Drift	0.984[1]		39.6	
R3 Stream	Drift	1.043[1]		37.4	
R4 Stream	Run-off	3.145[2]		12.4	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	39	11	10
R1 Pond	Drift	0.308[2]		127	
R1 Stream	Drift	2.264[1]		17.2	
R2 Stream	Drift	3.034[1]		12.8	
R3 Stream	Drift	3.190[1]		12.2	
R4 Stream	Run-off	2.263[1]		17.2	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme

The calculated TER_{LT} values are greater than the trigger value indicating an acceptable long-term risk to *Daphnia* from the proposed uses of Zoxium 240 SC in both potatoes and grapevines, without the need for risk mitigation.

Mysidopsis bahia

Under EU regulation 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, as data on the saltwater crustacean *Mysidopsis bahia* are available for zoxamide and the endpoint is lower than the *Daphnia* endpoint a risk assessment has been conducted. TER_{LT} values following applications of Zoxium 240 SC to potatoes and grapevines for *Mysidopsis bahia* have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple applications (see RAR Document B8, Point 8.5 for details) and the acute toxicity endpoint for the active substance. Results are listed in Table B.9.4.4-2.

Table B.9.4.4-2: Long-term TER values for *Mysidopsis bahia* from the proposed worst-case uses of Zoxium 240 SC using maximum initial Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Long-term endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	7.2	**7.64**	10
D4 Pond	Drift	0.047[2]		153	
D4 Stream	Drift	0.709[1]		10.1	
D6 Ditch (1st)	Drift	0.937[1]		**7.68**	

D6 Ditch (2nd)	Drainage	1.370 [2]		**5.28**	
R1 Pond	Run-off	0.534[2]		13.5	
R1 Stream	Run-off	2.415[2]		**2.98**	
R2 Stream	Drift	0.877[1]		**8.21**	
R3 Stream	Run-off	0.961[2]		**7.49**	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	7.2	**4.89**	10
R1 Pond	Drift	0.126[2]		57	
R1 Stream	Drift	1.666[2]		**4.32**	
R2 Stream	Drift	0.984[1]		**7.32**	
R3 Stream	Drift	1.043[1]		**6.90**	
R4 Stream	Run-off	3.145[2]		**2.29**	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	7.2	**2.03**	10
R1 Pond	Drift	0.308[2]		23.4	
R1 Stream	Drift	2.264[1]		**3.18**	
R2 Stream	Drift	3.034[1]		**2.37**	
R3 Stream	Drift	3.190[1]		**2.26**	
R4 Stream	Run-off	2.263[1]		**3.18**	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme
TER values below the trigger are highlighted **in bold**

Except for the D4 (pond), D4 (stream) and the R1 (pond) scenarios, the calculated TER_{LT} values are lower than the trigger value indicating a potential long-term risk to *Mysidopsis bahia* from the proposed uses of Zoxium 240 SC in potatoes. Except for the R1 (pond) scenario the calculated TER_{LT} values are lower than the trigger value, indicating a potential risk to *Mysidopsis bahia* from the proposed worst-case use of Zoxium 240 SC in grapevines (both early and late applications).

The initial refinement is to apply a 10 m buffer zone or a 20 m buffer zone as a risk mitigation measure depending on the main route of entry into the water body for each scenario (spray drift or run-off). Calculation of the subsequent TER values from the use of the initial FOCUS Step 4 PEC_{sw} for the failing scenarios are summarised in Table B.9.4.4-3.

Table B.9.4.4-3: Long-term TER values for *Mysidopsis bahia* the proposed worst-case uses of Zoxium 240 SC using the maximum FOCUS Step 4 PEC_{sw} values

Scenario	Maximum Step 4 PEC_{sw} (µg a.s./L)				Endpoint (µg a.s./L)	TER	Trigger value
	10 m *	10 m**	20 m*	20 m **			
Potatoes:							
D3 Ditch	0.164	-	-	-	7.2	43.9	10
D6 Ditch (1st)	0.163	-	-	-		44.1	
D6 Ditch (2nd)	**1.370[1]**					**5.27**	
R1 Stream	-	1.028	**-**	0.539		13.4	
R2 Stream	0.312	-	-	-		23.1	
R3 Stream	0.618	-	-	-		11.7	
Grapevines (early application):							

D6 Ditch	0.303	-	-	-		23.8	
R1 Stream	0.733	-	0.379	-		19	
R2 Stream	0.403	-	-	-	7.2	17.9	10
R3 Stream	0.265	-	-	-		27.2	
R4 Stream	0.674	-	-	-		10.7	
Grapevines (late application):							
D6 Ditch	0.776	-	0.272	-		26.8	
R1 Stream	0.597	-	-	-		12.1	
R2 Stream	0.801	-	0.281	-		25.6	
R3 Stream	1.579†[2] (10 m drift)	0.707†[2] (10 m*)	1.579† (20 m drift)	0.368 (20 m*)	7.2	10.2	10
R4 Stream	-	0.950	-	0.494		14.6	

[1]Drainage: no mitigation measures can be applied; value remains as calculated at Step 3
10 m *: Mitigation (spray drift): 10 m buffer zone
10 m **: Mitigation (spray drift and run-off): 10 m buffer zone and 10 m vegetative strip
20 m *: Mitigation (spray drift): 20 m buffer zone
20 m **: Mitigation (spray drift and run-off): 20 m buffer zone and 20 m vegetative strip
PECsw values highlighted **in bold** have been used in the calculation of TER values
TER values below the trigger are highlighted **in bold**

The TER_{LT} values for all scenarios except D6 Ditch (2^{nd}) are higher than the trigger demonstrating an acceptable risk to the marine crustacean *Mysidopsis bahia* when risk mitigation measures of a 20 m buffer with a 20 m vegetative strip are implemented.

A refined risk assessment for the D6 Ditch (2^{nd}) scenario is performed using the geomean approach as described in EFSA Guidance (2013). In this approach, the geomean NOEC value for species belonging to the same taxonomic group is calculated. In this case, a geomean NOEC value is calculated for crustaceans using the NOEC value of zoxamide for *Daphnia magna* and *Mysidopsis bahia*. This 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. In addition, the endpoints used from the two studies are biologically comparable (reproduction) and the resulting geomean NOEC (16.7 µg a.s./L) is of the same order of magnitude with the NOEC of the most sensitive species; therefore, it is considered that the geomean approach in this case is not biased by using data on insensitive species.

Using this refined NOEC value the resulting TER_{LT} for the D6 Ditch (2^{nd}) scenario is 12.2 (trigger = 10), therefore indicating that the long-term risks to aquatic invertebrates from the uses of Zoxium 240 SC is acceptable even for this exposure scenario for which risk mitigation measures such as buffer zones or vegetative strip zones have no effect.

<u>Sediment swelling aquatic invertebrates - *Chironomus riparius*</u>

TER_{LT} values following applications of Zoxium 240 SC to potatoes and grapevines for the sediment dwelling organism *Chironomus riparius* have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple applications (see RAR Document B Section 8, Point 8.5 for details) and the acute toxicity endpoint for the active substance. Results are listed in Table B.9.4.4-4.

Table B.9.4.4-4: Long-term TER values for *Chironomus riparius* from the proposed worst-case uses of Zoxium 240 SC using the maximum initial Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Long-term endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	450	477	10
D4 Pond	Drift	0.047[2]		9574	
D4 Stream	Drift	0.709[1]		634	
D6 Ditch (1st)	Drift	0.937[1]		480	
D6 Ditch (2nd)	Drainage	1.370 [2]		329	
R1 Pond	Run-off	0.534[2]		843	
R1 Stream	Run-off	2.415[2]		186	
R2 Stream	Drift	0.877[1]		513	
R3 Stream	Run-off	0.961[2]		468	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	450	306	10
R1 Pond	Drift	0.126[2]		3571	
R1 Stream	Drift	1.666[2]		270	
R2 Stream	Drift	0.984[1]		457	
R3 Stream	Drift	1.043[1]		431	
R4 Stream	Run-off	3.145[2]		143	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	450	127	10
R1 Pond	Drift	0.308[2]		1461	
R1 Stream	Drift	2.264[1]		199	
R2 Stream	Drift	3.034[1]		148	
R3 Stream	Drift	3.190[1]		141	
R4 Stream	Run-off	2.263[1]		199	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme

The calculated TER_{LT} values are greater than the trigger value indicating an acceptable long-term risk to *Chironomus riparius* from the proposed uses of Zoxium 240 SC in both potatoes and grapevines, without the need for risk mitigation.

B.9.4.5 Toxicity exposure ratios (TER) for algae

TER values following applications of Zoxium 240 SC to potatoes and grapevines for algae have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple application (see RAR Document B8, Point 8.5 for details) and the toxicity endpoint (E_bC_{50}) for the active substance. Results are listed in Table B.9.4.5-1.

Table B.9.4.5-1: TER values for algae from the proposed worst-case uses of Zoxium 240 SC using the maximum initial Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	11	11.7	10
D4 Pond	Drift	0.047[2]		234	
D4 Stream	Drift	0.709[1]		15.5	
D6 Ditch (1[st])	Drift	0.937[1]		11.7	
D6 Ditch (2[nd])	Drainage	1.370 [2]		**8.05**	
R1 Pond	Run-off	0.534[2]		20.6	
R1 Stream	Run-off	2.415[2]		**4.55**	
R2 Stream	Drift	0.877[1]		12.5	
R3 Stream	Run-off	0.961[2]		11.4	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	11	**7.48**	10
R1 Pond	Drift	0.126[2]		87	
R1 Stream	Drift	1.666[2]		6.60	
R2 Stream	Drift	0.984[1]		11.2	
R3 Stream	Drift	1.043[1]		10.5	
R4 Stream	Run-off	3.145[2]		**3.5**	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	11	**3.10**	10
R1 Pond	Drift	0.308[2]		35.7	
R1 Stream	Drift	2.264[1]		**4.86**	
R2 Stream	Drift	3.034[1]		**3.62**	
R3 Stream	Drift	3.190[1]		**3.45**	
R4 Stream	Run-off	2.263[1]		**4.86**	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme
TER values below the trigger are highlighted **in bold**

Except for the D6 (ditch 2[nd]) and the R1 (stream) scenarios for application to potatoes, the calculated TER values are greater than the trigger value indicating an acceptable risk to algae from the proposed uses of Zoxium 240 SC in potatoes. Except for the R4 (stream) and the D6 (ditch) scenario for early application to grapevines, the calculated TER values are greater than the trigger value indicating an acceptable risk to algae. However, except for the R1 (pond) scenario the calculated TER values are lower than the trigger value for late application in grapevines, indicating a potential risk to algae from the proposed worst-case use of Zoxium 240 SC.

The initial refinement is to apply a 10 m buffer zone or a 10 m buffer zone with a 10 m vegetative strip as a risk mitigation measure depending on the main route of entry into the water body for each scenario (spray drift or run-off). Calculation of the subsequent TER values with the use of the initial FOCUS Step 4 PEC_{sw} for the failing scenarios are summarised in Table B.9.4.5-2.

Table B.9.4.5-2: TER values for algae from the proposed worst-case uses of Zoxium 240 SC using the maximum FOCUS Step 4 PEC_{sw} values

Scenario	Maximum Step 4 PEC_{sw} (µg a.s./L)	Endpoint (µg a.s./L)	TER	Trigger

	10 m*	10 m**			value
Potatoes:					
D6 Ditch (2nd)	**1.370**[1]		11	**8.05**	10
R1 Stream	-	1.028		10.7	
Grapevines (early application):					
R4 Stream	0.674	-	11	16.3	10
D6 Ditch	0.303	-		36.3	
Grapevines (late application):					
D6 Ditch	0.776	-	11	14.2	10
R1 Stream	0.597	-		18.4	
R2 Stream	0.801	-		13.7	
R3 Stream	1.579† (10 m drift)	0.707† (10 m*)		15.6	
R4 Stream	-	0.950		11.6	

[1]Drainage: no mitigation measures can be applied; value remains as calculated at Step 3
10 m *: Mitigation (spray drift): 10 m buffer zone
10 m **: Mitigation (spray drift and run-off): 10 m buffer zone and 10 m vegetative strip
TER values below the trigger are highlighted **in bold**

Except for the D6 (ditch 2nd) for potatoes the calculated TER values are greater than the trigger value indicating an acceptable risk to algae from the proposed worst-case use of Zoxium 240 SC in potatoes and grapevines (both early and late applications). Due to drainage being the main route of entry it is not possible to further refine the scenario D6 (ditch 2nd) using FOCUS.

The current risk assessment is based on the guidance document SANCO/3268/2001 (final 2002). More up to date guidance related to the selection and use of preferable endpoints from toxicity studies with algae and aquatic plants has not been considered so far. According to the OECD guideline 201 (corrected in 2011) and also in line with the recently published EFSA Guidance (2013)[6] the preferred endpoint to be used in the risk assessment for algae at the first instance is the E_rC_{50} i.e. the EC_{50} value based on inhibition of growth rate, since it is more robust considering varying test conditions, and endpoints based on biomass or yield may be used if growth rate points are not provided. It is also stated that toxicity data based on specific growth rate are more informative and better suited than toxicity values based on biomass or yield for both algae and macrophytes. The reason is that direct use of the biomass concentration without logarithmic transformation cannot be applied to an analysis of result from a system in exponential growth. In addition, according to both EFSA Guidance (2013) and the OECD 201, EC_{50} values calculated for growth rate are usually greater than EC_{50} values calculated for biomass or yield for mathematical reasons, and this should not be interpreted as a difference in sensitivity between the response variables. It is also recommended that yield is not used for comparing the sensitivity to toxicants among algal or duckweed species. There is therefore a clear indication that endpoints based on biomass or yield are not necessarily more ecologically sensitive than endpoints based on growth rate.

