Renewal Assessment Report

under Regulation (EC) 1107/2009

Zoxamide

Volume 3

Active substance
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 1).

Table 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

No new studies provided.

The summaries from the original DAR were reported below and reviewed by the RMS for compliance with current requirements of guidance documents.

B.9.1.1.1 Acute oral toxicity to birds

Report: **CA, 8.1.1.1/01 [redacted] (1997a).**
RH-117,281 Technical: 14-day acute oral LD_{50} study in bobwhite quail.

Guidelines: US EPA Guidelines, Subdivision E, Series § 71-1
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: ISO common name: RH-117,281 Technical, Lot No. LG 3517, chemical purity: 92.9%
Test species: Bobwhite quails (*Colinus virginianus*)
Sex, weight, age: 36 male and 36 female birds, adult (at least 16 weeks)
Applied concentrations: 305, 488, 781, 1 250, and 2 000 mg a.i./kg
Type of application: single oral dose, by intubation in corn oil
Time of exposure: one single application, monitoring during 14 days

Results:
A study on the acute oral toxicity of technical RH-117,281 to bobwhite quail has been summarised in Table B.9.1.1.1.-1

Table B.9.1.1.1-1: Summary of acute oral toxicity to birds (data for technical RH-117,281)

Species	Purity	Acute oral LD50 (mg/kg bw)	Test Guideline	Reference
Bobwhite quail *Colinus virginianus*	92.9%	>2000	OPPTS 850.2100/FIFRA 71-1 and GLP	[redacted] (1997a), 94RC-0240, DP 82322

Six males and six females per dose level. Fourteen day observation period. No mortalities.

LD_{50} (*Colinus virginianus*) = > 2 000 mg a.s./kg bw
NOEL (*Colinus virginianus*) = 2 000 mg a.s./kg bw

RMS comments:
LD_{50} (*Colinus virginianus*) = > 2 000 mg a.s./kg bw
NOEL (*Colinus virginianus*) = 2 000 mg a.s./kg bw
This study is still considered acceptable and reliable for the risk assessment.

B.9.1.1.2 *Short-term dietary toxicity to birds*

Report:	**CA, 8.1.1.2/01 [redacted] (1997b)** **RH-117,281 Technical: 8-day acute dietary LC_{50} study in bobwhite quail.**

Guidelines: OECD Guideline 205, US EPA OPPTS 850.2200/ FIFRA Subdivision E, Subsection § 71-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: ISO common name: RH-117,281 Technical, Lot No. LG 3517, chemical purity: 92.9%
Test species: Bobwhite quails (*Colinus virginianus*)
Sex, weight, age: indeterminate sex, 10-day-old
Applied concentrations: 328, 656, 1 313, 2 625, 5 250 ppm zoxamide
Time of exposure: Short-term feeding test (5 days with exposition by the feed + 3 days recovery)

Results:
In a study on the dietary toxicity of technical RH-117,281 (purity 92.9%), five groups of 10-day-old bobwhite quail of indeterminate sex (10/group) were fed RH-117,281 in the diet at analytically confirmed concentrations of 328 ppm, 656 ppm, 1313 ppm, 2625 ppm, and 5250 ppm a.s., respectively, for a period of 5 days followed by a 3-day recovery period. A control group of ten 10-day-old bobwhite quail received untreated feed mixed with corn oil and acetone vehicle only.

No birds died during the study and no persistent or repeated clinical signs were noted in either control or treated birds.

Table B.9.1.1.2.-1: Cumulative mortality results of a short-term dietary study on bobwhite quail

8-Day Acute Dietary LC_{50} study in Bobwhite quail: RH 117,281 technical									
		Number dead/Number exposed Day of death							
Group	Concentration (ppm a.s.)	1	2	3	4	5	6	7	8
Control	0 ppm	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-1	328	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-2	656	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-3	1 313	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-4	2 625	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-5	5 250	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

Mean body weights in the test groups were not significantly different when compared to control birds on test day 5 and test day 8. Feed consumption in the treatment groups was not markedly different from the control during the 0-5 day test period or during the day 6-8 period, though no results of statistical analysis were presented.

Table B.9.1.1.2.-2: Results of mean body weight in short-term dietary study on bobwhite quail

8-day acute dietary LC_{50} study in Bobwhite quail: RH 117,281 technical					
			Mean body weight (g) of survivors		
Group	Concentration (ppm a.s.)		0 hour	Test day 5	Test day 8
Control	0	mean	18.2	34.3	44.8
		stdev	2.1	3.0	3.6

T-1	328	mean	18.3	34.3	44.0
		stdev	2.3	4.7	5.7
T-2	656	mean	18.5	33.5	42.5
		stdev	1.9	2.3	4.4
T-3	1 313	mean	18.5	33.4	42.3
		stdev	2.1	3.4	4.6
T-4	2 625	mean	18.6	33.2	42.8
		stdev	2.0	3.8	4.8
T-5	5 250	mean	18.5	31.4	41.9
		stdev	1.8	2.5	3.2

No abnormal pathological findings were noted in arbitrarily selected birds subjected to gross pathological examinations at study termination.

The 8-day dietary LC_{50} in bobwhite quail was > 5250 ppm zoxamide (>5250 mg/a.s. kg/food)
The 8-day NOEC was 5250 ppm zoxamide

RMS comments:

The study is considered acceptable. No conversion of the ppm to mg/kg bw/day was available in the original DAR. Based on study results, the RMS did the following conversion:

Daily dose [mg/kg bw/d] = (concentration in food [mg/kg diet] x daily food consumption [g/bird/d]) / body weight [g]

= (5250 x 11.3)/41.9
= 1415.9 mg/kg bw/d

The LD_{50} > 1415.9 mg/kg bw/day

Report: **CA, 8.1.1.2/02 [redacted] (1997c)**
RH-117,281 Technical: 8-day acute dietary LC_{50} study in mallard ducklings.

Guidelines: OECD Guideline 205, US EPA OPPTS 850.2200/ FIFRA Subdivision E, Subsection § 71-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: ISO common name: RH-117,281 Technical, Lot No. LG 3517, chemical purity: 92.9%
Test species: Mallard duck *(Anas platyrhynchos)*
Sex, weight, age: indeterminate sex, 8-day-old
Applied concentrations: 328, 656, 1 313, 2 625, 5 250 ppm zoxamide
Time of exposure: Short-term feeding test (5 days with exposition by the feed + 3 days recovery)

Results:
In a study on the dietary toxicity of technical RH-117,281 (purity 92.9%), five groups of 8-day-old mallard ducklings of indeterminate sex (10/group) were fed RH-117,281 in the diet at analytically confirmed concentrations of 328 ppm, 656 ppm, 1313 ppm, 2625 ppm, and 5250 ppm a.s., respectively,

for a period of 5 days followed by a 3-day recovery period. A control group of ten 8-day-old mallards received untreated feed mixed with corn oil and acetone vehicle only.

Table B.9.1.1.2.-3: Cumulative mortality results of a short-term dietary study on Mallard ducklings

8-Day Acute Dietary LC_{50} study in Mallard ducklings: RH 117,281 technical									
		Number dead/Number exposed Day of death							
Group	Concentration (ppm a.s.)	1	2	3	4	5	6	7	8
Control	0 ppm	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-1	328	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-2	656	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-3	1 313	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-4	2 625	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-5	5 250	0/10	0/10	1/10	1/10	1/10	1/10	1/10	1/10

One test bird died in the 5250 ppm a.s. group on day 3 of the study. Because no clinical signs were noted in this or any of the birds in this group, it is unlikely that this one mortality was treatment-related. No birds in the control group died.

Mean body weights in the test groups were not significantly different when compared to control birds on test day 5 and test day 8. All birds in all groups gained substantial weight by the end of day 8. Feed consumption in the treatment groups was not markedly different from the control during the 0-5 day test period or during the day 6-8 period, though no results of statistical analysis were presented.

Table B.9.1.1.2.-4: Results of mean body weight in study on Mallard ducklings

8-day acute dietary LC_{50} study in Mallard ducklings: RH 117,281 technical					
			Mean body weight (g) of survivors		
Group	Concentration (ppm a.s.)		0 hour	Test day 5	Test day 8
Control	0	mean	108.2	265.4	359.6
		stdev	9.6	21.6	29.9
T-1	328	mean	108.3	266.9	365.3
		stdev	10.5	25.5	34.3
T-2	656	mean	108.0	272.7	365.8
		stdev	9.4	21.2	32.0
T-3	1 313	mean	107.6	237.5	322.9
		stdev	8.8	52.0	60.4
T-4	2 625	mean	108.9	273.8	370.0
		stdev	8.1	20.7	34.2
T-5	5 250	mean	107.9	260.9	357.2
		stdev	8.8	19.5	30.2

No abnormal pathological findings were noted in the one bird that died or when arbitrarily selected survivors of all groups were subjected to gross pathological examinations at study termination.

The 8-day dietary LC_{50} in mallard ducklings was > 5250 ppm a.s. (>5250 mg/a.s. kg/food).
The 8-day NOEC was 2625 ppm a.s although the single mortality at 5250 ppm was not clearly treatment-related.

RMS comments:

The study is considered acceptable. No conversion of the ppm to mg/kg bw/day was available in the original DAR. Based on study results, the RMS did the following conversion:

Daily dose [mg/kg bw/d] = (concentration in food [mg/kg diet] x daily food consumption [g/bird/d]) / body weight [g]
= (5250 x 79.4)/357.2
= 1167 mg/kg bw/day
The LD_{50} > 1167 mg/kg bw/day

B.9.1.1.3 Sub-chronic toxicity and reproduction to birds

Report: **CA, 8.1.1.3/01 [redacted] (1998)**
Avian reproduction study of RH-117,281 Technical with northern bobwhite.

Guidelines: OECD Guideline 206, US EPA FIFRA Subdivision E Guideline, Subsection § 71-4
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: ISO common name: RH-117,281 Technical, Lot No. LG 3517, chemical purity: 92.3%
Test species: Bobwhite quails (*Colinus virginianus*)
Sex, weight, age: 150 bobwhite (75 males and 75 females)
Applied concentrations: 75, 200, 500, 1 000 ppm zoxamide
Time of exposure: 22 weeks

Results:
In a reproductive toxicity study, 22-week-old bobwhite quail were exposed to RH-117,281 (purity 92.3%) in the diet for 22 weeks. Nominal food concentrations were 0 (control), 75, 200, 500, and 1000 ppm a.s. (mg a.s./kg food). There were 15 pairs per dose level, one pair per cage. The birds were maintained in a controlled environmental test room and eggs were collected daily at the onset of egg production (week 12). Hatchlings were leg banded and maintained for 14 days in brooders. Reproduction endpoints were determined with eggs produced during the last 11 weeks of the study. Reproduction endpoints were: number of eggs laid, number of eggs set, number of eggs cracked, eggshell thickness, percent fertile eggs, number of live 18 day old embryos, number of hatchlings, percent hatchability, and number of 14 day old survivors, percent survival, and chick weights at day 0 and day 14 of age. Egg shell thickness was determined on five dates for eggs laid during a one-day period every two weeks.