On that basis it is considered appropriate to re-calculate the TER values for algae using the lowest available E_rC_{50} value of 18 µg a.s./L (Table B.9.4-1) and the maximum initial FOCUS Step 3 PEC_{sw} have been calculated. Results are listed in Table B.9.4.5-3.

Table B.9.4.5-3: TER values for algae (based on E_rC_{50} toxicity endpoint) from the proposed worst-case uses of Zoxium 240 SC using the maximum initial Step 3 PEC_{sw} values

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

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	18	19.1	10
D4 Pond	Drift	0.047[2]		383	
D4 Stream	Drift	0.709[1]		25.4	
D6 Ditch (1st)	Drift	0.937[1]		20.3	
D6 Ditch (2nd)	Drainage	1.370 [2]		13.2	
R1 Pond	Run-off	0.534[2]		33.7	
R1 Stream	Run-off	2.415[2]		**7.45**	
R2 Stream	Drift	0.877[1]		20.5	
R3 Stream	Run-off	0.961[2]		18.8	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	18	12.24	10
R1 Pond	Drift	0.126[2]		143	
R1 Stream	Drift	1.666[2]		10.8	
R2 Stream	Drift	0.984[1]		18.3	
R3 Stream	Drift	1.043[1]		17.2	
R4 Stream	Run-off	3.145[2]		**5.72**	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	18	**5.08**	10
R1 Pond	Drift	0.308[2]		58	
R1 Stream	Drift	2.264[1]		**7.95**	
R2 Stream	Drift	3.034[1]		**5.93**	
R3 Stream	Drift	3.190[1]		**5.64**	
R4 Stream	Run-off	2.263[1]		**7.95**	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme
TER values below the trigger are highlighted **in bold**

Except for D6 (ditch) and the R1 (stream) scenarios for application to potatoes, the calculated TER values are greater than the trigger value indicating an acceptable risk to algae from the proposed use of Zoxium 240 SC in potatoes for most scenarios. All TER values for early application except R4 (stream) in grapevines are greater than the trigger value; however, except for the R1 (pond) scenario the calculated TER values are still lower than the trigger value for late application in grapevines, indicating a potential risk to algae from the proposed worst-case use of Zoxium 240 SC in grapevines.

The initial refinement is to apply a 10 m buffer zone or a 10 m buffer zone with a 10 m vegetative strip as a risk mitigation measure depending on the main route of entry into water body for each scenario (spray drift or run-off). Calculation of the subsequent TER values from the use of the initial FOCUS Step 4 PEC_{sw} for the failing scenarios are summarised in Table B.9.4.5-4.

Table B.9.4.5-4: TER values for algae (based on E_rC_{50} toxicity endpoint) from the proposed worst-case of Zoxium 240 SC using maximum FOCUS Step 4 PEC_{sw} values

Scenario	Maximum Step 4 PEC_{sw} (µg a.s./L)		Endpoint (µg a.s./L)	TER	Trigger value
	10 m *	10 m **			
Potatoes:					
R1 Stream	-	1.028	18	17.5	10
Grapevines (early application):					
R4 Stream	0.674	0.305	18	27	10
Grapevines (late application):					
D6 Ditch	0.776	-	18	23.2	10
R1 Stream	0.597	-		30.1	
R2 Stream	0.801	-		22.5	
R3 Stream	1.579† (10 m drift)			11.4	
R4 Stream	-	0.950		19	

10 m *: Mitigation (spray drift): 10 m buffer zone
10 m **: Mitigation (spray drift and run-off): 10 m buffer zone and 10 m vegetative strip

All calculated TER values are greater than the trigger value indicating an acceptable risk to algae from the proposed uses of Zoxium 240 SC in both potatoes and grapevines when risk mitigation measures of a 10 m buffer with a 10 m vegetative strip are implemented.

TER values for algae following applications of Zoxium 240 SC to potatoes and grapevines have been determined using the maximum initial FOCUS Step 1 PEC_{sw} (see RAR Part B8 point 8.5 for details) and the acute toxicity endpoints for the metabolites of zoxamide RH-127450, RH-24549, RH-141455 and RH-163353. Results are listed in Table B.9.4.5-5.

Table B.9.4.5-5: TER values for algae from the proposed worst-case of Zoxium 240 SC using maximum initial FOCUS Step 1 PEC_{sw} values for surface water metabolites of zoxamide

Crops	Maximum initial Step 1 PEC_{sw} (µg a.s./L)	Endpoint (µg a.s./L)	TER	Trigger value
RH-127450				
Potatoes	24.4	>2800	>115	100
Grapevines	30.0		>**93.3**	100
RH-24549				
Potatoes	50.8	>21000	>413	100
Grapevines	53.0		>396	100
RH-141455				
Potatoes	17.3	>21000	>1214	100
Grapevines	18.5		>1135	100
RH-163353				
Potatoes	44.6	>23000	>516	100
Grapevines	52.1		>441	100

TER values below the trigger are highlighted **in bold**

The calculated TER values for RH-127450 (only for potatoes) RH-24549, RH-141455 and RH-163353 are greater than the trigger value indicating an acceptable risk from the proposed uses of Zoxium 240 SC. However, the TER value for RH-127450 (for grapevines) is lower than the trigger value indicating a potential concern. For this scenario the TER value for algae has been calculated using maximum FOCUS Step 2 PEC_{sw} value (see Table B.9.4.5-6).

Table B.9.4.5-6: TER value for algae from the proposed worst-case use of Zoxium 240 SC using maximum initial FOCUS Step 2 PECsw values surface water metabolites of zoxamide

Crops	Maximum initial Step 2 PEC_{sw} (µg a.s./L)	Endpoint (µg a.s./L)	TER	Trigger value
RH-127450				
Grapevines	1.59	>2800	>17610	100

The calculated TER value for RH-127450 greater than the trigger value indicating an acceptable risk from the proposed uses of Zoxium 240 SC in grapevines.

B.9.4.6 Toxicity exposure ratios (TER) for *Lemna*

TER values following applications of Zoxium 240 SC to potatoes and grapevines for *Lemna* have been determined using the maximum initial FOCUS Step 3 PEC_{sw} from either a single or multiple application (see RAR Document B8, Point 8.5 for details) and the acute toxicity endpoint for the active substance. Results are listed in Table B.9.4.6-1.

Table B.9.4.6-1: TER values for *Lemna* from the proposed worst-case uses of Zoxium 240 SC using the maximum initial Step 3 PEC_{sw} values

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Endpoint (µg a.s./L)	TER	Trigger value
Potatoes:					
D3 Ditch	Drift	0.943[1]	17	18.0	10
D4 Pond	Drift	0.047[2]		362	
D4 Stream	Drift	0.709[1]		24.0	
D6 Ditch (1st)	Drift	0.937[1]		18.1	
D6 Ditch (2nd)	Drainage	1.370 [2]		12.4	
R1 Pond	Run-off	0.534[2]		32	
R1 Stream	Drift	2.415[2]		**7.04**	
R2 Stream	Drift	0.877[1]		19.4	
R3 Stream	Run-off	0.961[2]		17.7	
Grapevines (early application):					
D6 Ditch	Drift	1.471[2]	17	11.6	10
R1 Pond	Drift	0.126[2]		135	
R1 Stream	Drift	1.666[2]		10.2	
R2 Stream	Drift	0.984[1]		17.3	
R3 Stream	Drift	1.043[1]		16.3	

Scenario	Main route of entry into water body at Step 3	Maximum initial Step 3 PEC_{sw} (µg a.s./L)	Endpoint (µg a.s./L)	TER	Trigger value
R4 Stream	Run-off	3.145[2]		**5.40**	
Grapevines (late application):					
D6 Ditch	Drift	3.546[2]	17	**4.8**	10
R1 Pond	Drift	0.308[2]		55.2	
R1 Stream	Drift	2.264[1]		**7.51**	
R2 Stream	Drift	3.034[1]		**5.60**	
R3 Stream	Drift	3.190[1]		**5.33**	
R4 Stream	Drift	2.263[1]		**7.51**	

[1] Calculated for a single application
[2] Calculated for multiple applications
‡ No mitigation can be performed for drainage within the programme
TER values below the trigger are highlighted **in bold**

Except for the R1 (stream) scenario for application to potatoes, the calculated TER values are greater than the trigger value indicating an acceptable risk to *Lemna* from the proposed use of Zoxium 240 SC in potatoes. All TER values for early application in grapevines except R4 (stream) are greater than the trigger value; however, except for the R1 (pond) scenario the calculated TER values are lower than the trigger value for late application in grapevines, indicating a potential risk to *Lemna* from the proposed worst-case use of Zoxium 240 SC in grapevines.

The initial refinement is to apply a 10 m buffer zone or a 10 m buffer zone with a 10 m vegetative strip as a risk mitigation measure depending on the main route of entry into the water body for each scenario (spray drift or run-off). Calculation of the subsequent TER values from the use of initial FOCUS Step 4 PEC_{sw} for the failing scenarios are summarised in Table B.9.4.6-2.

Table B.9.4.6-2: TER values for *Lemna* the proposed worst-case uses of Zoxium 240 SC using the maximum FOCUS Step 4 PEC_{sw} values

Scenario	Maximum Step 4 PEC_{sw} (µg a.s./L)		Endpoint (µg a.s./L)	TER	Trigger value
	10 m*	10 m**			
Potatoes:					
R1 Stream	-	1.028	17	16.5	10
Grapevines (early application):					
R4 Stream	0.674	0.305	17	25.2	10
Grapevines (late application):					
D6 Ditch	0.776	-	17	21.9	10
R1 Stream	0.597	-		28.5	
R2 Stream	0.801	-		21.2	
R3 Stream	1.579	-		10.8	
R4 Stream	-	0.950		17.9	

10 m *: Mitigation (spray drift): 10 m buffer zone
10 m **: Mitigation (spray drift and run-off): 10 m buffer zone and 10 m vegetative strip

The calculated TER values are greater than the trigger value indicating an acceptable risk to *Lemna* from the proposed uses of Zoxium 240 SC in both potatoes and grapevines when risk mitigation measures of a 10 m buffer with a 10 m vegetative strip are implemented.

RMS conclusion:
The acute and long-term risk of zoxamide is acceptable for aquatic organisms following the intended uses of Zoxium 240 SC in potatoes and in grapes using 20 m buffer zone.

B.9.5 EFFECTS ON ARTHROPODS

B.9.5.1 Effects on bees

Report:	**KCP, 10.3.1.1.1/01 Engelhard, E.K. (1998a)** **RH-117,281 2F (240SC): Laboratory oral and contact test with the honeybee, *Apis mellifera*.**

Guidelines: US EPA OPPTS 850.3020/FIFRA Guideline 141-1; EPPO 170
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i., TD No. 97-018)
Test species: honeybees (*Apis mellifera L.*)
Number of organism, ages: oral and contact test – 3 replicates for each treatment, 10 bees per replicate, young adults, 4-14 days old
Type of test: acute oral and contact toxicity test (72 hours)
Mean nominal test substance doses:
Oral test: 147 µg formulation per bee
Contact test: 200 µg formulation per bee
Test conditions :
temperature range from 25.0 to 26.5 ^{0}C
relative humidity range from 56 to 72%
photoperiod was 16 hours light and 8 hours dark

Results:

The 72-hour LD50 value for the oral toxicity test was estimated to be above 147 µg RH-117,281 2F (240 SC)/bee, since the test substance dose tested resulted in a mortality of less than 50%.

The 72-hour NOEC for oral toxicity test was determined as 147 µg RH-117,281 2F (240 SC)/bee since no significant effects on bee mortality or behaviour were observed. The oral 72-hour LOEC could not be established, as no significant difference between the control and the test substance treatment was observed.

The 72-hour LD50 value for the contact toxicity test with honeybees was estimated to be above 200 µg RH-117,281 2F (240 SC)/bee, since the test substance dose resulted in a mortality of less than 50%.

The 72-hour NOEC for the oral toxicity test was determined to be 200 µg RH-117,281 2F (240 SC)/bee, since this dose did not have any significant effects on bee mortality or behaviour. The contact 72-hour LOEC could not be established.

Table 9.5.1-1: Mean percent mortality and mean percent affected bees recorded during the 72-hour oral test with honeybees (*Apis mellifera*) exposed to RH-117,281 2F (240 SC)

Dose	Treatment	% Mortality	% Affected (Sublethal effects)

(µg/bee)[a]		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0	Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
100 – 200 (147[c])	TS[b]	0 ± 0	3.3 ± 5.8	10.0 ± 10.0	0 ± 0	3.3 ± 5.8	0 ± 0
0.14 – 0.16 (0.15[d])	Toxic standard	10.0 ± 17.3	16.7 ± 15.3	56.7 ± 20.8	13.3 ± 11.5	26.7 ± 25.2	6.7 ± 5.8

[a] test substance in µg RH-117,281 2F (240 SC)/bee and Toxic Standard in µg Dimethoate/bee.