Table B.9.1.1.3.-1: Results of a reproductive study on bobwhite quail

Nominal concentration (ppm a.s.)	0	75	200	500	1000
Measured concentration (ppm a.s.)	<LOQ[a]	75	203	506	974
Total eggs laid	651	755	698	812	555
Mean egg production (eggs/cage)	46.5	53.9	49.9	58.0	46.3
Total eggs set	587	686	622	737	502

Mean number of eggs set (eggs/cage)	41.9	49.0	44.4	52.6	41.8
Mean eggshell thickness (mm)	0.233	0.229	0.223	0.239	0.226[b]
Total eggs cracked	10	14	20	17	11
Mean number of cracked eggs (eggs/cage)	0.7	1.0	1.4	1.2	0.9
Percent of eggs laid that were cracked (%)	1.6	1.9	3.0	2.2	1.7
Total eggs viable	521	644	547	689	474
Mean number of viable eggs (eggs/cage)	37.2	46.0	39.1	49.2	39.5
Percent of eggs set that were viable (%)	89.0	93.7	87.3	93.7	86.8
Total 18 day embryos	521	641	545	689	472
Mean number of 18 day embryos (embryos/cage)	37.2	45.8	38.9	49.2	39.3
18 day embryos as percentage of fertile eggs (%)	100.0	99.5	99.7	100.0	99.6
18 day embryos as percentage of eggs set (%)	89.0	93.3	87.0	93.7	91.1
Total number of hatchlings	433	592	461	566	374
Mean number of hatchlings (hatchlings/cage)	30.9	42.3*	32.9	40.4	31.2
Number hatchlings as percent of fertile eggs (%)	83.6	91.9*	85.0	82.3	70.2
Number hatchlings as percent of eggs set (%)	74.9	86.1	74.1	77.2	66.5
Total number of 14 day chicks	344	475	366	464	288
Mean number of 14 day chicks (chicks/cage)	24.6	33.9*	26.1	33.1*	26.2[b]
Number 14 day chicks as percent of hatchlings (%)	80.5	79.6	80.9	82.2	67.5
Number 14 day chicks as percent of eggs set (%)	43.1	59.5	45.9	58.1	46.2
Mean body weight (g) of hatchlings	6.8	7.0	6.8	6.8	6.8
Mean body weight (g) of 14 day chicks	24.3	23.9	23.8	24.5	23.0[b]

[a] LOQ = 10 mg a.s./kg food.

[b] Number of cages = 11 because one cage had only one egg and no hatchlings.

* Significantly different from control, $p<0.05$ (Note: significant differences were actually increases with respect to the control and were not considered adverse effects. It should also be noted that no dose-related effect was apparent.) The footnote * indicating that the statistically significant differences are in fact increases compared to control and not negative effects.

No treatment-related effects were observed in the adult birds. Nine birds died during the study due to physical injuries from accidents in their cages or from pecking. The mates from seven of the birds that died during weeks 8-20 were euthanized during the week of the mate's death. The mates of the two birds that died during week 22 were kept until the final necropsy. The distribution of deaths among the treatment groups was one (control), one (75 ppm), two (200 ppm) one (500 ppm) and four (1000 ppm). There were no significant differences observed among treatments for total food consumption or average total food consumption. There were no treatment-related effects observed in food consumption, growth, survival, behavioural, or reproductive endpoints. There were no statistically significant or treatment

related effects (p >0.05) upon adult body weight among dietary concentrations at any time period for females or males.

Table B.9.1.1.3.-2: Summary of mortalities by treatment group during the 22-week study with RH-117,281 Technical

Treatment	0 ppm	75 ppm	200 pmm	500 ppm	1000 ppm
Starting number of cages	15	15	15	15	15
Ending number of cages	14	14	13	14	11
Deaths in week 1	0	0	0	0	0
Deaths in week 2	0	0	0	0	0
Deaths in week 3	0	0	0	0	0
Deaths in week 4	0	0	0	0	0
Deaths in week 5	0	0	0	0	0
Deaths in week 6	0	0	0	0	0
Deaths in week 7	0	0	0	0	0
Deaths in week 8	0	0	1	0	0
Deaths in week 9	0	0	0	0	0
Deaths in week 10	0	0	0	0	0
Deaths in week 11	0	0	0	0	0
Deaths in week 12					
Deaths in week 13	0	0	0	0	0
Deaths in week 14	0	0	0	0	1
Deaths in week 15	1	0	0	1	0
Deaths in week 16	0	0	0	0	0
Deaths in week 17	0	1	0	0	0
Deaths in week 18	0	0	0	0	1
Deaths in week 19	0	0	0	0	1
Deaths in week 20	0	0	0	0	0
Deaths in week 21	0	0	0	0	0
Deaths in week 22	0	0	1*	0	1*
* Deaths in week 22 occurred in the last few days and data from these cages used in analysis					

RMS comment - Data about mortality on week 12 is missing in the study report.

Based on the absence of effects on survival, growth, and reproductive parameters, the NOEC in bobwhite quail exposed to RH-117,281 in the diet was 1000 ppm zoxamide (mean measured concentration 974 ppm a.s.), the greatest concentration tested (NOEC = 1000 mg/a.s. kg/food).

RMS comments:
At 1000 ppm, no statistical differences were observed, however, 15 to 17% of reduction of total eggs laid, total eggs set, total number of hatchlings, number hatchlings as percent of fertile eggs (%), number hatchlings as percent of eggs set (%), total number of 14 day chicks and number 14 day chicks as percent of hatchlings (%) were observed. This is probably due to the fact that in one cage only one egg and no hatchlings were found. No differences compared to the control were observed for mean egg production (eggs/cage), mean number of eggs set (eggs/cage), mean number of hatchlings (hatchlings/cage) and mean number of 14 day chicks (chicks/cage). No EC_{10} can be derived.

Study is considered acceptable.

No conversion of the NOEC to NOEL was available in the original DAR. Based on study results, the RMS did the following conversion:

Daily dose [mg/kg bw/d] = (concentration in food [mg/kg diet] x daily food consumption [g/bird/d]) / body weight [g] = (1000 x 33.7)/213 = 158.2 mg/kg bw/d

The NOEL = 158.2 mg/kg bw/day

Report:	**CA, 8.1.1.3/02 [redacted] (1999) RH-117,281 technical: A reproduction study with the mallard (*Anas platyrhynchos*).**

Guidelines: OECD Guideline 206, US EPA FIFRA Subdivision E Guideline, Subsection § 71-4
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR 9510), chemical purity: 92.3%
Test species: Mallard duck (*Anas platyrhynchos*)
Sex, weight, age: 160 mallards (80 hens and 80 drakes), weight range from 866 – 1373 g, 18-week-old
Applied concentrations: 0, 75, 200, 500, 1 000 ppm zoxamide
Time of exposure: 22 weeks

Results:
In a reproductive toxicity study, breeding pairs of 18-week-old mallard ducks were exposed to RH-117,281 (purity 92.3%) in the diet for 22 weeks. Nominal food concentrations were 0 (control), 75, 200, 500, and 1000 ppm a.s. (mg a.s./kg food). There were 16 pairs per dose level, one pair per cage. The birds were observed daily for signs of toxicity, abnormal behaviour, and mortality. Adult body weights were measured at test initiation, on weeks 2, 4, 6, and at adult termination. Feed consumption was measured for each pen for a seven day period every week throughout the test. Necropsies were performed on all adults that died during the test and all adults surviving until test termination. The birds were maintained in a controlled environmental test room and eggs were collected daily at the beginning of Week 11 and set weekly for incubation. Reproduction endpoints were: number of eggs laid, number of eggs set, number of eggs cracked, eggshell thickness, percent viable embryos, number of live 21 day old embryos, number of hatchlings, bodyweight of hatchlings, percent hatchability, number of 14 day old survivors (chicks) and percent survival, and 14 day old chick weights. At concentration of 500 ppm a.s. 13 to 20% reduction from control are observed for the majority of the parameters, but such reductions were not observed at 75, 200 and 1000 ppm. No treatment related effects are observed.

Table B.9.1.3.-3: Results of a reproductive study on mallard

Nominal concentration (ppm a.s.)	0	75	200	500	1000
Measured concentration (ppm a.s.)	<LOQ[a]	77.6	215	517	1040
Total eggs laid	581	682	788	499	725
Eggs laid per hen	39	43	49	31	45
Eggs laid per hen per day (based on 94 days of egg production)	0.41	0.45	0.52	0.33	0.48

Nominal concentration (ppm a.s.)	0	75	200	500	1000
Eggs laid of maximum laid (%) (eggs/hen/maximum eggs laid by any hen in test)	47	52	60	38	55
Total eggs set	511	607	694	435	641
Mean eggshell thickness (mm) (n=number of eggs measured)	0.387 (n=55)	0.384 (n=59)	0.386 (n=65)	0.382 (n=48)	0.389 (n=64)
Total eggs cracked	4	8	12	10	11
Eggs cracked of eggs laid (%) (eggs cracked/eggs laid per pen)	1	1	2	2	1
Total viable embryos	462	568	615	404	596
Viable embryos of eggs set (%) (viable embryos/eggs set per pen)	89	93	90	81	93
Total 21 day embryos	459	566	610	401	593
Live 21 day embryos of viable embryos (%) (live 21 day embryos/viable embryos per pen)	99	100	99	99	100
Total number of hatchlings	375	496	488	324	488
Hatchlings of 21 day embryos (hatchlings/21 day embryos per pen)	80	87	81	78	81
Total 14 day chicks	371	493	475	316	476
14 day chicks of hatchlings (14 day chicks/hatchlings)	99	99	97	98	97
Hatchlings of eggs set (Hatchlings/eggs set per pen)	71	81	72	63	76
14 day chicks of eggs set (14 day chicks/egg set per pen)	71	80	70	62	74
14 day chicks per hen	25	31	30	20	30
Hatchlings of maximum set (hatchlings per pen/maximum set from any one hen)	35	44	43	29	43
14 day chicks of maximum set (14 day chicks per pen/maximum set from any one hen)	35	43	42	28	42
Mean body weight of hatchlings (g)	35	34	33	34	35
Mean body weight of 14 day chicks (g)	263	261	257	248	245

[a] LOQ= Limit of Quantitation = 5 mg a.s./kg food.

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the test concentrations. In addition, there were no treatment related effects upon any of the reproductive parameters measured at any of the concentrations tested.

Table B.9.1.1.3.-4: Mean food consumption (g/bird/day) for Mallard reproduction study with RH-117.281 Technical

Experimental group (ppm a.s.)	Weeks																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Control	110	134	123	102	136	129	118	141	103	126	136	120	149	157	190	174	189	184	196	191	174	162
75	104	124	110	79*	120	102*	105	132	81	104	120	121	140	140	166	151	190	172	185	179	178	164
200	115	102**	109	91	111*	107	100	120	79*	102	124	120	147	145	166	157	179	170	187	172	176	164
500	119	131	118	84	123	109	101	127	75*	86*	120	120	138	143	156	141	171	156	191	173	164	147
1000	121	107*	109	83	117	107	102	123	88	111	138	123	144	171	165	162	188	178	196	187	188	185
* Significantly different from the control at $p < 0.05$																						
** Significantly different from the control at $p<0.01$																						

Although some individual differences were statistically significant, the fact that they were neither consistent over the test period nor dose responsive, was considered to indicate that they were not related to treatment.

Due to excessive wastage by some birds, feed consumption was variable among pens. However, there were no apparent treatment-related effects upon feed consumption at any of the concentrations tested.

Comparing to the control group, there were slight, sporadic reductions in feed consumption at all test concentrations during the first ten week of the test. The slight decrease in feed consumption were statistically significant in the 75 ppm a.s. treatment during Weeks 4 (p=0.0207) and 6 (p=0.0271), in the 200 ppm a.s. treatment group during Week 5 (p=0.0202) and 9 (p=0.0447), in the 500 ppm a.s. treatment group during Week 9 (p=0.0113) and 10 (p=0.0137), and in the 100ppm a.s. treatment group during Week 2 (p=0.0289). There also was slight decrease in feed consumption in the 200 ppm a.s. treatment group during Week 2 that was statistically significant at p=0.0070. Since the differences observed were neither consistent over the test period nor dose responsive, the differences were not considered to be related to treatment. There were no other statistically significant differences between the control group and of the treatment groups at any of the other feed consumption intervals.
There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the test concentrations. There were no treatment-related effects upon any of the reproductive parameters measured at any of the concentrations tested. Based on the absence of effects on survival, growth, and reproductive parameters, the NOEC in mallard exposed to RH-117,281 in the diet was 1000 ppm a.s. (mean measured concentration 1040 ppm), the greatest concentration tested (NOEC = 1000 mg/a.s. kg/food).

RMS comments:
The study is considered acceptable.
No conversion of the NOEC to NOEL was available in the original DAR. Based on study results, the RMS did the following conversion:

Daily dose [mg/kg bw/d] = (concentration in food [mg/kg diet] x daily food consumption [g/bird/d]) / body weight [g] = (1000 x 140.6)/1230 = 114.3 mg/kg bw/d

The NOEL = 114.3 mg/kg bw/day

B.9.1.2 Effects on terrestrial vertebrates other than birds

B.9.1.2.1 Acute oral toxicity to mammals

The acute oral LD_{50} is estimated to be > 5000 mg a.s./kg b.w. for rat (see Vol 3, A.S. B.6.2.1 for further details).

B.9.1.2.2 Long-term and reproduction toxicity to mammals

The lowest NOEL is estimated to be 360 mg a.s./kg b.w./d from the multigeneration study on rat (see Vol. 3, A.S. B.6.6 for further details).

B.9.1.3 Active substance bioconcentration in prey of birds and mammals

According to the EU requirements, substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of bioaccumulation in prey of birds and mammals. Log Kow of zoxamide is greater than three (3.76) an assessment of secondary poisoning to earthworm-eating and fish-eating birds is required. Bioconcentration study in fish has been carried out (see B.9.2.1). Moreover, the risk from bioaccumulation to fish-eating and worm-eating birds has been carried out and is considered acceptable (TER>5) (see Volume 3, B.9 of the product).

Log Kow of metabolite RH-127,450 is greater than three (3.5) an assessment of secondary poisoning to earthworm-eating and fish-eating birds and mammals is required (see Volume 3, B.9 of the product).

RMS comments:

There are no toxicity endpoints for for birds and mammals for metabolite RH-127,450. Risk assessment of secondary poisioning to earthworm-eating and fish-eating birds and mammals for metabolite RH-127,450 could not be finalized and is considered as data gap.

B.9.1.4 Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No studies on other terrestrial vertebrates have been submitted. In the case of zoxamide, there are no studies in the literature on the toxicity of this active ingredient on amphibians and reptiles.

B.9.1.5 Potential for endocrine disruption

There are no indications of endocrine-disrupting effects from the existing database for active substance zoxamide. No conclusions regarding endocrine effects were made in the EU review.

Considering current data requirements, zoxamide is not expected to have an endocrine disrupting potential (see Vol. 3 A.S. B.6.8.3.)

B.9.2 EFFECTS ON AQUATIC ORGANISMS

B.9.2.1 Acute toxicity to fish

Report:	**CA, 8.2.1/01 [redacted] (1995a)** **Acute flow-through toxicity of RH-117,281 Technical to rainbow trout (*Oncorhynchus mykiss*).**

Guidelines: US EPA FIFRA/ OPPTS 850.1075 Subdivision E Guideline, Subsection § 72-1
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DK 2011), chemical purity: 94.2% a.s.
Test species: Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, loading:

140 rainbow trout, blotted wet weight: 1.2 ± 0.30 g, Min = 0.801 g, Max = 2.082 g; standard length: 44 ± 3 mm, Min = 39 mm, Max = 53mm; Biomass loading Rate: 0.11 g/L/day
Type of test: flow-through acute toxicity test (96 hours)
Applied and measured concentrations:
nominal test concentrations: Control (0.0), Vehicle Blank (0.10 mL/L acetone) 52, 86, 140, 240 and 400 µg a.i./L
The mean measured test concentrations: 0, Vechile Blank, 51, 71, 140, 210 and 380 µg a.i./L.
Test conditions:
temperature: 12 °C
pH: 8.1 to 8.2
oxygen content: 9.5 g/L
total hardness: 144 to 148 mg/L CaCO3
photoperiod: 16 hours daylight with 30-minute transition period

Results:

The 24- and 48-hour LC_{50} was >210 µg a.i./L. The 72- and 96-hour LC_{50} values were 260 and 160 µg a.i./L, respectively. The 95% confidence limits for the 96-hour LC_{50} were 130 and 180 µg a.i./L. Mortality and/or sublethal effects were noted in the 140-, 210-, and 380- µg a.i./L concentrations. The 96-hour no-observed effect concentration (NOEC) was 71 µg a.i./L based on the lack of >10% effects or mortality at this and all lower concentrations. The slope of the 96-hour dose response line was 5.7 as calculated by least-squares regression analysis of log concentration versus mortality transformed to probits.

96-hour LC_{50} 160 µg a.i./L (95% Confidence Limits of 130 and 180 µg a.i./L) (mean measured);

96-hour NOEC 71 µg a.i./L (mean measured).

Table 9.2.1.-1: Mortality and sublethal effects observations during the acute flow-through toxicity test of RH-117,281 Technical to Raibow trout (*Oncorhynchus mykiss*)

Mean measured test conc. (µg a.s./L)	Number placed in test	24 hours		48 hours		72 hours		96 hours	
		Cum. mort.	Obs.	Cum. mort.	Obs.	Cum. mort.	Obs.	Cum. mort.	Obs.
Control	20	0	20 N	0	20 N	0	20 N	0	20 N
Vehicle Blank	20	0	20 N	0	20 N	0	20 N	0	20 N
51	20	0	20 N	0	20 N	0	20 N	0	20 N
71	20	0	20 N	0	20 N	0	20 N	1	19 N
140	20	0	1 SUR; 16 N; 3 Q/OB	1	1 SUR; 18 Q/OB	3	5 DK/Q/OB; 12 Q/OB	5	4 DK/Q/OB; 11 Q/OB
210	20	2	7 DK/Q/OB; 2 Q/OB; 2 SUR; 7 N	4	1 SUR; 15 DK/Q/OB	7	1 SUR/DK; 12 DK/Q/OB	17	3 DK/OB/LR
380	20	8	12 DK/Q/OB	11	9 DK/Q/OB	15	5 DK/Q/OB/LR	20	---

Key to observations: N = Normal; SUR = Surfacing; Q = Quiescent; OB = Fish on the bottom of the test chamber; DK = Dark discoloration; LR = Labored respiration

RMS comments:

96-hour LC_{50} 160 µg a.i./L (mean measured);
96-hour NOEC 71 µg a.i./L (mean measured).
Study is considered acceptable.

Report:	**CA, 8.2.1/02 [redacted] (1995b)** **Acute flow-through toxicity of RH-117,281 Technical to bluegill (*Lepomis macrochirus*).**

Guidelines: US EPA FIFRA/ OPPTS 850.1075 Subdivision E Guideline, Subsection § 72-1
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DK 2011), chemical purity: 94.2% a.s.
Test species: bluegill (*Lepomis macrochirus*)
Number of organisms, weight, length, loading:
140 bluegill sunfish, blotted wet weight: 0.66 ± 0.23 g, Min = 0.368 g, Max = 1.134 g; standard length: 30 ± 4 mm, Min = 22 mm, Max = 37 mm; Biomass Loading Rate: 0.064 g/L/day
Type of test: flow-through acute toxicity test (96 hours)
Applied and measured concentrations:
nominal test concentrations: Control (0.0), Vehicle Blank (0.10 mL/L acetone) 0.13, 0.22, 0.36, 0.60, and 1.0 mg a.i./L
The mean measured test concentrations: 0, Vechile Blank, 0.10, 0.17, 0.29, 0.49 and 0.79 mg a.i./L.
Test conditions:
temperature: 23 °C
pH: 8.0 to 8.1
oxygen content: 8.3 g/L
total hardness: 142 to 158 mg/L CaCO3
photoperiod: 16 hours daylight with 30-minute transition period

Results:
The 24-, 48-, 72- and 96-hour LC_{50} values were all > 0.79 mg a.i./L (solubility limit in ABC hard blended water). The 96-hour no-observed effect concentration (NOEC) at the end of the study was 0.10 mg a.i./L. Dose-related sublethal effects were noted at 0.17-, 0.29-, 0.49-, and 0.79-mg a.i./L concentrations.

Exophtalmia was observed as a transient effect in a majority of the bluegill in all treated groups at 24 hr, but not at 48, 72 and 96 h, and was therefore not judged to be a treatment related effect.
96-hour LC_{50} > 0.79 mg a.i./L (mean measured)
96-hour NOEC = 0.10 mg a.i./L (mean measured)

Table 9.2.1-2: Mortality and sublethal effects observations during the acute flow-through toxicity test of RH-117,281 Technical to Bluegill (*Lepomis macrochirus*)

Mean measured test conc. (mg a.s./L)	Number placed in test	24 hours		48 hours		72 hours		96 hours	
		Cum. mort.	Obs.	Cum. mort.	Obs.	Cum. mort.	Obs.	Cum. mort.	Obs.
Control	20	0	20 N	0	20 N	0	20 N	0	20 N
Vehicle Blank	20	0	20 N	0	20 N	0	20 N	0	20 N
0.10	20	0	11 EX; 9 N	0	20 N	0	20 N	0	20 N
0.17	20	0	11 EX; 9 N	0	20 N	0	1 OB; 19 N	0	4 ES; 16 N
0.29	20	1	11 EX; 8 N	2	1 OB; 17 N	2	2 ES; 1 OB; 15 N	2	1 OB; 5 ES; 12 N
0.49	20	0	1 EX/OB; 13 EX; 6 N	0	1 LOE/OB/LR; 5 OB; 14 N	0	1 LOE/OB/LR; 8 OB; 1 ES; 10 N	0	1 LOE/OB/LR; 4 OB; 4 ES; 11 N
0.79	20	1	4 EX/OB; 11 EX; 4 N	2	2 LOE/OB/LR; 5 OB; 11 N	2	3 ES; 8 OB; 7 N	2	5 OB; 5 ES; 3 Q; 5 N

Key to observations: N = Normal; Q = Quiescent; OB = Fish on the bottom of the test chamber; LR = Labored respiration; EX = Exophtalmic (pop-eyed); ES = Erratic swimming; LOE = Loss of equilibrium

Note: Eyes equilibrated to te compound by the 48 hours observation, thus suggesting exophtalmia was transient effect.

RMS comments:
96-hour LC_{50} > 0.79 mg a.i./L (mean measured)
96-hour NOEC = 0.10 mg a.i./L (mean measured)
Study is considered acceptable.

Report: **CA, 8.2.1/04 [redacted] (1998a)**
RH-117,281 Technical: A 96-hour flow-through acute toxicity test with the zebra fish (*Brachydanio rerio*).