[b] TS = Test substance.

[c] Mean dose consumed (µg formulation/bee). The definitive oral exposure study was run at a target dose of 200 µg formulation/bee. Not all of the test substance dose was consumed (the bees within the replicates consumed 100 to 200 µg formulation/bee), however, so the actual mean nominal oral dose consumed was calculated as 147 µg formulation/bee.

[d] Mean dose consumed (µg formulation/bee). The definitive oral exposure study was run at a target dose of 0.18 µg dimethoate/bee. Not all of the toxic standard dose was consumed (the bees within the replicates consumed 0.14 to 0.16 µg dimethoate/bee), however, so the actual mean nominal oral dose was calculated as 0.15 µg dimethoate/bee.

Note: No statistically significant difference between the control and the test substance treatment level (Fisher Exact test, $p \leq 0.05$; only data from 72 hours were used for the statistical analysis).

Table 9.5.1-2: Mean percent mortality and mean percent affected bees recorded during the 72-hour Contact test with honeybees (*Apis mellifera*) exposed to RH-117,281 2F (240 SC)

Dose (µg/bee)[a]	Treatment	% Mortality			% Affected (Sublethal effects)		
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0	Control	0 ± 0	0 ± 0	3.3 ± 5.8	0 ± 0	0 ± 0	0 ± 0
200	TS[b]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.16	Toxic standard	83.3 ± 5.8	83.3 ± 5.8	83.3 ± 5.8	6.7 ± 5.8	10.0 ± 0.0	10.0 ± 0.0

[a] test substance in µg RH-117,281 2F (240 SC)/bee and Toxic Standard in µg Dimethoate/bee.

[b] TS = Test substance.

Acute oral, 48 and 72 hr LD_{50} >147 µg form./bee (NOEC = 147 µg form./bee).

Acute contact, 48 and 72 hr LD_{50} >200 µg form./bee (NOEC = 200 µg form./bee).

RMS comments:
Tests were carried out in accordance with GLP and study is considered acceptable.

Report:	**KCP, 10.3.1.2/01 Schmitzer, S. and Ehmke, A. (2014)**
	Chronic oral toxicity test of Zoxium 240 SC on the honey bee (*Apis mellifera*) in the laboratory.

Project no.: 80052136
Guidelines: OECD 213; CEB 230 (with modifications)
Deviations: None
GLP: Yes
Previous evaluation: No, submitted for the purpose of new data requirement

Executive Summary

The chronic oral toxicity of the honey bee exposed to the test item Zoxium 240 SC (a formulated product containing the active substance zoxamide) for a period of ten days was determined in a laboratory study. The chronic oral feeding test was conducted by daily administration of the test item to bees in sugar solution at a concentration of 5000 mg a.s./kg feeding solution. This concentration led to a daily mean dose of 174.8 µg a.s./bee/day after 10 days. Fifty freshly emerged worker bees (ten per replicate) were exposed to the test concentration and the control (50% aqueous sugar solution). A treatment with a reference item was also included in this study

Daily assessment of mortality and behavioural abnormalities was performed up to day ten.

No mortality occurred after daily exposure of 5000 mg a.s./kg feeding solution (corresponding to 174.8 µg a.s./bee/day) for ten days following the start of chronic exposure. There was 2.0% mortality in the control group at test end. There were no test item induced behavioural abnormalities at any time during the experiment.

The LC_{50} value (10 days) for honey bees exposed to Zoxium 240 SC was >5000 mg a.s./kg feeding solution corresponding to an LD_{50} value (10 days) of >174.8 µg a.s./bee/day. The NOEC and NOED (10 days) values were 5000 mg a.s./kg feeding solution and 174.8 µg a.s./bee/day, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	Zoxium 240 SC
	Batch no.:	OD0601
	Active ingredient/ content:	Zoxamide 240 g/L (nominal), 241.5 g/L (analytical)
	Description:	Suspension concentrate
2.	**Reference item:**	Perfekthion EC (BAS 152 11 I)
	Active ingredient/ content:	Dimethoate 400 g/L (nominal), 411.7 g/L (analytical)
	Description:	Blue liquid
3.	**Test organism:**	*Apis mellifera carnica* L.
	Stage and sex:	Freshly emerged (< 24 hours old) adult female worker bees
	Source:	Honey bee colonies, disease-free and queen-right, bred by IBACON
	Diet:	Fed continuously a*d libitum* with a 50% aqueous sugar solution made out of water and commercial ready-to-use syrup (Apiinvert; sugar content: 30% sucrose, 31% glucose, 39% fructose).
4.	**Treatment:**	0 (sugar solution) and 5000 mg a.s./kg feeding solution (the nominal dose per was 200 µg a.s./bee/day taking into account a mean uptake of feeding solution of 40 mg/bee/day; the actual dose 175.8 µg a.s./bee/day was calculated after the determination of food uptake by the bees at test end)
5.	**Test units:**	Stainless steel chambers 10 cm x 8.5 cm x 5.5 cm with a removable glass sheet and perforated bottom with 98 holes (1 mm diameter)
6.	**Environmental conditions:**	
	Temperature:	32 - 34°C
	Relative humidity:	39 – 79%
	Photoperiod:	Darkness (except during observation)

B. STUDY DESIGN AND METHODS

1. In-life phase: Aug 06 to 16, 2013

2. Test organism assignment and treatment

Freshly hatched bees remained for a day in an excluder box after collection from two brood combs. Then, the freshly emerged worker bees were taken out from the excluder box with forceps and were transferred to the test units. Zoxium 240 SC was tested as a limit dose of 5000 mg a.s./feeding solution resulting into an actual uptake of 174.8 µg a.s./bee/day. Additionally, honeybees were treated with Perfekthion EC (dimethoate) as the toxic reference at a concentration of 1 mg dimethoate/kg feeding solution (an actual uptake concentration of 0.027 µg a.s./bee/day) and with a 50% aqueous sugar solution as a control. Each test group had 5 replicates, which consisted of 10 bees in one cage. The treated and untreated food was offered *ad libitum* to each cage in syringes.

3. Dose preparation

Aqueous solutions of the test and reference item were prepared in such a way that they had the respective target concentration of the test item once they were subsequently mixed with sugar syrup at a ratio of 1 : 1. The concentration was calculated taking into account the nominal content of the a.s. in the product 240 g a.s./L and a density of 1.110 g/cm³. After mixing of these aqueous test solutions with ready-to-use sugar syrup the final concentration of sugar syrup in the test item solutions offered to the bees was 50 %. These final test solutions (test item solution in syrup) as well as the control and reference item solutions were prepared once for the entire time of the experiment (10 days), directly before start of the experiment and were kept cool in a refrigerator at 4°C ± 4°C in the dark. The treated and untreated food was replaced with fresh treated or untreated food every day.

4. Measurements and observations

The number of dead bees was assessed daily (±4 hours) until test end, ten days following start of exposure. Dead bees were removed from the test units on each assessment day.

Behavioural abnormalities were also assessed daily (±4 hours) until test end. The sub-lethal effects were categorised to as: food refusal/vomiting, affected (e.g. discoordinated movements), apathy, intensive cleaning and nervous.

Food uptake was recorded daily. The syringes were weighed daily before introduction into the cages and after the feeding interval (before replacement with fresh food). This difference of weight (amount of food) was divided by the number of living bees at the start of the corresponding exposure. The mean food consumption per bee per day over the whole testing period was calculated by summing up the daily food consumption and dividing by 10 (days).

5. Statistics

The NOEC/NOED of the test item was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. Mortality and behavioural abnormalities

No mortality occurred after daily exposure of 5000 mg a.s./kg (corresponding to 174.8 µg a.s./bee per day) 10 days following the start of chronic exposure. There was 2.0 % mortality in the control group (50 % aqueous sugar syrup solution) at test end, 10 days following start of exposure. There were no test item induced behavioural abnormalities at any time during the experiment.

The reference item (dimethoate) at a concentration of 1 ppm (1 mg dimethoate/kg feeding solution) corresponding to 0.027 µg a.s./bee per day caused 100 % mortality at day 10.

Table B.9.5.1-3: Mortality and behavioural abnormalities of the bees in the chronic oral toxicity test

		Test item (5000 mg a.s./kg equivalent to 174.8 µg a.s./bee/day*)	Reference item (1 mg a.s./kg equivalent to 0.027 µg a.s./bee/day*)	Untreated control
Day 1	**Mortality (mean %)**	0.0	0.0	0.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 2	**Mortality (mean %)**	0.0	0.0	0.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 3	**Mortality (mean %)**	0.0	2.0	0.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 4	**Mortality (mean %)**	0.0	10.0	0.0
	Behav. abnorm. (%)	0.0	2.0	0.0
Day 5	**Mortality (mean %)**	0.0	32.0	0.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 6	**Mortality (mean %)**	0.0	88.0	2.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 7	**Mortality (mean %)**	0.0	98.0	2.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 8	**Mortality (mean %)**	0.0	100.0	2.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 9	**Mortality (mean %)**	0.0	100.0	2.0
	Behav. abnorm. (%)	0.0	0.0	0.0
Day 10	**Mortality (mean %)**	0.0	100.0	2.0
	Behav. abnorm. (%)	0.0	0.0	0.0

Results are averages from five replicates (ten bees each) per concentration or untreated control
Behav. abnorm. = Behavioural abnormalities
*at test end, 10 days following application

B. Toxicity endpoints

The chronic oral toxicity endpoints of Zoxium 240 SC are provided in Table B.9.5.1-4 below.

Table B.9.5.1-4: Chronic oral toxicity of Zoxium 240 SC to young honey bees

Test item	Zoxium 240 SC	
Test organism	*Apis mellifera*	
Exposure	Oral 10 days chronic exposure *via* 50% aqueous sugar solution	
Application rate	Concentration (mg a.s./kg feeding solution	Dose (µg a.s./bee/day
	5000	174.8
Endpoints (day 10)*	LC_{50} >5000	LD_{50} >174.8
	NOEC >5000	NOED >174.8

III. CONCLUSION

In a chronic oral toxicity test to honey bees with Zoxium 240 SC, the LC_{50} value (10 days) for honey bees exposed to Zoxium 240 SC was > 5000 mg a.s./kg feeding solution corresponding to an LD_{50} value (10 days) of > 174.8 µg a.s./bee/day. The NOEC and NOED (10 days) values were 5000 mg a.s./kg feeding solution and 174.8 µg a.s./bee/day, respectively.

RMS comments:
The study is considered acceptable.

Report:	**KCP, 10.3.1.3/01 Schmitzer, S. (2014)**
	Effects of Zoxium 240 SC on honey bee brood (*Apis mellifera* L.) – Brood Feeding Test-

Project no.: 80051031
Guidelines: Oomen et al. 1992
Deviations: None
GLP: Yes
Previous evaluation: No, submitted for the purpose of new data requirement

Executive Summary
A bee brood test was conducted, in order to assess the effect of Zoxium 240 SC (a formulated product containing the active substance zoxamide) to the honey bee brood in natural field conditions. Two rates of Zoxium 240 SC were tested, 0.833 g/L sugar solution and 3.47 g/L sugar solution, equivalent to 0.18 g a.s./L sugar solution and 0.75 g a.s./L sugar solution, respectively. An untreated control (pure sugar syrup (Apiinvert)) and a toxic reference (Insegar, 0.75 g fenoxycarb/L sugar solution) were included in the study. Three bee colonies were used per treatment group. The test item (2 treatments) and reference item solutions were mixed with ready-to-use sugar syrup (Apiinvert) and applied to the bee colonies via a feeding trough, which was put directly into the colony on top of the second magazine.

Ontogenesis of a defined number of honey bee eggs, young and old larvae was observed for a period of 21 days (one day before application, and 4, 8, 15 and 21 days after application) for each treatment group and colony, the results were presented as the measured mean termination rates of eggs, young larvae and old larvae. Mortality of adult bees and pupae was also assessed.

The mean termination rates of the eggs in the both Zoxium 240 SC rates were not statistically significantly different compared to the control value. The development success of the young larvae and the mean termination rates of old larvae in both test item treatment groups were not statistically significantly different compared to the control values. Adult bee mortality in both test item treatment groups was lower and thus not statistically significantly different when compared to the control group.

Overall, it was concluded according to the results of this study that the administration of Zoxium 240 SC fortified sugar syrup at both rates (0.833 and 3.47 g/L Zoxium 240 SC, corresponding to 0.18 and 0.75 g a.s./L) to honey bee colonies does not adversely affect honey bee colonies or bee brood development.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	Zoxium 240 SC
	Batch no.:	OD0601
	Active ingredient/ content:	Zoxamide 240 g/L (nominal), 241.5 g/L (analytical)
	Description:	Suspension concentrate
2.	**Reference item:**	Insegar
	Active ingredient/ content:	Fenoxycarb 250 g/kg (nominal), 257 g/L (analytical)
	Description:	Solid grey to brown
3.	**Test organism:**	*Apis mellifera carnica* L.
	Stage:	All ages and all stages
	Source:	Honey bee colonies, maintained by IBACON's responsible beekeeper
	Diet:	Natural food and water sources. No additional food was provided during the course of the study.
4.	**Treatment:**	0.833 g of the formulated product Zoxium 240 SC/L ready-to-use sugar syrup corresponding to 0.18 g a.s./L and 3.47 g of the formulated product Zoxium 240 SC/L ready-to-use sugar syrup corresponding to 0.75 g a.s./L. Positive control: 3.0 g reference item (Insegar; 25 % fenoxycarb)/L ready-to-use sugar syrup equivalent to a nominal active substance concentration of 0.75 g fenoxycarb/L Negative control: Ready–to-use sugar syrup.
5.	**Test colonies:**	Colonies were well fed and queen-right, each colony occupied two magazines with 11 frames each. At the start of the experiment, each colony had 7 - 16 brood combs containing eggs, larvae and capped cells and a sufficient amount of honey and pollen. The colonies were assembled at the same time with healthy queens in order to guarantee uniform bee material in all treatments. 1 year old sister queens were used. The colonies contained about 10035 - 15030 adult honey bees.
6.	**Climatic conditions:**	The experimental phase of this study took place at a settled, constant weather period with sunny and warm days. Mean temperatures over the course of the study ranged from 19.7 °C to 28.6 °C. Rain occurred only on a few occasions.

B. STUDY DESIGN AND METHODS

1. In-life phase: Jul 15 to Aug 08, 2013

2. Study design

Colonies were set up 7 days before application at the same location. The bee colonies were placed in a meadow in order to enable the bees to get familiar with the new environment and to lower the set-up related mortality to a normal extent. Bees had free access to natural food sources. Due to the season, there was no bee attractive crops or flowering weeds in the surrounding area. The bee colonies remained at the test site until the end of the trial.

Three bee colonies were used per treatment group. 1 L contaminated, test item at 2 rates (0.18 g a.s./L and 0.75 g a.s./L) and reference item (0.75 g fenoxycarb/L), or untreated commercial ready-to-use sugar syrup (Apiinvert) per colony were offered per colony in a feeding trough (for feeding bees according to routine bee keeping practice). This procedure was suitable to feed each colony. The trough was put into an empty magazine on top of the populated bee magazines. One single application per colony was performed during the afternoon in order to prevent robbery. The bees had free access to the feeding trough. Feeding was started during the afternoon (13:40 - to 14:50 h). The feeding troughs and empty magazines were removed after complete uptake of the feeding solutions. Thereafter, the exact uptake of food was determined by reweighing the feeder . 25 hours after application food uptake was complete in the colonies of the control and the 0.18 g a.s./L test item treated group. In the 0.75 g a.s./L test item and reference item treated colonies food uptake lasted up to 46 hours.

3. Dose preparation

For the preparation of the low test item rate corresponding to 0.18 g zoxamide/L, 0.833 g of the formulated product Zoxium 240 SC was dissolved in 1 L ready-to-use sugar syrup (Apiinvert). For the preparation of the high test item rate corresponding to 0.75 g zoxamide/L, 3.47 g of the formulated product Zoxium 240 SC was dissolved in 1 L ready-to-use sugar syrup (Apiinvert). Apiinvert is a ready-to-use sugar syrup, the sugar content of the sugar syrup is made up by 30 % sucrose, 31 % glucose and 39 % fructose.

3.0 g reference item (Insegar; 25 % fenoxycarb) was dissolved in 1 L ready-to-use sugar syrup (Apiinvert) per colony, equivalent to a nominal active substance concentration of 0.75 g fenoxycarb/L.

4. Measurements and observations

Mortality

To evaluate bee mortality, dead bees were collected from dead-bee traps placed in front of each colony. Dead bees, removed from the colonies by worker bees, were dropped in the trap as they try to fly through the mesh cover. The collected dead bees were separated during counting into adult worker bees, larvae and pupae. Inspection intervals were carried once per day from day -3 to day 21 after application.

Behavioural abnormalities

Behavioural abnormalities of the bees at the colony entrance *e.g.* intensive cleaning, restlessness or moving coordination problems were recorded. Observations were conducted during the assessments of mortality.

Brood development

The honey bee brood was assessed at different expected stages during the development, covering one complete development period of the honey bee (*i.e.* one complete honey bee brood cycle, 21 days). The development of the bee brood in individually marked cells was observed by photo-graphing the combs. At the assessment before the application (=BFD0; Brood Area Fixing Day), one brood comb with an appropriate amount of eggs, young- and old larvae was selected from of each colony under investigation and a digital photo of this brood comb was taken, respectively. The comb was labelled with two pins, which served as a hallmark for orientation and retrieval of marked positions on the comb. After saving each photo-file on a computer, 150 cells containing eggs, 140 - 150 cells with young larvae and 150 cells with old larvae were selected, automatically numbered and marked by using an image analysis program (ImageJ). For each subsequent brood assessment (BFDn), the same comb per individual colony was selected, and another digital photo was taken and the photo-file was saved, respectively. For each photo, the orientation points were marked again, in order to allow for an automated allocation of the previous marked cells. After retrieval of the cells for each assessment date, the cell content was assigned. This allowed a continuous photo-documentation, starting with the first brood fixing date (BFD0) and continuing until the end of the assessment period. After completion of the cell assessments, a gallery of the cells was automatically generated. Therefore, the development of each individually marked cell throughout the duration of the test could be determined.
The different brood stages on the assessment dates were transcribed into indices (*e.g.* 0=empty; 1=egg; 2=young larvae, 3=old larvae; 4=pupa; 5=nectar; 6=pollen; 7=dead; 8=not classified) to calculate the termination rate. If not enough development stages were found on one side of the selected comb per individual colony, the brood on the second side of this comb or an additional comb was selected, photographed and inspected accordingly. In most treatment groups and replicates, 150 cells were marked.

Assessment of the development took place according to the following scheme:

- For cells that contained eggs (1) on BFD0 the expected brood stages were young (2) to old larvae (3) or capped cells (4) +5 days after BFD0, capped cells (4) +9 days after BFD0, capped cells (4) shortly before hatch +16 days after BFD0 and empty or eggs, young larvae or food +22 days after BFD0.
- For cells that contained young larvae (2) on BFD0 the expected brood stages were old larvae (3) or capped cells (4) +5 days after BFD0, capped cells (4) +9 days after BFD0, capped cells (4) or empty cells, or cells with eggs, larvae or food +16 days after BFD0 and all development stages, empty or food +22 days after BFD0.
- For cells that contained old larvae (2) on BFD0 the expected contents brood stages were capped cells (4) +5 days after BFD0, capped cells (4) +9 days after BFD0, empty cells or cells containing eggs, young larvae or food +16 days after BFD0; assessment was not necessary +22 days after BFD0.

5. Statistics

The data were tested for normal distribution using Shapiro-Wilk’s test and homogeneity of variance using Levene’s test. A pairwise comparison (α = 0.05) was conducted for the mortality data (two-sided before application and one-sided greater, after application) using Student t-test for homogeneous variances. A pairwise comparison (one-sided greater, α = 0.05) was con-ducted for the comparison of the brood data (egg and larvae termination rates), using Student t-test for homogenous variances. The software used to perform the statistical analysis was Tox-Rat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. Mortality and behavioural abnormalities

Following both treatments with Zoxium 240 SC, no direct (acute) toxicity occurred after ingestion of the test item treated sugar syrup. There was no clear increased mortality level in the test item treated replicates at any time of the test (on days 17 to 19 increased mortality levels were found in one of the three test item treated colonies of the higher rate, which was explainable by robbery [robbery = bees are fighting at the entrance hole of the hives, because bees try to “steal” honey from other hives]). Over the entire post-application period from day 0 to day 21, a mean of 7.5 dead bees/colony/day was found in the dead bee traps of the lower rate test item treated colonies (0.18 g a.s./L). A mean of 16.8 dead bees/colony/day was found in the dead bee traps of the higher rate test item treated colonies (0.75 g a.s./L) for the same period. This was in both cases lower compared to a mean of 21.2 dead bees per colony/day, which was found in the control group. A comparison of the overall mean number of dead bees per treatment group for the entire post-application period (day 0 to day 21) did not show a statistically significant difference between the control and both test item treatments (Student t-test, pair-wise comparison to the control, one-sided greater, α = 0.05).

Treatment with the reference item Insegar (0.75 g a.s./L fenoxycarb) resulted during the post-application period (day 0 to day 21) in a slightly increased number of dead bees (25.4 dead bees per day per colony). This slightly increased number of dead bees was not statistically significantly higher when compared to the control (Student t-test, pairwise, one-sided greater, α = 0.05).

During the entire period from day 0 following the application until day 21, a mean of 0.09 dead pupae/larvae per day and colony was found in the lower rate Zoxium 240 SC treatment group (0.18 g a.s./L, respectively). Treatment with 0.75 g a.s./L led to a mean of 0.03 dead pupae/larvae per day and colony. In the control group, during the same time period, a mean of 0.9 dead pupae/larvae per day and colony was found. There was no statistically significant difference in the number of dead pupae/larvae between the colonies of both test item groups and the colonies of the control group (Student t-test, pairwise comparison, α = 0.05, one-sided greater).

Application of the reference item Insegar (0.75 g fenoxycarb/L) did result in an increased number of dead pupae/larvae after application. During the same time period, a mean of 1.3 dead pupae/larvae per day and colony was found. This was not statistically significant compared to the value of the control colonies (Student t-test, pairwise comparison, α = 0.05, one-sided greater).

No behavioural impairments were noted at any time in any of the test or reference item treatment groups until test end. Also no behavioural abnormalities were observed in the control group.

Table 9.5.1-5 summarizes the mortality of worker bees, pupae and larvae.

Table 9.5.1-5: Summarized mortality data of worker bees, pupae and larvae

Time[a]	**Untreated control dead bees (mean[b] ± SD)**	**Zoxium 240 SC (0.18 g a.s./L) dead bees (mean[b] ± SD)**	**Zoxium 240 SC (0.75 g a.s./L) dead bees (mean[b] ± SD)**	**Reference item Insegar (fenoxycarb) dead bees (mean[b] ± SD)**
Mean day -3 to -1 b.a. [c]	20.3 ± 21.9	7.4 ± 2.0	2.0 ± 1.8	5.8 ± 1.8
Mean day 0 to 21 a.a. [d]	21.2 ± 17.0	7.5 ± 4.1 [n s]	16.8 ± 27.0 [n s]	25.4 ± 17.9 [n s]
Time	**Untreated control dead larvae/pupae (mean[b] ± SD)**	**Zoxium 240 SC (0.18 g a.s./L) dead larvae/pupae (mean[b] ± SD)**	**Zoxium 240 SC (0.75 g a.s./L) dead larvae/pupae (mean[b] ± SD)**	**Reference item Insegar (fenoxycarb) dead larvae/pupae (mean[b] ± SD)**
Mean day -3 to -1 b.a. [c]	0.9 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 2.2
Mean day 0 to 21 a.a. [d]	0.9 ± 1.8	0.09 ± 0.2 [n s]	0.03 ± 0.1 [n s]	1.3 ± 1.8 [n s]

[a] days -3 to -1 = days before application; day 0 to 21 = days after application (= day 22 after BFD0); [b] mean values (rounded) of three colonies per treatment group; [c] b.a.= before application; [d] a.a.= after application
n.s. = not statistically significant compared to the control (Student t-test, pairwise comparison, two-sided (before application); one-sided greater (after application), α = 0.05)

B. Development of the bee brood

Following the assessment of single cells from the egg stage to the successfully hatched worker bee, the mean termination rate in the lower rate test item treatment group (0.18 g a.s./L) was slightly lower compared to the control group (17.8 %) with 17.3 %. Termination rate in the higher treatment group (0.75 g a.s./L) was 26.9 %. This slight increase in mean termination rate in this test item group was not statistically significantly different (Student t-test, pairwise comparison, one-sided greater) when compared to control.

Comparing the development success of the young larvae after treatment with the test item to the corresponding control values, a very slight increase in mean termination rate in the lower test item rate (0.18 g a.s./L) was observed (termination rate test item: 10.7 % vs 10.2 % in the control group.). The higher rate (0.75 g a.s./L) caused a lower termination rate of the young larvae (6.7 %) when compared to the control group (10.2 %). When subjecting the data to statistical analysis (Student t-test, pairwise comparison, one-sided greater), the difference in both Zoxium 240 SC rates was found not to be statistically significant compared to the control.

Also no effect of the test item on old larvae was found: 2.9 % and 5.6 % of the marked old larvae in the 0.18 g a.s./L and 0.75 g a.s./L test item treated colonies have not completed their development, compared to 6.4 % in the control group, which was not statistically significantly different (Student t-test, pairwise comparison, one-sided greater).

Treatment with the reference item Insegar resulted in a statistically significant loss of brood development of the marked eggs, young- and old larvae, finally resulting in a termination rate of 100 % (eggs), 99.6 % (young larvae) and 43.8 % (old larvae) compared to the control. The termination rates of the young and old larvae were statistically significantly different when compared to the corresponding control values (Student t-test, pairwise comparison, one-sided greater).