Guidelines: OECD 203
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: zebra fish (*Brachydanio rerio*)
Number of organisms, weight, length, age, loading:
140 zebra fish, mean weight (g) 0.20, range 0.14 to 0.28; total length (mm) 29, range 27 to 30, juveniles.
Type of test: flow-through acute toxicity test (96 hours)
Applied and measured concentrations:
Nominal test concentrations: Negative control, solvent control (0.10 mL/L acetone) 0.16, 0.26, 0.43, 0.72 and 1.2 mg a.i./L;
The mean measured test concentrations: Negative control, solvent control, 0.16, 0.25, 0.40, 0.61 and 0.73 mg a.i./L
Test conditions:
temperature: 22 °C
pH: 8.1 to 8.4
oxygen content: 5.2g/L
photoperiod: 16 hours of light and 8 hours of darkness

Results:
Groups of 20 zebra fish (10 per replicate) were exposed to a geometric series of five test concentrations, a solvent control (1.0 mL Acetone/L) and negative (well water) control for 96-hours. Nominal test concentrations used in the study were 0.16, 0.26, 0.43, 0.72 and 1.2 mg a.i./L. The test concentrations were adjusted for purity of the active ingredient (92.3%) in the test substance.

Table 9.2.1.-3: Cumulative percent mortality and treatment-related effects of Zebra fish exposed to RH-117,281 Technical under flow-through conditions

Mean measured test conc. (mg a.s./L)	Replicate		3 hours		24 hours		48 hours		72 hours		96 hour		Cumulative percent mort.
		No. exposed	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	
Negative control	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0

Solvent control	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
0.16	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
0.25	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
0.40	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
0.61	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
0.73	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0

[1] Cumulative number of dead fish

[2] Observed Effects: AN = Appear normal.

In the mixing chambers, the 0.72 and 1.2 mg a.i./L solutions had slight white precipitate noted at the water surface and covering the mixing caps. All other solutions in the mixing chambers were clear and colorless with no precipitate noted. Test solutions in the actual test chambers remained clear and colorless throughout the test. Concentrations of RH-117,281 technical in the test solutions were measured prior to the test initiation and at the beginning and end of the test. Mean measured concentrations were 0.16, 0.25, 0.40, 0.61 and 0.73 mg a.i./L. These mean values represent 100, 96, 93, 85 and 61 % of nominal test concentrations, respectively.

Temperature measurements were within acceptable limits (22±1 ^{0}C) throughout the 96-hour exposure period. Water pH measurements ranged from 8.1 to 8.4 and were typical of moderately-hard freshwater. Measurements of dissolved oxygen concentrations remained in excess of 60% saturation (i.e. > 5.2 mg/L) throughout the test period.

Zebra fish in the negative control, solvent control and all RH-117,281 treatment groups appeared healthy and normal throughout the test. The 96-hour LC_{50} was >0.73 mg a.i./L, the highest concentration achievable under the conditions of administration. The no mortality concentration and the no-observed-effect-concentration (NOEC) were 0.73 mg a.i./L.

96-hour LC_{50} > 0.73 mg a.i./L (mean measured).

NOEC = 0.73 mg a.i./L (mean measured).

RMS comments:

96-hour LC_{50} > 0.73 mg a.i./L (mean measured).

NOEC = 0.73 mg a.i./L (mean measured).

Study is considered acceptable.

Report:	**CA, 8.2.1/05 [redacted] (1997)** **RH-117,281 Technical: a 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*).**

Guidelines: US EPA OPPTS 850.1075/FIFRA/ Subdivision E Guideline, Subsection § 72-3 (a)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: sheepshead minnow (*Cyprinodon variegatus*)
Number of organisms, weight, length, loading:
140 sheepshead minnows, mean weight (g) 0.30, range = 0.14 to 0.47; mean standard length (mm) 0.22, range = 18 to 26, juveniles.
Type of test: flow-through acute toxicity test (96 hours)
Applied and measured concentrations:
nominal test concentrations: Negative control, solvent control(0.5 mL/L acetone) 0.13, 0.22, 0.36, 0.60, and 1.0 mg a.i./L
The mean measured test concentrations: <LOQ, <LOQ, 0.136 0.235, 0.349, 0.482 and 0.855 mg a.i./L.
Test conditions:
temperature: 22 °C
pH: 8.4 to 8.5
oxygen content: >4.7 mg/L

Results:
Sheepshead minnows in the negative and solvent control groups appeared healthy and normal throughout the test. Te 96-hour LC50 was >0.855 mg a.i./L, the highest concentration achievable. The no mortality concentration and the no-observed-effect-concentration (NOEC) were 0.855 mg a.i./L.

Table 9.2.1.-4: Cumulative percent mortality and treatment-releated effects

Mean measured test conc. (mg a.s./L)	Replicate		20 hours		24 hours		48 hours		72 hours		96 hour		Cumulative percent mortality
		No. exposed	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	No. dead[1]	Effects[2]	
Negative control	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
Solvent control	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.136	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.235	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	

0.349	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.482	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.855	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	

[1] Cumulative number of dead fish

[2] Observed Effects: AN = Appear normal.

96-hour LC_{50} > 0.855 mg a.i./L (mean measured).
NOEC = 0.855 mg a.i./L (mean measured).

RMS comments:
96-hour LC_{50} > 0.855 mg a.i./L (mean measured)
NOEC = 0.855 mg a.i./L (mean measured)
Study is considered acceptable.

Report: **CA, 8.2.1/06 [redacted] (1998a)**
Acute toxicity of RH-127,450 to the rainbow trout (*Oncorhynchus mykiss)* in a range-finding test under static conditions.

Guidelines: OECD 203
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-127,450 (Lot No. BM3933), chemical purity: 99.27% a.s.
Test species: Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, loading:
100 rainbow trout, blotted wet weight: from 0.255 to 0.540 g, mean = 0.375 ± 0.0974 g; standard length: 33 ± 2.3 mm, range from 30 to 37 mm; loading: 0.25 g fish tissue per liter of test solution.
Type of test: static acute toxicity test (96 hours)
Applied and measured concentrations:
Nominal test concentrations: 0.0 (control), 0.0 (500 µL./L acetone control), 43.8, 87.5, 175, 350 and 700 mg a.i./L
Nominal test concentrations for range-finding exposure: 0.0 (control), 0.0 (500 µL/L acetone control), 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.i./L.
Test conditions:
temperature: range from 11.2 to 12.4 °C

pH: range from 7.5 to 8.2
oxygen content: range from 9.6 to 9.8 mg/L
total hardness: 130 to 160 mg/L CaCO3
photoperiod: 16 hours daylight with 30-minute transition period
solubility of metabolite RH-127,450: water - 0%, acetone – 100%

Results:
During the first 48 hours of exposure, test solutions appeared clear with no visible precipitate or surface film. At 72 hours, crystals of what was believed to be RH 127,450 were observed floating on the surface of the 5.0 mg a.i./L test solution. By 96 hours, crystals were observed floating on the surface of the 2.5 and 5.0 mg a.i./L test solutions. These observations suggest functional water solubility under the test conditions had been achieved. Water quality measurements were within acceptable limits throughout the exposure.

Atfer 96 hours of exposure, no mortality was observed at any concentration tested. Sublethal effects observed included dark discoloration, fish resting on or near the bottom of the test chamber, loss of equilibrium, and fish remaining in the top quarter of the water column. These observations were most prevalent in the 1.3, 2.5 and 5.0 mg a.i./L treatments. One individual in each of the 0.31 and 0.63 mg a.i./L treatments was observed remaining in the top quarter of the water column at 96 hours.

Table 9.2.1.-5: Behavioural observations during a 96-hour static exposure of Rainbow trout (*Oncorhynchus mykiss*) to RH-127, 450

Nominal conc. (mg a.s./L)	Behavioral observations (% affected)			
	24-hours	48-hours	72-hours	96-hours
Control	10 N (0)	10 N (0)	10 N (0)	10 N (0)
Acetone control	10 N (0)	10 N (0)	10 N (0)	10 N (0)
0.31	10 N (0)	10 N (0)	10 N (0)	9 N; 1 S 910)
0.63	10 N (0)	10 N (0)	10 N (0)	9 N; 1 S (10)
1.3	4 N; 6 S (60)	6 N, 4 S (40)	8 N; 2 S (20)	4 N; 6 S (60)
2.5	1 N; 9 S (90)	2 N; 8 S (80)	3 N; 6 S; 1 B (70)	1 N; 9 S (90)
5.0	7 D; 3 D/B (100)	4 D/L; 6 D/B (100)	7 D/B; 3 D/L (100)	1 D; 7 D/B; 2 D/L (100)

Key to observations: N = Normal; S = Fish remaining in the top quarter of the water column; L = Loss of equilibrium; D = Dark discoloration; B = Resting on or near the bottom of the test chamber.

The 96-hour LC_{50} for rainbow trout exposed to RH-127,450 is estimated to be > 5.0 mg a.i./L. The no-observed-effect concentration (NOEC) was 5.0 mg a.i./L based on lack of mortality.

LC_{50} >5.0 mg a.i./L (nominal)
NOEC = 5.0 mg a.i./L (nominal)

RMS comments:
The study with metabolite RH-127,450 is considered acceptable.

No information in the study about how crystals impacted the results of the study, solubility of metabolite in water is 0%, and acetone was used as solvent (solubility in acetone 100%) . No mortality has been observed in the study; some sublethal effects appered in treatments groups like dark discoloration, fish remaining in the top quarter of the water column (see Table 9.2.1.-5). RMS thinks crystals have not impacted the study results; probably crystals left impact on sublethal effects but there is no information about it in the study.

RMS agrees with endpoints LC_{50} >5.0 mg a.i./L (nominal) and NOEC = 5.0 mg a.i./L (nominal)

Report:	**CA 8.2.1/07 [redacted] (2002)** **Zoxamide metabolite RH-139,432 – Acute toxicity to Rainbow Trout (*Oncorhynchus mykiss)* under static conditions**

Guidelines: OECD Guideline 203, US EPA FIFRA Guideline 72-1
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Materials and Methods:
Test substance: Zoxamide metabolite RH-139,432, Lot No. LJG&-39A, Identification No. TSN103194, purity 99.8% a.s.
Test species: Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, loading:
mean total length = 48 mm (range; 41 to 56 mm), N=30; mean wet weight = 1.3 g (range: 0.69 to 2.04 g), N=30
Type of test: static acute toxicity test (96 hours)
Applied and measured concentrations:
Nominal test concentrations: 0.38, 0.75, 1.5, 3.0 and 6.0 mg a.i./L
Mean measured concentrations: 0.19, 0.39, 0.76, 1.5 and 3.1 mg a.i./L.
Test conditions:
temperature: range from 13 to 14 °C
pH: range from 7.4 to 7.6
dilution water: well water
dissolved oxygen concentration: range of 92 to 94% saturation
total hardness: 40 to 54 mg/L CaCO3
total alkalinity: 32 mg/L
photoperiod: 16 hours light and 8 hours darkness
solubility of metabolite RH-127,450:

Results:

The water quality parameters were unaffected by the concentrations of zoxamide metabolite RH-139,432 tested and remained within acceptable ranges for the survival of rainbow trout. Daily measurement of the temperature in the test solutions and continuous temperature monitoring established that the exposure solution temperature ranged from 13 to 14 ^{o}C during the definitive study.

Measured concentrations were consistent over the exposure period and were defined as 0.19, 0.39, 0.76, 1.5 and 3.1 mg a.s./L. Analysis of the quality control samples resulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 92.9 to 112% (n=6) of the nominal fortified levels (0.25, 0.75, 3.13 and 6.25 mg a.s./L) recoveries of approximately 50% of the nominal concentrations were expected based on the amount of undissolved material observed in the water accommodated fractions. Based on the resukts of these analyses, it was established that the appropriate precision and quality control was maintained during the analyses of the exposure solutions.

The 96-hour LC_{50} value for zoxamide metabolite RH-139,432 and *Oncorhynchus mykiss* was estimated by nonlinear interpolation to be 2.0 mg a.s./L, with 95% confidence intervals, calculated by binomial probability, of 1.5 to 3.1 mg a.s./L. The No-Observed-Effect concentration (NOEC) was determined to be 0.76 mg a.s./L. The highest concentration producing 0% mortality was 0.76 mg a.s./L. The lowest concentration producing 100% mortality was 3.1 mg a.s./L. No mortality was observed in any of the remaining treatment levels or the control. Sublethal effects (i.e., partial and complete loss of equilibrium, etc.) were observed among surviving organisms exposed to the 1.5 mg a.s./L exposure level.

Table B.9.2.1-6: Concentrations of zoxamide metabolite RH-139, 432 measured in the exposure solutions during 96-hour static acute exposure of rainbow trout (*Oncorhynchus mykiss)*

Nominal concentration (mg a.s./L)	Measuerd concentration (mg a.s./L)			
	0-hour	96 hour	Mean[a]	Percent of nominal
Control	<0.032	<0.033	NA[b]	NA
0.38	0.18	0.19	0.19	49
0.75	0.39	0.39	0.39	52
1.5	0.76	0.76	0.76	51
3.0	1.6	1.4	1.5	51
6.0	2.9	3.2	3.1	51
QC[c] #1 0.250	0.253 (101)	0.268 (107)		
QC #2 0.750	0.811 (108)	0.697 (92.9)		
QC #3 3.13/6.25[d]	3.49 (112)	6.34 (101)		

[a] Values are based on actual analytical results and not on rounded values (two significant figures) presented in this table.

[b] NA = Not applicable.

[c] QC = Quality control sample, with percent of nominal presented in parentheses.

[d] Prepared concentrations at 0-hour/96-hour.

Table B.9.2.1-7: Mean measured concentrations tested, corresponding mortalities and observations made during the 96-hour static acute toxicity test exposing rainbow trout (*Oncorhynchus mykiss)* to zoxamide metabolite RH-139,432

Mean measured concentration (mg a.s./L)	Cumulative percent mortality[a]						
	2-hour	3-hour	6-hour	24-hour	48-hour	72-hour	96-hour
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.19	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

0.39	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.76	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.5	0[b] (0)	0[bd] (0)	0[be] (0)	10[bg] (1)	10[ce] (1)	10[cd] (1)	10[f] (1)
3.1	0[cd] (0)	0[cd] (0)	0[f] (0)	90[b] (9)	100 (10)	100 (10)	100 (10)

[a] Actual number of mortalities is presented in parentheses.

[b] One surviving fish exhibited a complete loss of equilibrium.

[c] several surviving fish exhibited a complete loss of equilibrium.

[d] One surviving fish exhibited a partial loss of equilibrium.

[e] Two surviving fish exhibited a partial loss of equilibrium.

[f] All fish exhibited a complete loss of equilibrium.

[g] Several fish exhibited a partial loss of equilibrium.

Tables B.9.2.1-8: The LC_{50} values, corresponding 95% confidence intervals and No-observed-effect concentration (NOEC) established during the 96-hour static acute exposure of rainbow trout (*Oncorhynchus mykiss)* to zoxamide metabolite RH-139,432

	LC_{50} (mg a.s./L)	95% Confidence intervals	
		Lower (mg a.s./L)	Upper (mg a.s./L)
24-hour[a]	2.2	1.7	2.8
48-hour[b]	2.0	1.5	3.1
72-hour[b]	2.0	1.5	3.1
96-hour[b]	2.0	1.5	3.1

[a] LC_{50} value and 95% confidence intervals were calculated by probit analysis.

[b] LC_{50} value estimated by nonlinear interpolation. Corresponding 95% confidence intervals calculated by binomial probability.

96-hour LC_{50} = 2.0 mg a.s./L (nominal)
NOEC = 0.76 mg a.s./L (nominal)

RMS comments:

No information about solubility of metabolite RH-139,432 and used solvent in the study, just mentioned that the exposure solutions were prepared from dilutions of 50 mg a.s./L water accommodated fraction. It is noted that resultant solution was observed to be clear and colorless with undissolved test substance present on the surface and a very small amount at the bottom of the mixing vessel. No information how undissolved substance have influenced the results of study. RMS still considers study acceptable.

96-hour LC_{50} = 2.0 mg a.s./L (nominal)
NOEC = 0.76 mg a.s./L (nominal)

B.9.2.2 Long-term and chronic toxicity to fish

Report:	**CA, 8.2.2.1/01 [redacted] (1996)** **Early life-stage toxicity of RH-117,281 Technical to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions.**

Guidelines: OECD Guideline 210, US EPA OPPTS 850.1400/FIFRA Subsection § 72-4
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. LG3517), chemical purity: 92.9%
Test species: Rainbow trout (*Oncorhynchus mykiss*)

Results:
A 95-day fish early life stage flow-through study was undertaken with eggs, larvae and juveniles of rainbow trout (*Oncorhynchus mykiss*). Newly fertilised eggs (<4 hours post-fertilisation, four replicates/concentration, 30 eggs/replicate) were exposed to nominal concentrations of 0.00038, 0.00075, 0.0015, 0.0030, and 0.0060 mg a.s./L plus control and acetone solvent control. Mean measured concentrations of RH-117,281 were 0.000403, 0.000882, 0.00170, 0.00348, and 0.00687 mg a.s./L, representing 106 to 118% of nominal. Water quality parameters were: temperature (10 ± 1°C), dissolved oxygen (>91% of saturation) and pH (8.1 to 8.5). The total duration of the exposure period was 95 days. Sac-fry were thinned to 15 per replicate at day 39 to give a total of 60 individuals per concentration. Survival and growth parameters were monitored throughout the 61 day post-hatch period.

Time to hatch and time to initiation of sustained swim-up was not affected at any test concentration when compared to the pooled control group. Morphological and behavioural abnormalities were observed in the control, vehicle control, and all test concentrations but the 0.00687 mg a.s./L group exhibited significantly more abnormalities and with a higher frequency. The abnormalities at this concentration were therefore considered to be related to exposure to RH-117,281.

Egg hatchability was not affected at any test concentration when compared to the pooled control group. Survival was significantly reduced in the 0.00687 mg a.s./L treatment when compared to the pooled control group on day 35 post-hatch (75% reduction), and the vehicle blank on day 61 post-hatch (84% reduction). A statistically significant survival effect was also observed in the 0.000403 mg a.s./L treatment on day 61 post-hatch when compared to the vehicle blank. This is unlikely to be due to the exposure to RH-117,281 as no significant adverse effects on survival were seen at three higher doses. At 35 and 61 days post-hatch, mean standard length was not affected at any test concentration when compared to the pooled control group. At 61 days post-hatch, blotted wet weight was not affected at any test concentration when compared to the pooled control (0.00687 mg a.s./L was excluded from day 61 post-hatch analyses due to a significant survival effect).

Table 9.2.2.-1: Egg hatchability and survival of Rainbow trout (*Oncorhynchus mykiss*) exposed to RH-117,281 Technical

Mean measured RH-117,281 concentration (µg a.s./L)	Replicate	Egg hatch (%)	35-day post-hatch survival (%)	61-day post-hatch survival (%)
Dilution water control	A	100	93.3	85.7
	B	100	100	100
	C	95.5	73.3	73.3
	D	100	80.0	80.0
	Mean	**98.9**	**86.7**	**84.7**

Vehicle Blank	A	100	100	100
	B	100	100	100
	C	100	100	100
	D	95.5	86.7	86.7
	Mean	**98.9**	**96.6**	**96.5**
Pooled control	**Mean**	**98.9**	**91.7**	**----**
Level 1 (0.403)	A	90.9	80.0	80.0
	B	95.5	61.5	53.8
	C	95.5	93.3	86.7
	D	100	93.3	93.3
	Mean	**95.5**	**82.7**	**79.3*[a]**
Level 2 (0.882)	A	100	100	100
	B	100	93.3	93.3
	C	100	93.3	93.3
	D	95.5	80.0	80.0
	Mean	**98.9**	**91.7**	**91.5**
Level 3 (1.70)	A	100	100	100
	B	90.9	93.3	93.3
	C	100	100	100
	D	100	93.3	93.3
	Mean	**97.7**	**96.7**	**96.7**
Level 4 (3.48)	A	100	100	100
	B	100	100	100
	C	100	100	93.3
	D	86.4	100	100
	Mean	**96.6**	**100**	**98.3**
Level 5 (6.87)	A	95.5	20.0	6.7
	B	95.5	26.7	20.0
	C	100	20.0	13.3
	D	100	20.0	20.0
	Mean	**97.8**	**21.7***	**15.0***

***** Statistically significant reduction ($p \leq 0.05$) when compared to the pooled control for hatch and 35-day post-hatch survival and when compared to the vehicle blank for 61-day post-hatch survival.

[a] Not considered to be due to the RH-117,281, because mortality in replicate B contributed primarily to this value and the three next higher concentrations did not have survival effects.

Table B.9.2.2-2: Time to 95% embryo hatch and time to inition of swim-up behaviour by Rainbow Trout (*Oncorhynchus mykiss*) exposed to RH-117,281 Technical

Mean measured RH-117,281 concentration (µg/L)	Replicate	Time to 95% hatch (days)	Time to initiation of sustained swim-up (days)
Dilution water control	A	34	48
	B	34	48
	C	34	51
	D	34	48
	Mean	**34**	**49**
Vehicle Blank	A	35	52
	B	35	51
	C	34	49
	D	35	51
	Mean	**35**	**51**
Pooled control	**Mean**	**34**	**50**
Level 1 (0.403)	A	34	51
	B	35	51

	C	35	51
	D	34	51
	Mean	**35**	**51**
Level 2 (0.882)	A	34	48
	B	35	49
	C	34	51
	D	34	51
	Mean	**34**	**50**
Level 3 (1.70)	A	34	51
	B	33	51
	C	35	48
	D	35	50
	Mean	**34**	**50**
Level 4 (3.48)	A	34	51
	B	33	50
	C	33	51
	D	33	51
	Mean	**33**	**51**
Level 5 (6.87)	A	33	51
	B	33	51
	C	33	51
	D	33	51
	Mean	**33**	**51**

A range of morphological and behavioural effects were observed in the control and at all dose levels but these were not considered dose-related at doses up to and including 0.00348 mg a.s./L. Effects seen at 0.00687 mg a.s./L included discolouration and abnormal body posture. The NOEC was therefore considered to be 0.00348 mg a.s./L based on survival and other sublethal effects (discolouration and abnormal body posture).
The NOEC = 0.00348 mg a.s./L (mean measured)

RMS comments:
EC_{10} value is not given in the study, no argumentation about absence of value, results considered not sufficient. The study is considered acceptable.
The NOEC = 0.00348 mg a.s./L (mean measured)

Report:	**CA, 8.2.2.2/01 (8.2.1/03) [redacted] (1998d)** **RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*).**

Guidelines: US EPA OPPTS 850.1500/FIFRA Subdivision E Guideline, Subsection § 72-5 and OECD 203
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: fathead minnow (*Pimephales promelas*)
Number of organisms, weight, length, age, loading: eggs <24 hours old at test initiation.