A summary of the bee brood termination rate is presented in the following table.

Table 9.5.1-6: Bee brood termination rate

Treatment group	Eggs BFD0	22 days after BFD0 Brood terminated				Young larvae BFD0	22 days after BFD0 Brood terminated				Old larvae BFD0	16 days after BFD0 Brood terminated			
		#	%	Mean %	Stat.		#	%	Mean %	Stat.		#	%	Mean %	Stat.
Control	150 150 150	29 11 40	19.3 7.3 26.7	17.8	-	150 150 150	10 26 10	6.7 17.3 6.7	10.2	-	150 150 150	9 12 8	6.0 8.0 5.3	6.4	-
Zoxium 240 SC (0.18 a.s./L)	150 150 150	12 35 31	8.0 23.3 20.7	17.3	n.s.	150 140 150	8 28 10	5.3 20.0 6.7	10.7	n.s.	150 150 150	6 5 2	4.0 3.3 1.3	2.9	n.s.
Zoxium 240 SC (0.75 a.s./L)	150 150 150	23 32 66	15.3 21.3 44.0	26.9	n.s.	150 150 150	4 14 12	2.7 9.3 8.0	6.7	n.s.	150 150 150	12 8 5	8.0 5.3 3.3	5.6	n.s.
Reference item	150 150 150	150 150 150	100 100 100	100.0	n.d.	150 150 150	150 150 148	100 100 98.7	99.6	*	150 150 150	71 80 46	47.3 53.3 30.7	43.8	*

BFD0 = Brood Fixing Day 0; # = number of terminated cells
Stat. = statistic, Student t-test, pairwise comparison, one-sided greater, α=0.05
n.s. = not statistically significant compared to the control; *= statistically significant compared to the control; n.d. = not determined

III. CONCLUSION

In a bee brood test, under natural conditions, it was concluded that Zoxium 240 SC fortified sugar syrup at both rates (0.833 and 3.47 g/L Zoxium 240 SC, corresponding to 0.18 and 0.75 g a.s./L) to honey bee colonies does not adversely affect honey bee colonies or bee brood development.

RMS comments:
The study is considered acceptable.

B.9.5.2 Effects on non-target arthropods other than bees

Report:	**KCP, 10.3.2.1/01 Engelhard, E.K. (1998b)** **RH-117,281 2F (240SC): Laboratory acute toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae).**

Guidelines: IOBC Guideline, Polgar (1988) and Mead-Briggs (1992)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i.
Test species: parasitic wasp, *Aphidius rhopalosiphi*
Number of organisms, age: 3 replicates per treatment, each with 10 wasps for the exposure phase of the study (mortality assessment); 10 replicates per treatment, with 1 wasp each, for the reproductive phase (fecundity assessment), adult females, less than 72 hours old
Type of test: laboratory acute toxicity test using glass plate
Applied concentrations:
control (shared with Springborn stdy #1007.030.270), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 S) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha), and a toxic standard treatment (Perfekthion, applied at 0.85 µL/L, shared with Springborn study #1007.030.270)
Exposure route: 48-hour test
Test conditions:
temperature: 16.5 to 22.0 °C
relative humidity: 70 to 85%
light regime: 16:8 hours light:dark, light intensity: 850 to 1050 lux, 2250 lux during the fecundity phase

Results:
During two-phase laboratory study, adult *Aphidius rhopalosiphi* survival and behaviour was established during a period of 48 hours of exposure to the test substance. The surviving wasps were allowed to parasitive aphids over 24 hours and after 10 ± 2 days their fecundity (or parasitization efficiency) was assessed. Mortality and parasitization rates were used to calculate the effect of the test substance on the beneficial capacity (E-value) under worst-case conditions. The E-values forms the criterion to assess the hazard according to the IOBC hazard rating scheme.

During the exposure phase of two-phase test, four treatments were established: a control (shared with Springborn stdy #1007.030.270), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 S) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha), and a toxic standard treatment (Perfekthion, applied at 0.85 µL/L, shared with Springborn study #1007.030.270). The test substance concentrations were chosen based on 100% of the recommended use rates in arable crops. It is important to note, the test substance will also be used in orchard and vineyard crops for which the ESCORT guideline (1994) recommends testing 40% of the recommended use rate. Therefore, the test substance concentrations used in this study represent 2.5x and 5x of expected exposure in orchards and vineyards. All treatments were replicated 3 times with each replicate consisting of 10 female wasps (total 30 wasps per treatment). During the fecundity phase of the test, 10 female wasps randomly selected from each treatment were used.

The cumulative mortality after 48 hours of exposure was 0%, 0%, 0% and 100% for the control, RH-117,281 2F (240 SC) 1x, RH-117,281 2F (240 S) 2x, and a toxic standard (Perfekthion) treatment. The mortality of the control and the toxic standard treatment was within the expected range.

The parasitization rate between the control and both test substance concentrations were not statistically significant. Fecundity for the control, RH-117,281 2F (240 SC) 1x and RH-117,281 2F (240 S) 2x treatments was 21.2, 19.6 and 13.6 mummies per survival female. No fecundity data for the toxic standard treatment was recorded due to 100% mortality of the wasps during the exposure phase of the study.

Based on E-value (7.8%), the RH-117,281 2F (240 SC) applied at 1x (2.5x for orchard and vineyard crops) of the proposed maximum labelled application rate (625 mL formulation/ha, corresponding to 150 g a.i./ha) is classified according to the IOBC as “harmless” to *Aphidius rhopalosiphi* under worst-case laboratory conditions.

Based on E-value (36.1%), the RH-117,281 2F (240 SC) applied at 2x (5x for orchard and vineyard crops) of the proposed maximum labelled application rate (1250 mL formulation/ha, corresponding to 300 g a.i./ha) is classified according to the IOBC as “slightly harmful” to *Aphidius rhopalosiphi* under worst-case laboratory conditions.

The formulation RH-117,281 2F (240 SC) is slightly harmful to *Aphidius rhopalosiphi* at the application rate of 300 g a.i./ha.

RMS comments:
LR_{50}: >300 g a.s./ha
ER_{50} (reproduction): > 300 g a.s./ha
Study is considered acceptable.

Report:	**KCP, 10.3.2.1/02 Engelhard, E.K. (1998c)** **RH-117,281 2F (240SC): Laboratory toxicity test with the predacious mite, *Typhlodromus pyr*i Scheuten (Acari: Phytoseiidae).**

Guidelines: IOBC Guideline, Overmeer (1998)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i.
Test species: predacious mite *Typhlodromus pyr*i
Number of organisms, age: 5 replicates per treatment, each containing 20 protonymhs, lifestage: protonymph to adult
Type of test: laboratory toxicity test using glass plate
Applied concentrations:
control (shared with Springborn study #1007.030.268), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 SC) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha) and toxic Standard treatment (Ethyl Parathion 500 g/L EC, applied at 0.036%, shared with Springborn study #1007.030.268)
Exposure route: 14 days laboratory test
Test conditions:
temperature: 24.0 to 26.5 °C
relative humidity: 70 to 85%
light regime: 16:8 hours light:dark, light intensity: 850 to 1050 lux

Results:

Four treatments were established for this study, a control (shared with Springborn study #1007.030.268), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 SC) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha) and toxic Standard treatment (Ethyl Parathion 500 g/L EC, applied at 0.036%, shared with Springborn study #1007.030.268). The test substance concentrations were chosen based on 100% of the recommended use rates in arable crops. It is important to note, the test substance will also be used in orchard and vineyard crops for which the ESCORT guideline (1994) recommends testing 40% of the recommended use rate. Therefore, the test substance concentrations used in this study represent 2.5x and 5x of expected exposure in orchards and vineyards. Five replicates per treatment (20 protonymphs per replicate) were set up. Mortality was recorded on test Days 3, 7, 10 and 14. Assessment of egg production was performed on Days 7, 10 and 14.

At test termination (Day 14), 8.0%, 23.0%, 29.0% and 83% mortality was observed in the control, the RH-117,281 2F (240 SC) 1x, the RH-117,281 2F (240 SC) 2x, and the toxic Standard treatment. The differences in per cent cumulative mortality between the control and RH-117,281 2F (240 SC) 1x and the control the RH-117,281 2F (240 SC) 2x treatment were statistically significant on Day 7, 10 and 14. The mortality observed among mites exposed to the control and the toxic standard was within the range expected for these treatments. No significant difference in per cent missing mites between the control and RH-117,281 2F (240 SC) 1x or RH-117,281 2F (240 SC) 2x treatment was observed. At test termination, only the RH-117,281 2F (240 SC) 2x treatment had missing mites (2%).

The females treated with RH-117,281 2F (240 SC) 1x produced significantly less eggs per female per day when compared to the control on Days 7, 10, and14. Average egg laying during the entire study for the RH-117,281 2F (240 SC) 1x treatment was significantly lower when compared to the control. The mean number of eggs laid per female per day for the entire study was 1.23, 0.57, 0.91, and 0 for the control, the RH-117,281 2F (240SC) 1x, the RH-117,281 2F (240 SC) 2x and the toxic standard treatment. The number of eggs laid in the control treatment was within the range expected for this treatment.

The effect of RH-117,281 2F (240 SC) applied at 1x (2.5x for orchard or vineyard crops) of the maximum application rate on *Typhlodromus pyr*i under worst-case laboratory conditions is classified as „slightly harmful" according to the IOBC hazard assessment scheme (Hassan, 1992). However, the effect of RH-117,281 2F (240 SC) applied at 2x (5x for orchard and vineyard crops) of the maximum application rate on *Typhlodromus pyr*i under worst-case laboratory conditions is classified as "harmless". This classification difference at two different treatment rates is due to the significant reduction in eggs produced per female in the 1x treatment, but not in the 2x treatment. Since no effect on reproduction was observed at the higher RH-117,281 2F (240 SC) 2x treatment and no dose-response relationship could be established, the overall rating for RH-117,281 2F (240 SC) on *Typhlodromus pyr*i under worst-case laboratory conditions can be considered "harmless".

Since no effect on reproduction was observed at the higher RH-117,281 2F (240 SC) 2x treatment and no dose-response relationship could be established, formulation RH-117,281 2F (240 SC) on *Typhlodromus pyr*i under worst-case laboratory conditions can be considered "harmless".

RMS comments:
LR_{50}: >300 g a.s./ha
ER_{50} (reproduction): > 300 g a.s./ha
Study is considered acceptable.

Report:	**KCP, 10.3.2.1/03 Engelhard, E.K. (1998d)** **RH-117,281 2F (240SC): Laboratory toxicity test with the predacious mite, *Amblyseius andersoni* Chant (Acari: Phytoseiidae)**

Guidelines: IOBC Guideline, Overmeer (1998)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i.
Test species: predacious mite *Amblyseius andersoni*
Number of organisms, age: 5 replicates per treatment, each containing 20 protonymhs, lifestage: protonymph to adult
Type of test: laboratory toxicity test using glass plate
Applied concentrations:
control (shared with Springborn study #1007.030.268), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 SC) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha) and toxic Standard treatment (Ethyl Parathion 500 g/L EC, applied at 0.036%, shared with Springborn study #1007.030.268)
Exposure route: 14 days laboratory test
Test conditions:
temperature: 24.0 to 26.0 °C
relative humidity: 70 to 85%
light regime: 16:8 hours light: dark, light intensity: 1003 to 1148 lux

Results:
The following results were obtained for M-(corrected mortality), R_r-(effect on reproduction), E-(beneficial capacity) values and the hazard rating according to the IOBC hazard assessment scheme (Hassan, 1992) of RH-117,281 2F (240SC):

Table B.9.5.2-1: Summary of the laboratory toxicity test with the predacious mite

Application rate (mL formulation/ha)	M (%)	R_r	E (%)	Hazard rating
625[1]	8.4	1.06	3.12	harmless
1250[2]	4.1	1.02	1.7	harmless

[1] This value represents either 1x (for arable crops) or 2.5x (for orchard or vineyard crops) of the recommended test concentration.
[2] This value represents either 2x (for arable crops) or 5x (for orchard or vineyard crops) of the recommended test concentration.

Formulation RH-117,281 2F (240 SC) on *Amblyseius andersoni* under worst-case laboratory conditions can be considered “harmless”.

RMS comments:
LR_{50}: >300 g a.s./ha
ER_{50} (reproduction): > 300 g a.s./ha
Study is considered acceptable.

Report:	**KCP, 10.3.2.1/04 Engelhard, E.K. (1998e)** **RH-117,281 2F (240SC): Laboratory toxicity test with the spiders, *Pardosa sp.* (Araneae: Lycosidae).**

Guidelines: BBA Guideline Part VI 23-2.1.9 (Wehling and Heimbach, 1994)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i.
Test species: spiders *Pardosa sp.*
Number of organisms, age: 20 replicates per treatment (10 replicates each with 1 female spider and 10 replicates each with 1 male spider)
Type of test: laboratory toxicity test
Applied concentrations:
control (shared with Springborn study #1007.030.272), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 SC) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha) and toxic Standard treatment (Karate 5 EC, applied at 2 g a.i./ha, shared with Springborn study #1007.030.272)
Exposure route: 14 days laboratory test
Test conditions:
temperature: 19.0 to 21.0 °C
relative humidity: 65 to 83%
light regime: 16:8 hours light:dark, light intensity: 948 lux

Results:
Based on the effects of RH-117,281 2F (240 SC) on *Paradosa sp.* mortality (M = 0% for both concentrations) and the feeding rate (reduction of <30% for both concentrations), the test substance applied at 1x (2.5x for orchard or vineyard crops) and 2x (5x for orchard or vineyard crops) of the maximum proposed field application rate is classified according to the IOBC scheme as „harmless" to *Paradosa sp.* under worst case laboratory conditions.