Type of test: flow-through acute toxicity test (96 hours)
Applied and measured concentrations: nominal test concentrations: Negative control, solvent control (0.10 mL/L acetone) 7.5, 15, 29, 60 and 121 µg a.i./L; mean measured test concentrations: Negative control, solvent control, 7.8, 15, 29, 60 and 121 µg a.i./L
Length of test: 219 days (202 parental generation exposure)
Test conditions:
temperature: 25 °C
pH: 7.7-8.2
oxygen content: 5.2-6.4 g/L
total hardness: 128 - 140 mg/L CaCO3
photoperiod: 16 hours of light and 8 hours of darkness

Results:
A flow-through full life-cycle study was undertaken on fathead minnow (*Pimephales promelas*). Minnow embryos (<24 hours old, 50/replicate) were exposed to nominal concentrations of 0.0075, 0.015, 0.03, 0.06, 0.12 mg a.s./L plus control and acetone solvent control. Mean measured concentrations in the parental exposure were 0.0078, 0.015, 0.029, 0.06, and 0.121 mg a.s./L, representing 97 to 104% of nominal. Mean measured concentrations during the second generation exposure were 0.0081, 0.016, 0.028, 0.064 and 0.127 mg a.s./L, representing 93 to 108% of nominal. The full duration of the parental exposure was 202 days. Timings of individual life-stages (in days) were: First generation embryos (0-4); First generation larval/juvenile (5-60); First generation juveniles (61-166); First generation adult reproduction (167-202); Second generation embryos (180-191); Second generation larval/juvenile (185-219). With the exception of dissolved oxygen which dropped to 63% of saturation on one day, all water quality parameters remained within acceptable levels; temperature (25 ± 1°C), pH (7.7 to 8.3), dissolved oxygen (>73% dissolved oxygen).

Observations of mortality and other clinical signs of toxicity were made daily. Growth and survival of embryos, larvae and juveniles were monitored in each treatment and the controls. Additionally, embryo viability and time to hatch were monitored for both the first and second generations. First generation adult survival, growth, spawning frequency, number of eggs deposited, and fertilisation success were also monitored.

The results of the study are based on mean measured concentrations of RH-117,281. Water samples were collected on a weekly basis from both the parental and second generation exposures. Water samples and stock solution samples were analysed for 14C radioactivity using liquid scintillation counting (LSC). Selected water samples were also collected at the test initiation and termination to measure concentrations of RH-117,281 using high performance liquid chromatography (HPLC). Mean measured concentrations in the parental exposure were 7.8, 15, 29, 60 and 121 µg a.i./L (97 to 104% of nominal). Mean measured concentrations during the second generation exposure were 8.1, 16, 28, 64 and 127 µg a.i./L (93 to 108% of nominal).

Key biological parameters were evaluated over the eight month period in order to assess effects upon first and second generation fish. There were no apparent treatment-related effects on time to hatch, hatching success, survival, reproduction or growth of fathead minnows exposed to RH-117,281 at concentrations up to 60 µg a.i./L during the life-cycle toxicity test. Fathead minnows exposed to RH-117,281 at a concentration of 121 µg a.i./L showed statistically significant ($p \leq 0.05$)reductions in:
1) First generation survival (Day 4-60);
2) First generation length on Day 32;
3) Length and weight of male parental generation fish thinned on Day 166;
4) Survival of second generation fish

Table 9.2.2.-3: Survival of parental generation larvae and juveniles Day 4 to Day 60

Mean measured concentration (µg a.s./L)	Replicate	Initial number of larvae	Approximate live counts (test day)									Replicate percent survival[1]	Treatment percent survival
			4	11	18	25	32	39	46	53	60		
Negative control	A	25	25	25	25	25	24	24	24	24	24	96	93
	B	25	25	25	25	24	22	22	22	22	22	88	
	C	25	25	25	25	25	24	24	24	24	24	96	
	D	25	25	24	24	24	23	23	23	23	23	92	
Solvent control	A	25	25	24	24	24	24	24	24	24	24	96	89
	B	25	25	24	24	24	23	23	23	22	21	84	
	C	25	25	25	25	25	23	23	23	23	22	88	
	D	25	25	25	24	24	22	22	22	22	22	88	
7,8	A	25	25	25	25	25	24	24	24	24	24	96	95
	B	25	25	25	24	24	24	24	24	24	24	96	
	C	25	25	25	25	25	24	24	24	24	24	96	
	D	25	25	24	24	24	24	23	23	23	23	92	
15	A	25	25	25	25	25	25	25	25	25	25	100	93
	B	25	25	24	24	24	24	24	24	23	22	88	
	C	25	25	24	24	24	24	24	24	24	24	96	
	D	25	25	24	23	23	23	23	23	23	22	88	
29	A	25	25	25	25	25	24	24	24	24	24	96	93
	B	25	25	24	24	24	24	24	24	24	24	96	
	C	25	25	25	25	25	25	25	25	24	23	92	
	D	25	25	24	24	24	24	24	24	24	22	88	
60	A	25	25	24	24	24	20	20	20	20	20	80	93
	B	25	25	24	24	24	24	24	24	24	24	96	
	C	25	25	25	25	25	24	24	24	24	24	96	
	D	25	25	25	25	25	25	25	25	25	25	100	
121	A	25	25	22	20	20	20	20	20	20	18	72	81*
	B	25	25	23	23	23	19	19	19	19	19	76	
	C	25	25	25	25	25	24	24	24	24	23	92	
	D	25	25	25	23	23	21	21	21	21	21	84	

[1] Percent Survival = $(Live\ count\ on\ day\ 60\ \div initial\ number\ of\ larvae) \times 100$

* Statistically significant ($p \leq 0.05$) from the pooled control group. Percent survival for the pooled controls was 91%

Table 9.2.2.-4: Survival of parental generation juveniles and adults Day 61 to Day 166

Mean measured concentration (µg a.s./L)	Replicate	Initial number of larvae	Approximate live counts (test day)															Replicate percent survival[1]	Treatment percent survival
			61	68	75	82	89	96	103	110	117	124	131	138	145	152	159	166	
Negative control	A	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	24	24	96	98
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	
Solvent control	A	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	24	96	98
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	
7,8	A	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	100
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	
15	A	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	100
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	
29	A	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	98
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	24	96	
60	A	25	25	25	25	25	25	25	25	25	25	25	25	24	23	23	23	22	88	94
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	
121	A	25	25	25	25	25	25	25	25	25	25	24	24	24	24	24	24	23	92	96
	B	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	100	

[1] Percent Survival = $(Live\ count\ on\ day\ 166\ \div initial\ number\ of\ larvae\ on\ day\ 61) \times 100$

Table 9.2.2.-5: Survival of parental generation adults Day 167 to Day 202

Mean measured concentration (µg a.s./L)	Replicate	Initial # after thinning	Approximate live counts (test day)							Replicate percent survival[1]	Treatment percent survival
			167	173	180	187	194	201	202		
Negative control	A	15	15	15	15	15	15	14	14	93	93
	B	15	15	15	15	15	15	15	14	93	
Solvent control	A	15	15	15	15	15	15	14	14	93	93
	B	15	15	15	14	14	14	14	14	93	
7,8	A	15	15	15	14	14	14	13	13	87	87
	B	15	15	15	15	15	14	13	13	87	
15	A	15	15	15	15	15	15	15	15	100	97
	B	15	15	15	15	15	15	14	14	93	
29	A	15	15	15	15	15	15	14	13	87	94
	B	15	15	15	15	15	15	15	15	100	
60	A	15	15	15	14	14	14	13	13	87	90
	B	15	15	15	15	15	15	15	14	93	
121	A	15	15	14	14	14	14	13	12	80	84
	B	15	15	15	14	14	14	13	13	87	

[1] Percent Survival = $(Live\ count\ on\ day\ 202\ \div initial\ number\ of\ adults\ after\ thinning) \times 100$

All other biological parameters, including reproduction, evaluated in the RH-117,281 treatment groups at concentrations ≤ 121 µg a.i./L were comparable and not statistically different ($p \geq 0.05$) from fish exposed in the negative control or solvent control. Consequently, the NOEC for this study was 60 µg a.i./L. The LOEC, based on the effects listed above, was 121 µg a.i./L and MATC was calculated to be 85 µg a.i./L.

A 96-hour flow-through acute toxicity test was also conducted with RH-117,281 Technical in an attempt to determine the acute/chronic ratio. Juvenile fathead minnows were exposed to a negative control (dilution water), a solvent control (0.10 mL acetone/L) and mean measured concentrations of 37, 62, 112, 173, 208 µg a.i./L. A white precipitate was present in the mixing chambers of the 173 and 208 µg a.i./L treatment groups indicating that these test concentrations were at or above the maximum limit of water solubility under conditions of administration. All test solutions were clear and colorless without visible signs of precipitate. After 96-hours of exposure, survival was 100% in both the controls and all RH-117,281 Technical treatment groups. Consequently, the 96-hour LC_{50} for fathead minnows exposed to RH-117-281 Technical was >208 µg a.i./L, the highest concentration tested. The acute/chronic ratio, based on the 96-hour LC_{50} and the NOEC, was calculated to be >3.5.

The NOEC was 0.06 mg a.s./l. The LOEC, based on first generation survival, first generation length on Day 32, length and weight of male parental generation fish thinned on Day 166, and survival of second generation fish was 0.121 mg a.s./l. The acute/chronic ratio (ACR) was >3.5 based on the 96-hour LC_{50} and the NOEC.

There were no apparent treatment-related effects on time to hatch, hatching success, survival, reproduction or growth of fathead minnows exposed to RH-117,281 at concentrations up to 60 µg a.s./L during a life-cycle toxicity test. Key biological parameters were assessed over the eight month period in order to assess effects upon first and second generation fish. Fathead minnows exposed to RH-117,281 at a concentration of 121 µg a.s./L showed statistically significant reduction in survival and growth.

96-hour LC_{50} > 208 µg a.s./L (mean measured)
LOEC = 121 µg a.s./L (mean measured)
NOEC = 60 µg a.s./L (mean measured)

RMS comments:
EC_{10} value is not given in the study, no argumentation about absence of value, results considered not sufficient. The study is considered acceptable.
96-hour LC_{50} > 208 µg a.s./L (mean measured)
NOEC = 60 µg a.s./L (mean measured)

B.9.2.3 Potential for endocrine disruption

There are no indications of endocrine-disrupting effects from the existing database for active substance zoxamide. No conclusions regarding endocrine effects were made in the EU review.

Considering current data requirements, zoxamide is not expected to have an endocrine disrupting potential (see Vol. 3 A.S. B.6.8.3.)

B.9.2.4 Acute toxicity to aquatic invertebrates

Report:	**CA, 8.2.4.1/01 Sword, M.C., Gardner, C. (1995c)** **Acute flow-through toxicity of RH-117,281 Technical to *Daphnia magna.***

Guidelines: US EPA OPPTS 850.1010/FIFRA Subdivision E Guideline, Subsection § 72-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DK 2011), chemical purity: 94.2% a.s.
Test species: Daphnia magna
Number of organisms, weight, length, age, loading:
20 Daphnids/concentration
Type of test: acute flow-through toxicity test (48 hours)
Applied and measured concentrations:
Nominal test concentrations: Control, Vehicle Blank (acetone), 0.075, 0.15, 0.30, 0.60 and 1.2 mg a.i./L;
The mean measured test concentrations: Control, Vehicle Blank (0.1 mL/L acetone), 0.092, 0.15, 0.25, 0.42 and 0.78 mg a.i./L
Test conditions:
temperature: 21 °C
pH: 8.1
oxygen content: 8.3 mg/L
total hardness: 144 to 148 mg/L $CaCO_3$

Results:
Twenty (10/replicate) *Daphnia magna* were exposed to each concentration and control. The control and levels 1 through 4 were clear and free of precipitate during the test. The mixing box and the level 5 (0.78 mg a.i./L) test chamber developed a small amount of precipitate before the completion of the study indicating that maximum water solubility had been achieved.
The following results are based on the mean measured concentrations of RH-117,281 Technical. The 24- and 48-hour EC_{50} values were >0.78 mg a.i./L (solubility limit in ABC`s hard blended water) based on 0% immobility throughout the exposure period. The 48-hour no-observed effect concentration (NOEC) was 0.78 mg a.i./L. All daphnids were normal throughout the test.
48-hour EC_{50} > 0.78 mg a.i./L (mean measured).
48-hour NOEC = 0.78 mg a.i./L (mean measured).

RMS comments:
Study is considerd acceptable.
48-hour EC_{50} > 0.78 mg a.i./L (mean measured).
48-hour NOEC = 0.78 mg a.i./L (mean measured).

Report:	**CA, 8.2.4.1/02 Rhodes, J.E., Williams, S. (1998b)** **Acute toxicity of RH-127,450 to *Daphnia magna* in a range-finding test under static conditions.**

Guidelines: US EPA OPPTS 850.1010/FIFRA Subdivision E Guideline, Subsection § 72-2, OECD 202
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-127.450 Technical (Lot No. BM 3933), chemical purity: 99.27% a.s.
Test species: Daphnia magna
Number of organisms, weight, length, age, loading:
20 Daphnids/concentration, < 24 hours old
Type of test: acute flow-through toxicity test (48 hours)
Applied and measured concentrations:
Nominal test concentrations (June 19-21): 0.0 control, 0.0 (50 µL/L acetone control), 43.8, 87.5, 175, 350 and 700 µg a.i./L;
Nominal test concentrations (June 29-July1): 0.0 (control), 0.0 (50 µL/L acetone control), 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.i./L.
Test conditions:
temperature: 20 °C
pH: 8.0 to 8.4
oxygen content: 7.8 to 8.2 mg/L
total hardness: 130 to 160 mg/L $CaCO_3$
photoperiod: 16 hours of daylight and 30-minute dusk/dawn transition period

Results:
The control, acetone control, 0.31, 0.63 and 1.3 mg a.i./L test solutions appeared clear with no visible precipitate or surface film. The 2.5 and 5 mg a.i./L test solutions appeared clear with no visible precipitate. However, numerous small crystalline particles were observed floating on the surface at initiation. At 24 and 48 hours, the material floating on the surface appeared white colour. These observations indicate that functional water solubility was achieved. Water quality measurements were within acceptable limits throughout the exposure.
After 48 hours of exposure, no immobility/mortality or sublethal effects were observed at any concentration tested. The 48-hour EC_{50} for *Dapnia magna* exposed to RH-127,450 is estimated to be > 5.0 mg a.i./L The no-observed-effect concentration (NOEC) was 5.0 mg a.i./L based on upon the lack of immobilization/mortality and sublethal effects at this and all lower concentrations tested.
The 48-hour EC_{50} for *Daphnia magna* exposed to RH-127,450 is estimated to be > 5.0 mg a.i./L. The 48-hour NOEC was 5.0 mg a.i./L based on an absence of immobilization/mortality and sublethal effects at this and all lower concentrations.
48-hour EC_{50} > 5.0 mg asi./L (nominal).

RMS comments:
Study is considered acceptable.

No information in the study about how crystals impacted the results of the study, solubility of metabolite RH-127,450 in water is 0%, and acetone was used as solvent (solubility in acetone 100%). No mortality or sublethal effects has been observed in the study, RMS thinks crystals have not impacted the study results. RMS agrees with endpoint 48-hour EC_{50} >5.0 mg a.s./L.

Report:	**8.2.4.2/01 Roberts, C.A., and Swigert, J.P., (1997) RH-117,281 Technical: A 96-Hour-Flow Through Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)**
Guidelines:	USEPA FIFRA Subdivision E 540/9-82-024; USEPA OPP 540/9-85-010; ASTM Standard E729-88a
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal of a.s. approval

Executive Summary

In an acute toxicity study, saltwater mysid (*Mysidopsis bahia*) were exposed to the test item RH-117,281 Technical. The test was conducted under flow-through conditions over a period of 96 hours with the following nominal test item concentrations: 0 (negative and solvent controls), 0.016, 0.026, 0.043, 0.072 and 0.12 mg a.s./L (mean measured 0.0170, 0.0306, 0.0469, 0.0763 and 0.132 mg a.s./L, respectively). Twenty test organisms (10 per replicate) were exposed to each test concentration and the controls.

All mysids in the negative control and solvent control groups appeared healthy and normal throughout the test. No mortalities were observed in the 0.0170 and 0.12 mg a.s./L treatment groups. Percent mortality at test termination in the 0.0469, 0.0763 and 0.132 mg a.s./L was 5, 45 and 100%, respectively.

Measured concentrations of RH-117,281 Technical ranged from 101 to 122% of nominal (at the beginning of the test) and.from 108 to 113% (at the end of the test). The mean measured test concentrations were 0.0170, 0.0306, 0.0469, 0.0763 and 0.132 mg a.s./L, representing, 106, 118, 109, 106 and 110% of nominal concentrations, respectively.

The 96 hours LC_{50} was determined to be 0.076 mg a.s./L, with 95% confidence limits of 0.067 and 0.087 mg a.s./L. The 96-hour no-mortality concentration and the NOEC were 0.0306 mg a.s./L.

Material and Methods:

Materials

1.	**Test material:**	RH-117,281 Technical
	Batch number:	DSR 9510
	Purity:	92.3%
	Description:	White powder
2.	**Reference item:**	None
3.	**Dilution water:**	Natural seawater from Indian River Inlet, Delaware (filtered and diluted to a salinity of approximately 20‰ with well water)
	Vehicle:	Acetone
4.	**Test organism**	
	Species:	Saltwater mysid, *Mysidopsis bahia*
	Age at test initiation:	< 24 hours old
	Source:	Mysids used in the test were obtained as juveniles from cultures maintained by Wildlife International, Ltd., Easton, Maryland.
	Diet:	During the test, the mysids were fed live brine shrimp nauplii (*Artemia* sp.) daily.

Test vessels:	Test chambers were 8-L polyethylene aquaria filled with 6.5 L of test water. Each test chamber contained 500-mL glass beakers (~8 cm diameter and 13 cm in height) with nylon screen attached to an opening of each side of the beakers.

Study design and methods

1. Environmental conditions

Temperature:	24.8 – 25.1°C
pH:	8.3 – 8.4
Dissolved oxygen:	5.4 – 6.3 mg/L
Salinity:	20‰ (0-hour dilution water measurement salinity)
Photoperiod:	16/8 hour light/dark cycle (232 lux at test initiation)

2. Animal assignment and treatment:

Saltwater mysids were exposed to a geometric series of five test concentrations (nominal test concentrations were 0.016, 0.026, 0.043, 0.072 and 0.12 mg a.s./L), a negative control (dilution water) and a solvent control (0.5 mL/L acetone) for 96 hours under flow-through conditions. At test initiation juvenile mysids were collected from the cultures and transferred to 20 mL plastic cups. Mysids were transferred from the cups to the test compartments with minimal handling (using a wide-bore pipette). At test initiation two replicate chambers were maintained in each treatment and control group, with 10 mysids in each chamber, for a total of 20 mysids per concentration.

3. Dose preparation:

A primary stock solution was prepared by mixing a calculated amount of test substance into acetone at a nominal concentration of 0.26 mg RH-117,281 Technical/mL. Four secondary solutions (at nominal concentrations of 0.034, 0.056, 0.094 and 0.16 mg RH-117,281 Technical /mL) were prepared in acetone by proportional dilution of the primary stock. Stock solutions were prepared one time during the test period. The five stocks were injected into the diluter mixing chambers where they were mixed with dilution water to achieve the desired test concentrations. The resulting test concentrations were adjusted for purity of the active substance in the test substance.

4. Measurements/observations:

Observations were made to determine the number of mortalities. The number of individuals exhibiting clinical signs of toxicity or abnormal behaviour was also evaluated. Observations were made approximately 3, 24, 48, 72 and 96 hours after test initiation. Samples were collected from each replicate test chamber in each treatment and control group prior to the test to evaluate diluter performance. Samples were also collected from each replicate test chamber in each treatment and control group at the beginning and end of the test to measure concentrations of the test substance. Samples were analysed by HPLC with UV detection.

5. Statistics:

When the dose response pattern allowed for the calculation of an LC_{50} value, the data were analysed using the computer program of C, E. Stephan. The program was designed to estimate or calculate the LC_{50} value and the 95% confidence interval by probit analysis, the moving average method, or binomial probability with nonlinear interpolation. In this study, the probit method was used to evaluate mortality at 72, and 96 hours. The no mortality concentration and NOEC were determined by visual interpretation of the mortality and clinical observation data.

Results and discussion

A. Mortality and signs of toxicity

Daily observations of mortality and other signs of toxicity observed during the test are shown in Table 9.2.4.-1. All mysids in the negative control and solvent control groups appeared healthy and normal throughout the test with the exception of one dead mysid in the negative control at 96 hours.

Mysids in the 0.0170 mg a.s./L, treatment group also appeared normal throughout the test with no mortalities or overt signs of toxicity. In the 0.0306 mg a.s./L treatment, no mortalities were observed throughout the test and all mysids appeared healthy and normal except one mysid at 96 hours that was observed swimming erratically. Percent mortalities in the 0.0469 and 0.0763 mg a.s./L treatment groups were 5% and 45%, respectively, at test termination. The clinical sign of toxicity observed in mysids from those levels included erratic swimming. Mortality in the 0.132 mg a.s./L treatment group, the highest concentration tested, was 5% within 24 hours of test initiation, and 100% by 96 hours. Clinical signs of toxicity observed in mysids from this treatment included erratic swimming behaviour and lethargy.

Table 9.2.4.-1: Mortality and treatment-related effects

Mean measured concentration	3 hours		24 hours		48 hours		72 hours		96 hours	
	No. Dead [1]	Effects [2]	No. Dead[1]	Effects [2]	No. Dead [1]	Effects [2]	No. Dead [1]	Effects[2]	No. Dead [1]	Effects [2]
Negative control	0	20 AN	0	20 AN	0	20 AN	0	20 AN	1	19 AN
Solvent control	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.0170	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	20 AN
0.306	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	19 AN; 1E
0.0469	0	20 AN	0	20 AN	0	20 AN	0	20 AN	1	18 AN; 1E
0.0763	0	20 AN	0	20 AN	0	20 AN	5	15 AN	9	7 AN; 4E
0.132	0	20 AN	1	18 AN; 1E	5	12E; 3C	19	1E	20	-

[1] Cumulative number of dead mysids

[2] Observations: AN = appear normal; C = lethargy; E = erratic swimming; N = loss of equilibrium

B. Toxicity endpoint

LC_{50} values and 95% confidence limits at 24, 48, 72, and 96 hours were calculated from the mortality data, and are shown in Table 9.2.4.-2.