RMS comments:
LR_{50}: >300 g a.s./ha
ER_{50} (feeding): > 300 g a.s./ha
Study is considered acceptable.

Report:	**KCP, 10.3.2.1/05 Engelhard, E.K. (1998f)** **RH-117,281 2F (240SC): Laboratory acute toxicity test with the ground beetle, *Poecilus cupreus L.* (Coleoptera: Carabidae).**

Guidelines: IOBC (Heimbach, 1994)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i.
Test species: ground beetle *Poecilus cupreus*
Number of organisms, age: 5 replicates per treatment, each with 6 beetles 93 males and 3 females), 3 to 4 week old adult beetles
Type of test: laboratory acute toxicity test
Applied concentrations:
control (shared with Springborn study #1007.030.267), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 SC) 2x (1250 mL formulation/ha,

corresponding to 300 g a.i./ha) and toxic Standard treatment (Afugan 30 EC, applied at 1 L/ha, shared with Springborn study #1007.030.267)
Exposure route: 14 days laboratory test
Test conditions:
temperature: 19.0 to 21.0 °C
relative humidity: 66 to 85%
light regime: 16:8 hours light:dark, light intensity: 502 to 574 lux

Results:
No mortality was observed in the control or RH-117,281 2F (240SC) treatments. Mortality in the toxic standard treatment was 96.7%. This mortality was within the range expected for the toxic standard.
No difference in beetle behaviour was observed between the control and RH-117,281 2F (240SC) 1x and between the control and 2x treatment. All beetles behave normally. The first signs of detrimental changes in the behaviour of the beetles treated with toxic standard were observed at 24 hours after treatment application. Most of these beetles died by Test Day 2.
No difference in beetle behaviour was observed between the control and the RH-117,281 2F (240 SC) 1x and between the control and the 2x treatment. All the beetles behaved normally. The first signs of detrimental changes in the behaviour of the beetles treated with the toxic standard were observed at 24 hours after treatment application. Most of the beetles died by test Day 2.
No significant effects on the average feeding rate per beetle per day were observed in the RH-117,281 2F (240SC) 1x and 2x treatment when compared to the control. The average feeding rate per surviving beetle per day was 0.05, 0.07, 0.03 and 0 fly pupae for the control, RH-117,281 2F (240SC) 1x, the RH-117,281 2F (240SC) 2x and the toxic standard treatment.
Based on the effect on mortality (no mortality) and on the feeding rate (no reduction for 1x; no significant reduction (42.8%) for the 2x treatment when compared to the control), the test substance RH-117,281 2F (240SC) applied at 1x (2.5x for orchard or vineyard crops) of the maximum proposed application rate is classified according to the IOBC scheme as “harmless” to *Poecilus cupreus* under the worst-case laboratory conditions.

RMS comments:
LR_{50}: >300 g a.s./ha
ER_{50} (feeding): > 300 g a.s./ha
Study is considered acceptable.

Report:	**KCP, 10.3.2.1/06 Engelhard, E.K. (1998g)** **RH-117,281 2F (240SC): Laboratory toxicity test with the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae).**

Guidelines: IOBC (Bigler, 1988)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i.
Test species: green lacewing, *Chrysoperla carnea*
Number of organisms, age:
-*Exposure phase:* 4 replicates per treatment (glass plates), each containing 10 *Chrysoperla carnea* larvae (one per cylinder);

- *Reproductive phase:* 2 replicates per treatment, each containing *Chrysoperla carnea* impartially pooled from each treatment to form groups of ≤ 20 adults with sex ratio of approximately 1:1;
lifestage: 24-hour old larvae.
Type of test: laboratory toxicity test using glass plate
Applied concentrations:
control (shared with Springborn study #1007.030.281), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 SC) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha) and toxic Standard treatment (Perfekthion, applied at 0.75 L/ha, shared with Springborn study #1007.030.281)
Exposure route: 14 days laboratory test
Test conditions:
temperature: 21.0 to 24.0 °C
relative humidity: 67 to 80%
light regime: 16:8 hours light:dark, light intensity: 3030 to 3850 lux

Results:
The following results were obtained for corrected mortality (M-value), the reproduction parameter (R-value), the beneficial capacity (E-value) and the hazard rating according to the IOBC:

Table B.9.5.2-2: Summary of the laboratory toxicity test with the green lacewing

Treatment	application rate	M (%) [a]	R (%)	E (%) [b]	Hazard rating
RH-117,281 2F (240SC) 1x[1]	150 g a.i./ha	2.56	0.86	16.2	harmless
RH-117,281 2F (240SC) 2x[2]	300 g a.i./ha	2.56	0.64	37.8	slightly harmful

[a] A positive value means higher a negative value means lower mortality in the test substance treatment than in the control.
[b] A positive value means a reduction and a negative value means an increase in beneficial capacity.
[1] This value represents either 1x (for arable crops) or 2.5x (for orchard or vineyard crops) of the recommended test concentration.
[2] This value represents either 2x (for arable crops) or 5x (for orchard or vineyard crops) of the recommended test concentration.

RMS comments:
LR_{50}: >300 g a.s./ha
ER_{50} (reproduction): > 300 g a.s./ha
Study is considered acceptable.

Report:	**KCP, 10.3.2.1/07 Engelhard, E.K. (1998h)** **RH-117,281 2F (240SC): Laboratory contact toxicity test with the predator, *Orius insidiosus* (Heteroptera: Anthocoridae)**

Guidelines: IOBC (1988)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC), (Lot No. YS-1239), chemical purity: 22.35% a.i.
Test species: predator, *Orius insidiosus*

Number of organisms, age: 10 replicates per treatment, each containing 10 *Orius insidiosus*, age - second nymph stage to adult.
Type of test: laboratory contact toxicity test using glass cells
Applied concentrations:
control (shared with Springborn study #1007.030.269), RH-117,281 2F (240 SC) 1x (625 mL formulation/ha, corresponding to 150 g a.i./ha), RH-117,281 2F (240 SC) 2x (1250 mL formulation/ha, corresponding to 300 g a.i./ha) and toxic Standard treatment (Ethyl Parathion 500 g/L EC, applied at 5.186 mL/ha, shared with Springborn study #1007.030.269)
Exposure route: 14 days laboratory test
Test conditions:
temperature: 24.0 to 26.0 °C
relative humidity: 70 to 80%
light regime: 16:8 hours light:dark, light intensity: 2680 to 3190 lux
air flow: 0.1 to 0.16 m/s

Results:
The following results were obtained for M-(cumulative nymphal mortalities, corrected according to Schneider-Orelli), R_r-(effect on reproduction), E-(beneficial capacity) values and the hazard rating according to the IOBC hazard assessment scheme (Hassan, 1992) of RH-117,281 2F (240SC):

Table B.9.5.2-3: Summary of laboratory contact toxicity test with the predator, *Orius insidiosus*

Application rate (mL formulation/ha)	M (%)	R_r	E (%) [a]	Hazard rating
625[1]	4.1	0.97	6.7	harmless
1250[2]	4.1	1.07	-2.2	harmless

[a] A positive value means a reduction and negative value means an increase in beneficial capacity.
[1] This value represents either 1x (for arable crops) or 2.5x (for orchard or vineyard crops) of the recommended test concentration.
[2] This value represents either 2x (for arable crops) or 5x (for orchard or vineyard crops) of the recommended test concentration.

RMS comments:

LR_{50}: >300 g a.s./ha
ER_{50} (reproduction): > 300 g a.s./ha
Study is considered acceptable.

B.9.6 RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1 Risk assessment for bees

The risk assessment for bees has been conducted in line with the Terrestrial Guidance Document (SANCO/10329/2002).

Table B.9.4.7.1-1: Acute hazard quotients for the proposed uses of Zoxium 240 SC

Test substance	Route of exposure	Maximum single application rate	Endpoint	HQ	Trigger value
Zoxium 240 SC	Oral	180 g a.s./ha	>33 µg a.s./bee	< 5.4	50
Zoxium 240 SC	Contact	180 g a.s./ha	>43.2 µg a.s./bee	< 4.2	

The above acute oral and contact hazard quotients are less than the trigger value, indicating an acceptable acute risk to bees from oral exposure to Zoxium 240 SC.

According to the results from the adult chronic oral toxicity test (CP 10.3.1.2), exposure to bees for 10 days at worst case concentrations (5000 mg Zoxium 240 SC/L feeding solution equivalent to 174.8 µg a.s./bee/day) has no adverse effects (i.e. mortality, sublethal effects).

According to the results from the honey bee development test (CP 10.3.1.3) exposure of bee colonies at the highest concentration of zoxamide in the spray solution (0.18 g a.s./L) and a concentration 4 fold higher (0.75 g a.s./L) has no adverse effects on the honey bee colonies and the bee brood development.

It is therefore considered that the risk to honey bees from the proposed uses of Zoxium 240 SC is acceptable.

RMS comments:

The oral and contact risk of zoxamide is acceptable for bees following the intended uses of Zoxium 240 SC in potatoes and grapes.

B.9.6.2 Risk assessment for non-target arthropods

Estimation of in- and off- crop exposure

Table B.9.6.2-1 summarises the in- and off-crop exposure of zoxamide following the maximum application of Zoxium 240 SC to potatoes and grapes at 180 g a.s./ha.

Table B.9.6.2-1: Estimated exposure levels from the proposed uses of Zoxium 240 SC

Crop	Application rate	MAF	In-field exposure	Drift rate	Off-field exposure
Potatoes	180 g a.s./ha	3.0	540 g a.s./ha	0.0175	9.45 g a.s./ha
Grapevines	180 g a.s./ha	3.0	540 g a.s./ha	0.0659	35.586 g a.s./ha

MAF: Multiple application factor (3.0 for 5 applications)

First tier risk assessment: In- and off- crop hazard quotients for indicator species

The LR_{50} values from the laboratory standard glass plate studies with the standard indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* are used in the first tier risk assessment.

Table B.9.6.2-2: In-field and off-field risk hazard quotient for terrestrial arthropods, from the proposed uses of Zoxium 240 SC

Species	Exposure		Endpoint	In-field HQ	Off-field HQ	Trigger value
	In-field	Off-field				
Potatoes:						
Aphidius rhopalosiphi	540 g a.s./ha	9.45 g a.s./ha	>300 g a.s./ha	<1.8	<0.0315	2
Typhlodromus pyri	540 g a.s./ha	9.45 g a.s./ha	>300 g a.s./ha	<1.8	<0.0315	
Grapevines:						
Aphidius rhopalosiphi	540 g a.s./ha	35.586 g a.s./ha	>300 g a.s./ha	<1.8	<0.119	2
Typhlodromus pyri	540 g a.s./ha	35.586 g a.s./ha	>300 g a.s./ha	<1.8	<0.119	

The resulting hazard quotients for both indicator species demonstrate that the risk to non-target arthropod species from the proposed uses of Zoxium 240 SC in potatoes and grapes is acceptable and no higher-tier risk assessment is required.

RMS comments:
The acute and long-term risk of zoxamide is acceptable for non-target arthropods following the intended uses of Zoxium 240 SC in potatoes and grapes.

B.9.7 EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.7.1 Earthworms

None. The risk assessment conducted for the active substance is acceptable (see B.9.8.1 below).

B.9.7.2 Effects on non-target soil meso and macrofauna (other than earthworms)

See Vol. 3, Part B.9.4.2. for active substance.

B.9.8 RISK ASSESSMENT FOR NON-TARGET SOIL MESO- AND MACROFAUNA

The risk to earthworms posed by the intended uses of Zoxium 240 SC has been evaluated using the recommendations presented in the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 of October 2002).

The calculated soil PEC values are given in RAR Document Section B8, Point 8.2.

<u>Metabolites</u>

According to the environmental fate section the metabolites of zoxamide: RH-127450, RH-24549, RH-163353 and RH-141455 have been identified as potentially relevant for the soil risk assessment.

No data were submitted on RH-24549, RH-163353 or RH-141455. As stated in the part B.8 AS, the metabolite RH-24549 is formed via a hydrolytic process and is therefore expected to be present in sufficient levels even in the artificial substrate used in the earthworm studies. Although present at a low level, the biological activity in the artificial substrate is expected to be sufficient even for metabolites RH-127450, RH-163353 and RH-141455 to be formed during the reproductive study. Earthworms present in the reproductive study are therefore likely to have been exposed to all four metabolites, at levels which, while low, would still exceed environmentally relevant levels.

It is most probable that RH-24549, RH-127450, RH-163353 and RH-141455 would have occurred in the test soils during the 56-day studies, since, the parent has a half-life of <10 days in natural soil and that metabolites RH-24549, RH-127450 and RH-163353 all reached peak concentrations within 7-10 days and RH-141455 within 28 days. Therefore, it is considered that the above assessment of parent also addresses the potential risk posed by metabolites.

Furthermore, all four metabolites lack the haloketone toxophore associated with the parent compound's mode of toxic action. The metabolite RH-141455 has been shown not to have any fungicidal activity. Metabolite RH-127450 was of very low acute toxicity with a 14-day LC_{50} of >1000 mg/kg. Metabolite RH-163353 is structurally similar to RH-127450 and even more polar so it can be assumed that it is very unlikely that it would be more toxic than RH-163353 or the parent active substance. For information on structure of the metabolites and degradation scheme please refer to Vol. 3 part B.8 AS point B.8.1 and figure B.8.1.1.1-1.

To address any further uncertainties a worst-case approach has been taken, assuming that the soil metabolites of zoxamide are 10 fold more toxic than then parent compound and comparing these calculated endpoints to the maximum PEC_{soil} values for all relevant metabolites except RH-141455, based on Part B.8 point 8.2. TER calculations for metabolite RH-141455 is made considering PEC accumulation values (Section Vol. 3, B.8, CP, point 8.2).

The details of these TERs are given in Table B.9.8-1.

Table B.9.8 -1: Acute and chronic toxicity exposure ratios for the risk to earthworms from the proposed uses of Zoxium 240 SC

Species	Test substance	Toxicity endpoint (mg a.s./kg soil dw)	Maximum initial PEC_{soil} (mg/kg soil dw)***	TER	Trigger value
Acute					
Eisenia foetida	Zoxamide	>535*	0.467	1146	10
	RH-127450	>500	0.039	12820	
	RH-24549	>53.5**	0.070	764	
	RH-163353	>53.5**	0.073	733	
	RH-141455		PEC soil accumulation **(mg/kg soil dw)**		
		>53.5**	no tillage – potatoe 0.0505	1059.4	
		>53.5**	no tillage – vineyard 0.0404	1324.3	

		>53.5**	tillage – potatoe 0.0366	1461.7	
		>53.5**	tillage – vineyard 0.0293	1825.9	
Chronic					
Eisenia foetida	Zoxamide	0.5* (artificial soil)	0.467	**1.07**	5
		7* (natural soil)	0.467	15	
	RH-127450	0.7**	0.039	17.9	
	RH-24549	0.7**	0.070	10.0	
	RH-163353	0.7**	0.073	9.60	
	RH-141455		PEC soil accumulation **(mg/kg soil dw)**		
		0.7**	no tillage – potatoe 0.0505	13.9	
		0.7**	no tillage – vineyard 0.0404	17.3	
		0.7**	tillage – potatoe 0.0366	19.1	
		0.7**	tillage – vineyard 0.0293	23.9	

* Since zoxamide has a log P_{ow} of 3.76 (>2) it is necessary to reduce the LC_{50} and NOEC values by a factor of 2 for the studies conducted using artificial soil in line with EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 of October 2002). For the natural soil study, no adjustment of the toxicity value is required because the natural soil used in this study is typical of agricultural soils and contained only 2.7% organic carbon.
** Acute and chronic toxicity endpoints used for metabolites assuming that each metabolite is 10 fold more toxic than the parent to earthworms
*** All maximum initial PEC_{soil} values were calculated for application in potatoes
Values in **bold** are below the trigger value

All resulting acute and chronic TERs (with consideration to earthworm exposure via natural soil) demonstrate that the risk to earthworms from the proposed uses of Zoxium 240 SC in potatoes and grapes is acceptable.

RMS comments:
For chronic risk assessment the NOEC of zoxamide was divided by 2 only for the study performed with artificial soil and not for the study with natural soil. In the present case, the factor of 2 for the test with natural soil was not considered relevant as the LUFA soil contains only 2% organic matter, which is considered to be more representative of natural soil conditions.
The acute and long-term risk of zoxamide is acceptable for earthworms following the intended uses of Zoxium 240 SC in potatoes and grapes.

B.9.9 EFFECTS ON SOIL NITROGEN TRANSFORMATION

Refer to Volume 3, Section B.9 for the active substance.

B.9.10 RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

For the first EU review it was concluded that zoxamide had no impact on soil respiration and nitrogen mineralisation at soil concentrations equivalent to a rate of 1.5 kg a.s./ha (2 mg a.s./kg soil).

A summary of the existing endpoints is provided in Table B.9.10-1.

No further data are considered necessary.

Table B.9.10-1: Summary of data on the toxicity of zoxamide to soil nitrogen transformation

Test	Endpoint	Reference
Zoxamide		
Nitrogen mineralisation	<25% after 42 days at 2 mg a.s./kg soil	CA 8.5/01 van der Kolk, J. 1998b
Carbon transformation	<25% after 28 days at 2 mg a.s./kg soil	

The potential risk to soil microbial processes from the proposed uses of Zoxium 240 SC has been evaluated using the recommendations presented in the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 of October 2002).

The results of the available study showed no effect of $\geq$ 25% on soil microbial process, at a soil concentration of 2 mg a.s./kg soil, which is significantly higher than the maximum calculated PEC_{soil} of 0.467 mg a.s./kg soil (highest PECs is for application in potatoes, in vines PECs is 0.374 mg a.s./kg soil) (Vol. 3, Part B 8 PPP, point 8.2). Therefore, the risk to soil microbial processes from the proposed uses of Zoxium 240 SC is considered to be acceptable.

Metabolites

According to the environmental fate section the metabolites of zoxamide: RH-127450, RH-24549, RH-163353 and RH-141455 have been identified as potentially relevant for the soil risk assessment. No data were submitted on either of these metabolites on soil nitrogen transformation. For more details for soil metabolite please refer to Vol. 3, Part B.8 AS study Callow & Hilton, 2013a and Table B.8.1.1.16 to B.8.1.1.1-13.

It is most probable that RH-24549, RH-127450, RH-163353 and RH-141455 would have occurred in the test soil during the 42-day study, since, the parent has a half-life of <10 days in natural soil and that metabolites RH-24549, RH-127450 and RH-163353 all reached peak concentrations within 7-10 days and RH-141455 within 28 days. Furthermore, all four metabolites lack the haloketone toxophore associated with the parent compound's mode of toxic action. The metabolite RH-141455 has been shown not to have any fungicidal activity.

Therefore, it is considered that the above assessment of parent also addresses the potential risk posed by metabolites.

B.9.11 EFFECTS ON TERRESTRIAL NON-TARGET HIGHER TIER PLANTS

B.9.11.1 Summary of screening data

For the first EU review, only screening test data on terrestrial vascular plants were presented. No adverse effects were seen at dose rates up to 500 g a.s./ha. Therefore, no studies on seedling emergence and vegetative vigour were required.

As the active substance is not a herbicide and/or plant growth regulator additional studies examining the effects on seedling emergence and vegetative vigour are not required.

No further data are considered necessary.

B.9.11.2 Testing on non-target plants

Report:	**KCP, 10.6.2/01 Nunez, M.V. (1998a)** **Greenhouse phytotoxicity tests with RH-117,281 2F.**

Guidelines: Rohm and Haas Company, Greenhouse phytotoxicity test method
GLP: No
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC) Lot No. YS-1239, chemical purity: 22.35% a.i.
Type of test: phytotoxicity test
Test concentrations: 125, 250 and 500 g/a.i./ha

Results:
In a glasshouse screening study, RH-117,281 (240 SC formulation, 22.35% a.s.) was applied to a range of broad-leaved and grass weeds and crop plants at doses up to 500 g a.s./ha (=3.3 times the maximum individual dose on potatoes). Applications were made pre- and post-emergence of target plants. Assessments were made of growth inhibition, chlorosis and necrosis at 14 days after treatment (DAT). In total eight species of broad-leaved weeds, eight species of grass weeds, two species of broad-leaved crops and three species of cereal crops were tested. No adverse effects were seen on any species at any dose.

Table B.9.11.2-1: % Pre and postemergence injury (*) on broadleaf weeds 14 DAT

Broadleaf Weeds	Rate g/Ha	Growth Inhibition % PRE	Growth Inhibition % POST	Necrosis % PRE	Necrosis % POST	Chlorosis % PRE	Chlorosis % POST
Xanthium pensylvanicum	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Bidens pilosa	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Ipomoea hederacea	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Solanum nigrum	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Amaranthus retroflexus	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Polygonum lapathifolium	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Sida spinosa	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Abutilon theophrasti	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0

Table B.9.11.2-2: % Pre and postemergence injury (*) on grassy weeds 14 DAT

Grassy Weeds	Rate g/Ha	Growth Inhibition % PRE	Growth Inhibition % POST	Necrosis % PRE	Necrosis % POST	Chlorosis % PRE	Chlorosis % POST
Echinochloa crus-galli	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Panicum antidotale	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Digitaria sanguinalis	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Setaria viridis	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Cyperus esculentus	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Lolium multiflorum	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Brachiaria platyphylla	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Avena fatua	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0

* Average of four replicates
Date Treated: 08/05/98
Evaluation: 08/19/98

Table B.9.11.2-3: % Pre and postemergence injury (*) on grassy weeds 14 DAT

Crops	Rate g/Ha	Growth Inhibition % PRE	Growth Inhibition % POST	Necrosis % PRE	Necrosis % POST	Chlorosis % PRE	Chlorosis % POST
Zea mays	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Gossypium hirsutum	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Triticum spp.	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Oryza sativa	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0
Glycine max	125	0	0	0	0	0	0
"	250	0	0	0	0	0	0
"	500	0	0	0	0	0	0

* Average of four replicates
Date Treated: 08/05/98
Evaluation: 08/19/98

RH-117,281 did not cause any injury when applied at 125, 250 and 500 g a.s./ha pre or postemergence to selected weeds and crops.
Based upon this greenhouse test results, RH-117,281 is not expected to cause injury when applied at ≤ 500 g a.s./ha under field conditions to the weeds and crops listed above. As a consequence, not expect to cause injury to adjacent crops in field application of RH-117,281 at rates ≤ 500 g a.s./ha or less.

RMS comments:
Study is considered acceptable.

Report:	**KCP, 10.6.2/02 Nunez, M.V. (1998b)** **Greenhouse crop phytotoxicity tests with RH-117,281 2F.**

Guidelines: Rohm and Haas Company, Greenhouse phytotoxicity test method
GLP: No
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 2F (240 SC) Lot No. YS-1239, chemical purity: 22.35% a.i.
Type of test: phytotoxicity test
Test concentrations: 125, 250 and 500 g/a.i./ha

Results:
In a glasshouse screening study, RH-117,281 (240 SC formulation, 22.35% a.s.) was applied to 17 species of crop plants at doses up to 500 g a.s./ha (=3.3 times the maximum individual dose on potatoes). Applications were made pre- and post-emergence of target plants. Assessments were made of growth inhibition, chlorosis and necrosis at 14 days after treatment (DAT). In total five species of cereals/grasses and 12 species of broad-leaved crops were tested. No adverse effects were seen on any species at any dose.

Table B.9.11.2-5: Growth inhibition, greenhouse data summary of plant response to preemergence treatments (height average of four replicates)

Means followed by same letter do not significantly differ (P=.05, Duncan's New MRT)

Crops	Application Rates	Plant Ht (cm)*	Statistical Analysis	
Barley	125 g/Ha	38.0 a	LSD (P=.05)	3.84
"	250 g/Ha	37.0 a	Std. Deviation.	2.40
"	500 g/Ha	39.5 a	CV	6.33
"	*Untreated*	37.5 a		
Cabbage	125 g/Ha	15.0 a	LSD (P=.05)	1.99
"	250 g/Ha	15.3 a	Std. Deviation.	1.25
"	500 g/Ha	15.3 a	CV	8.31
"	*Untreated*	14.5 a		
Carrot	125 g/Ha	8.3 a	LSD (P=.05)	1.61
"	250 g/Ha	9.3 a	Std. Deviation.	1.00
"	500 g/Ha	8.5 a	CV	11.23
"	*Untreated*	9.8 a		
Corn	125 g/Ha	59.0 a	LSD (P=.05)	2.65
"	250 g/Ha	59.8 a	Std. Deviation.	1.66
"	500 g/Ha	61.0 a	CV	2.77
"	*Untreated*	59.8 a		
Cotton	125 g/Ha	18.8 a	LSD (P=.05)	2.63
"	250 g/Ha	18.8 a	Std. Deviation.	1.64

"	500 g/Ha	17.3 a	CV	8.93
"	*Untreated*	18.8 a		
Lettuce	125 g/Ha	14.5 a	LSD (P=.05)	2.82
"	250 g/Ha	14.5 a	Std. Deviation.	1.77
"	500 g/Ha	16.0 a	CV	11.72
"	*Untreated*	15.3 a		
Melon	125 g/Ha	14.5 a	LSD (P=.05)	2.42
"	250 g/Ha	15.8 a	Std. Deviation.	1.51
"	500 g/Ha	15.0 a	CV	10.04
"	*Untreated*	15.0 a		
Onion	125 g/Ha	12.5 a	LSD (P=.05)	4.58
"	250 g/Ha	11.5 a	Std. Deviation.	2.86
"	500 g/Ha	13.0 a	CV	25.17
"	*Untreated*	8.5 a		
Peas	125 g/Ha	12.0 b	LSD (P=.05)	3.43
"	250 g/Ha	16.0 a	Std. Deviation.	2.05
"	500 g/Ha	12.9 ab	CV	15.21
"	*Untreated*	13.0 ab		
Rape	125 g/Ha	19.5 a	LSD (P=.05)	2.02
"	250 g/Ha	17.9 a	Std. Deviation.	1.24
"	500 g/Ha	18.0 a	CV	6.64
"	*Untreated*	19.0 a		
Rice	125 g/Ha	29.5 a	LSD (P=.05)	2.95
"	250 g/Ha	32.0 a	Std. Deviation.	1.84
"	500 g/Ha	31.8 a	CV	5.91
"	*Untreated*	31.5 a		
Rye	125 g/Ha	27.0 a	LSD (P=.05)	2.20
"	250 g/Ha	27.0 a	Std. Deviation.	1.37
"	500 g/Ha	26.5 a	CV	5.14
"	*Untreated*	26.5 a		
Soybean	125 g/Ha	22.8 a	LSD (P=.05)	1.73
"	250 g/Ha	22.3 a	Std. Deviation.	1.08
"	500 g/Ha	23.5 a	CV	4.78
"	*Untreated*	22.3 a		
Sugarbeet	125 g/Ha	13.3 a	LSD (P=.05)	3.00
"	250 g/Ha	15.0 a	Std. Deviation.	1.87
"	500 g/Ha	14.8 a	CV	12.86
"	*Untreated*	15.3 a		
Sunflower	125 g/Ha	29.8 a	LSD (P=.05)	7.11
"	250 g/Ha	27.3 a	Std. Deviation.	4.44
"	500 g/Ha	29.8 a	CV	15.53
"	*Untreated*	27.8 a		
Tomato	125 g/Ha	11.0 a	LSD (P=.05)	5.78
"	250 g/Ha	13.0 a	Std. Deviation.	3.61
"	500 g/Ha	10.8 a	CV	28.34
"	*Untreated*	16.3 a		
Wheat	125 g/Ha	34.5 a	LSD (P=.05)	3.60
"	250 g/Ha	34.0 a	Std. Deviation.	2.25
"	500 g/Ha	34.8 a	CV	6.53
"	*Untreated*	34.5 a		

Table B.9.11.2-4: Necrosis, chlorosis and malformation, greenhouse data summary of plant response to pre and postemergence treatments (average readings of four replicates)

CROPS /	Application Rates of RH-117281	% Necrosis* PRE	% Necrosis* POST	% Chlorosis* PRE	% Chlorosis* POST	% Malformation* PRE	% Malformation* POST
Barley	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Cabbage	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Carrot	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Corn	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Cotton	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Lettuce	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Melon	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Onion	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Pea	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Rape	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Rice	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Rye	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Soybean	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Sugarbeet	125 g/Ha	0	0	0	0	0	0

"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Sunflower	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Tomato	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0
Wheat	125 g/Ha	0	0	0	0	0	0
"	250 g/Ha	0	0	0	0	0	0
"	500 g/Ha	0	0	0	0	0	0
"	*Untreated*	0	0	0	0	0	0

Based upon these greenhouse test results, RH-177,281 is not expected to cause injury to the crop listed in tables B.9.11.2-3 and Table B.9.11.2-4.

RMS comments:

The study was not GLP compliant. Study is considered acceptable.

B.9.11.3 Extended laboratory studies on non-target plants

None. Not required.

B.9.11.4 Semi-field and field tests on non-target plants

None. Not required.

B.9.12 RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

According to the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (SANCO/10329/2002) as a general rule, at the risk assessment step based on initial screening data, the risk should be considered acceptable if there are no data indicating more than 50 % phytotoxic effect at the maximum application rate. In this case, no adverse effects were seen at dose rates up to 500 g a.s./ha (2.77 times the maximum individual proposed dose in potatoes and grapes) in glasshouse screening studies on 21 species from 4 different taxonomic groups.

It is therefore concluded that the risk to non-target plants from the proposed uses of Zoxium 240 SC is acceptable.

B.9.13 EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No data on other terrestrial organisms have been generated with Zoxium 240 SC. During the previous EU review adequate data were submitted to assess the potential impact of the active substance zoxamide on a range of insect species (screening data). No adverse effects on insects (2 species of *Lepidoptera*, 1 species of mite, 2 species of *Homoptera*) were seen at dose rates up to 600 g a.s./ha. On a third species of *Homoptera* 40% mortality was seen at the same rates and when applied to soil, zoxamide was harmless to one species of *Coleoptera* at 11.4 kg a.s./ha. Please refer to the DAR dated May 2001, Point B.9.9.1 (IIA 8.6/03 Sames, B.A., 1998) for further details.

No further data are considered necessary.

B.9.14 RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

On six of the seven insect species tested, no adverse effects were seen at doses up to 600 g a.s./ha. More detailed studies of the effects on non-target fauna have been addressed in other sections of this assessment.

B.9.15 REFERENCES RELIED ON

<u>Public literature</u>

A literature review report on zoxamide has been submitted by the applicant in the framework of this renewal. Twenty two databases have been searched (AGRICOLA, AGRIS International, Aqualine, ASFA, BIOSIS® Toxicology , BIOSIS Previews® , CAB Abstracts, EMBASE, Environment Abstracts, Foodline®: SCIENCE, FSTA®, GEOBASE, GeoRef, MEDLINE, Meteorological and Geoastrophysical Abstracts, PASCAL, Pollution Abstracts, ToxFile, Toxicology Abstracts, TOXLINE, Water Resources Abstracts) and their choices have been justify.

The following key words were searched: zoxamide, CAS number 156052-68-5, company developmental name RH-117,281 and RH-117,281, PPP Zoxium 240 SC, IUPAC names, chemical names and primary metabolites of concern, plus synonyms (RH-141,452, RH-141,455, RH-150,721, RH-24549, RH-139432, RH-127450, RH-163353).

Studies published since 2004 have been looked for in order to include the most recent scientific peer-reviewed open literature.

Following assessment of titles and abstracts two articles were obtained for review of the full text to assess their reliability and detailed relevance. Publications meeting the relevance criteria were those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, its relevant metabolites and/or the plant protection product on human health, animal health and/or the environment, which could impact the endpoints or the risk assessment parameters.

Following assessment of the output from these searches, none of the results met the relevant criteria for ecotoxicology section.

References relied on:

New studies

Data point	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data protection claimed (Y/N)	Justification if data protection claimed	Owner
KCP, 10.2.1.1/01	[redacted]	2010	GOW 008: Acute toxicity to zebra fish (*Danio rerio*) in a 96-hour study under static exposure. [redacted] GLP Unpublished report.	Y	Y	Data to support new representative formulation	Gowan
KCP, 10.2.1.2/01	Mantilacci S.	2010	Acute toxicity of product GOW008 on *Daphnia magna* in a 48-hour immobilization test under static exposure. Biotecnologie BT srl GLP Unpublished report.	Y	Y	Data to support new representative formulation	Gowan
KCP, 10.3.1.2/01	Schmitzer, S. and Ehmke, A.	2014	Chronic oral toxicity test of Zoxium 240 SC on the honey bee (*Apis mellifera*) in the laboratory. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany Project 80052136 GLP, Not published	N	Y	New data requirement	Gowan
KCP, 10.3.1.3/01	Schmitzer, S.	2014	Effects of Zoxium 240 SC on honey bee brood. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany Project 80051031 GLP, Not published	N	Y	New data requirement	Gowan

Data point	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data protection claimed (Y/N)	Justification if data protection claimed	Owner
KCP, 10.3.1.4/01	Schmitzer, S.	2014	Effects of Zoxium 240 SC on honey bee brood. Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany Project 80051031 GLP, Not published	N	Y	New data requirement	Gowan

Studies relied on for the first inclusion of zoxamide in Annex I to Directive 91/414/EEC and for renewal of approval under Regulation (EC) 1107/2009.

Data point	Annex point (Original dossier)	Author(s)	Year	Title, Source (where different from company), Company, Report No, GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection claimed	Owner
KCP, 10.2.1.3/01 KCA, 8.2.6.1/06	IIA, 8.2.6/06	Ward, S.C., Murdock, C.W.	1998	Toxicity of RH-117,281 2F (240SC) to S*elenastrum capricornutum* Printz ABC Laboratories Report No. 44196 ER Ref No: 14.6 US Ref No: 97RC-0094 GLP, Unpublished	N	N	NA	Gowan
KCP, 10.3.1.1.1/01 KCP, 10.3.1.1.2 /01 KCA, 8.3.1.1.2/01	IIA, 8.3.1.1/02	Engelhard, E.K.	1998a	RH-117,281 2F (240SC): Laboratory oral and contact test with the honeybee, *Apis mellifera*. Springborn Laboratories (Europe) AG Report No. 97-066-1007. ER Ref No: 11.6 US Ref No: 97RC-0095 GLP, Unpublished	N	N	NA	Gowan

Data point	Annex point (Original dossier)	Author(s)	Year	Title, Source (where different from company), Company, Report No, GLP or GEP status (where relevant), Published or not	Vertebrate study Y/N	Data Protection Claimed Y/N	Justification if data protection claimed	Owner
KCP, 10.3.2.1/01 KCA, 8.3.2.1/01	IIA, 8.3.2/01	Engelhard, E.K.	1998b	RH-117,281 2F (240SC): Laboratory acute toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae). Springborn Laboratories (Europe) AG Report No. 97-062-1007. ER Ref No: 11.8 US Ref No: 97RC-0106 GLP, Unpublished	N	N	NA	Gowan
KCP, 10.3.2.1/02 KCA, 8.3.2.2/01	IIA, 8.3.2/02	Engelhard, E.K.	1998c	RH-117,281 2F (240SC): Laboratory toxicity test with the predacious mite, *Typhlodromus pyr*i Scheuten (Acari: Phytoseiidae). Springborn Laboratories (Europe) AG Report No. 97-070-1007. ER Ref No: 11.3 US Ref No: 97RC-0105 GLP, Unpublished	N	N	NA	Gowan
KCP, 10.3.2.1/03 KCA, 8.3.2.2/02	IIA, 8.3.2/03	Engelhard, E.K.	1998d	RH-117,281 2F (240SC): Laboratory toxicity test with the predacious mite, *Amblyseius andersoni* Chant (Acari: Phytoseiidae) Springborn Laboratories (Europe) AG Report No. 97-075-1007. ER Ref No: 11.7 US Ref No: 97RC-0111 GLP, Unpublished	N	N	NA	Gowan
KCP, 10.3.2.1/04 KCA, 8.3.2.2/03	IIA, 8.3.2/04	Engelhard, E.K.	1998e	RH-117,281 2F (240SC): Laboratory toxicity test with the spiders, *Pardosa sp.* (Araneae: Lycosidae). Springborn Laboratories (Europe) AG Report No. 97-059-1007. ER Ref No: 11.9 US Ref No: 97RC-0107 GLP, Unpublished	N	N	NA	Gowan

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KCP, 10.3.2.1/05 KCA, 8.3.2.2/04	IIA, 8.3.2/05	Engelhard, E.K.	1998f	RH-117,281 2F (240SC): Laboratory acute toxicity test with the ground beetle, *Poecilus cupreus L.* (Coleoptera: Carabidae). Springborn Laboratories (Europe) AG Report No. 97-064-1007. ER Ref No: 11.2 US Ref No: 97RC-0108 GLP, Unpublished	N	N	NA	Gowan
KCP, 10.3.2.1/06 KCA, 8.3.2.2/05	IIA, 8.3.2/06	Engelhard, E.K.	1998g	RH-117,281 2F (240SC): Laboratory toxicity test with the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae). Springborn Laboratories (Europe) AG Report No. 97-068-1007. ER Ref No: 11.11 US Ref No: 97RC-0109 GLP, Unpublished	N	N	NA	Gowan
KCP, 10.3.2.1/07 KCA, 8.3.2.2/06	IIA, 8.3.2/07	Engelhard, E.K.	1998h	RH-117,281 2F (240SC): Laboratory contact toxicity test with the predator, *Orius insidiosus* (Heteroptera: Anthocoridae) Springborn Laboratories (Europe) AG Report No. 97-077-1007. ER Ref No: 11.10 US Ref No: 97RC-0110 GLP, Unpublished	N	N	NA	Gowan
KCP, 10.6.2/01 KCA, 8.6/01	IIA, 8.6/01	Nunez, M.V.	1998a	Greenhouse phytotoxicity tests with RH-117,281 2F. Rohm and Haas Report No. 98R-1092 ER Ref No: 28.4 US Ref No: 98R-1092 GLP, Unpublished	N	N	NA	Gowan

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KCP, 10..2/02 KCA, 8.6/02	IIA, 8.6/02	Nunez, M.V.	1998b	Greenhouse crop phytotoxicity tests with RH-117,281 2F. Rohm and Haas Report No. 98R-1114 ER Ref No: 28.5 US Ref No: 98R-1114 GLP, Unpublished	N	N	NA	Gowan