Table 9.2.4.-2: Summary of endpoints for RH-117,281 Technical

Time	LC_{50} (mg a.s./L)	95% confidence intervals	96 hour-NOEC (mg a.s./L)
24 hours	>0.132	-*	0.0306**
48 hours	>0.132	-*	
72 hours	0.09	0.079-0.103	
96 hours	0.076	0.067-0.087	

* Confidence limits could not be calculated with the mortality data obtained

**NOEC as determined by visual inspection of the cumulative percent mortality and treatment-related effects data.

C. Analytical verification

Results of analyses to measure concentrations of RH-117,281 Technical in water samples collected during the test are presented in Table 9.2.4.-3. All test solutions in the mixing chambers and test chambers appeared clear and colourless. Samples collected at the beginning of the test had measured values that

ranged from 101 to 122% of nominal values. Measured values for samples taken at 96 hours ranged from 108 to 113% of nominal values. When measured concentrations of samples collected at test initiation and at test termination were averaged, the mean measured concentrations for the study were 0.0170, 0.0306, 0.0469, 0.0763, and 0.132 mg a.s./L. Mean measured concentrations were used in the estimation or calculation of LC_{50} values.

Table 9.2.4.-3: Measured concentrations of RH-117,281 Technical in the exposure solutions

Nominal concentration	Sampling time (hours)	Replicate mean measured concentration (µg a.s./L)[1]	% nominal	Mean measured concentration (µg a.s./L)[2]	Mean % nominal
Negative control	0	< LOQ	-	-	-
	96	< LOQ	-		
Solvent control	0	< LOQ	-	-	-
	96	< LOQ	-		
0.016	0	0.0169	104	0.0170	106
	96	0.0173	108		
0.026	0	0.0317	122	0.0306	118
	96	0.0295	112		
0.043	0	0.0458	107	0.0469	109
	96	0.0479	111		
0.072	0	0.0726	101	0.0763	106
	96	0.0799	111		
0.12	0	0.128	107	0.132	110
	96	0.135	113		

[1] The limit of quantitation (LOQ) was based upon the lowest matrix fortification level, 0.005 mg a.s./L, analysed during the study.
[2] The overall mean is the average value of the replicate mean.

Conclusions:
96 hours LC_{50} (*Mysidopsis bahia*) = 0.076 mg a.s./L, with 95% confidence limits of 0.067 and 0.087 mg a.s./L
The 96-hour no-mortality concentration and the NOEC (*Mysidopsis bahia*) = 0.0306 mg a.s./L

RMS comments:
Study is considered acceptable.
96 hours LC_{50} = 0.076 mg a.s./L
NOEC = 0.0306 mg a.s./L

Report:	**CA, 8.2.4.1/03 Zoxamide metabolite RH-139,432 – Acute toxicity to Daphnids (*Daphnia magna*) under static conditions**

Guidelines: US EPA FIFRA Guideline, Subsection § 72-2, OECD 202

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

Material and Methods:
Test substance: RH-139,432 Lot No. LJG7-39A, Identification No. TSN 103194, purity 99.8% a.s.
Test species: Daphnia magna
Number of organisms, weight, length, age, loading:
< 24 hours old
Type of test: static acute toxicity test (48 hours)
Applied and measured concentrations:
Nominal test concentrations: 3.1, 6.3, 13, 25 and 50 mg a.i./L;
Nominal test concentrations: 1.8, 3.4, 7.3, 14 and 28 mg a.i./L.
Test conditions:
temperature: from 20 to 21 °C
pH: 7.9
oxygen content: 8.6 to 8.8 mg/L
total hardness: 160 mg/L $CaCO_3$
toatal alkalinity: 110 mg/L $CaCO_3$
photoperiod: 16 hours of light and 8 hours darkness at a light intensity range 60 to 90 footcandles

Results:

During the preliminary test, daphnids were exposed under static conditions to nominal zoxamide metabolite RH-139,432 concentrations of 3.1, 6.3, 13, 25 and 50 mg a.s./L and dilution water control. Four replicate test vessels containing five daphnids per replicate were established for each treatment level and the control. Following 48 hours of exposure, 75 and 100% immobilization was observed in the 25 and 50 mg a.s./L treatment levels. All surviving daphnids in the 25 mg a.s./L treatment level were observed to be lethargic and appeared to have particulates caught on appendages. No immobilization or sub-lethal effects were observed among daphnids exposed to the remaining treatment levels tested (3.1, 6.3 and 13 ma a.s./L) or the control. Based on these results, nominal zoxamide metabolite RH-139,432 concentrations of 3.1, 6.3, 13, 25 and 50 mg a.s./L were selected for the definitive exposure.

Water quality parameters were unaffected by the concentrations of zoxamide metabolite RH-139,432 tested and remained within acceptable ranges for the survival of daphnids. Daily measurements of the temperature in the test solutions and continuous temperature monitoring of replicate A of the 50 mg a.s./L treatment level established that the exposure solution temperature was 20 to 21 ºC during the definitive study.

Throughout the exposure period, analytical measurements of the test solutions established the expected concentration gradient and were consistent throughout the exposure. Mean measured concentrations were defined as 1.8, 3.4, 7.3, 14 and 28 mg a.s./L. recoveries of approximately 55% of the nominal concentrationwere expected based on the amount of undissolved test substance observed in the WAF.
Analysis of the quality control samples rsulted in measured concentrations which were consistent with the predetermined recovery range and ranged from 93 to 115% (N=6) of the nominal fortified levels (1.00 to 10.00 mg a.s./L) Based on the results of these analyses, it was established that the appropriate precision and quality control were maintained during the analyses of the exposure solutions.

Following 48-hours of exposure, 25 and 100% immobilization was observed among daphnids exposed to the 14 and 28 mg a.s./L treatment levels. All but one surviving daphnid exposed to the 14 mg a.s./L treatment level were observed to be lethargic.No immobilization or sublethal effects were observed among daphnids exposed to the remaining treatment levels tested (1.8, 3.4 and 7.3 mg a.s./L) or the control.

The 48-hour EC_{50} value for zoxamide metabolite RH-139,432 and *Daphnia magna* was estimated by non-linear interpolation to be 17 mg a.s./L, with 95% confidence intervals (calculated by binomial probability) of 14 to 28 mg a.s./L. The NOEC was determined to be 7.3 mg a.s./L. The highest concentration producing 0% immobilization was 7.3 mg a.s./L. The lowest concentration producing 100% immobilization was 28 mg/L.

Table B.9.2.4-4: Concentrations of zoxamide metabolite RH-139,432 measured in the exposure solutions during 48-hour static acute exposure of daphnids (*Daphnia magna*)

Nominal concentration (mg a.s./L)	Measuerd concentration (mg a.s./L)			
	0-hour	96 hour	Mean[a]	Percent of nominal
Control	<0.17	<0.16	NA[b]	NA
3.1	2.0	1.5	1.8	57
6.3	3.7	3.0	3.4	53
13	7.2	7.3	7.3	56
25	15	13	14	56
50	30	26	28	55
QC[c] #1 1.00	1.14 (114)[d]	0.995 (99.5)		
QC #2 5.00	5.51 (110)	4.65 (93.0)		
QC #3 10.0	11.5 (115)	11.3 (113)		

[a] Calculated results are based on the original raw data and not the rounded results presented in this table.

[b] NA = Not applicable.

[c] QC = Quality control sample

[d] Percent recovery is presented in parentheses.

Table B.9.2.4-5: Mean measured concentrations tested, corresponding cumulative percent and number of immobilized organisms, and observations made during the 48-hour static exposure of daphnids (*Daphnia magna*) to zoxamide metabolite RH-139,432

Mean measured concentration (mg a.s./L)	Cumulative percent of immobilized organisms[a]									
	24 hour					48 hour				
	A	B	C	D	Mean	A	B	C	D	Mean
Control	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
1.8	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
3.4	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
7.3	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)	0
14	0 (0)	0 (0)	0 (0)	20 (1)	5[b]	0 (0)	0 (0)	40 (2)	60 (3)	25[c]
28	80 (4)	100 (5)	60 (3)	80 (4)	80[d]	100 (5)	100 (5)	100 (5)	100 (5)	100

[a] The actual number of immobilized daphnids is presented in parentheses.

[b] Several surviving daphnids were observed to be lethargic.

[c] All but one surviving daphnid were observed to be lethargic.

[d] All surviving daphnids were observed to be lethargic.

Tables B.9.2.4-6: The EC_{50} values (corresponding 95% confidence intervals) and No-observed-effect concentration (NOEC) for zoxamide metabolite RH-139,432 and daphnids (*Daphnia magna*) under static conditions

	EC_{50} (mg a.s./L)	95% Confidence intervals	
		Lower (mg a.s./L)	Upper (mg a.s./L)
24-hour[a]	22	19	26
48-hour[b]	17	14	28

[a] EC_{50} value and 95% confidence intervals were calculated by probit analysis.

[b] EC_{50} value estimated by nonlinear interpolation with 95% confidence intervals calculated by binomial probability.

NOEC throughout 48 hours = 7.3 mg a.s./L
EC_{50} = 17 mg a.s./L (nominal)

RMS comments:

No information about solubility of metabolite RH-139,432 and used solvent in the study. It is mentioned in the study that solution was mixed for two hours with magnetic stir bar and stir plate, the resultant solution solution was observed to be clear and colorless with undissolved material in suspension and on the surface. The WAF (water accommodated fraction) was allowed to settle for 1 hour and the contents were withdrawn from the vessel and used to prepare the nominal concentations. Exposure solutions were observed to be clear and colorless with no visible sign of undissolved test substance after preparation. No information how undissolved substance have influenced the results of study. RMS still considers study acceptable.

NOEC = 7.3 mg a.s./L
EC_{50} = 17 mg a.s./L (nominal)

B.9.2.5 Long-term and chronic toxicity to aquatic invertebrates

Report:	**CA, 8.2.5.1/01 Murrell, H., Rhodes, J.E., Stewart, S. (1997) Chronic toxicity of RH-117,281 Technical to *Daphnia magna* under flow-through test conditions.**

Guidelines: OECD 211, US EPA OPPTS 850.1300/FIFRA Subdivision E Guideline, Subsection § 72-4
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR 9510), chemical purity: 92.3% a.s.
Test species: Daphnia magna
Number of organisms, weight, length, age, loading:

40 Daphnids/treatment (10 per replicate), < 24 hours old
Type of test: under flow-through test, 21 day
Applied and measured concentrations:
Nominal test concentrations: control (0.0), acetone vehicle Blank (0.10 mL/L), 0.011, 0.023, 0.045, 0.090 and 0.18 mg a.i./L; Mean measured test concentrations: Control (<MQL), acetone vehicle Blank (<MQL), 0.0090, 0.020, 0.039, 0.074 and 0.16 mg a.i./L.
Test conditions:
temperature: 20.5 °C; pH: 8.18-8.50; oxygen content: 6.2-7.9 mg/L; total hardness: 142-158 mg/L $CaCO_3$; photoperiod: 16 hours of daylight, 8-hour dark period preceded by 30-minute stimulated dusk/dawn transition period

Results:

The chronic toxicity of RH-117,281 (purity 92.3%) to *Daphnia magna* was assessed in a 21-day flow-through study. First instar daphnids (<24 hours old, 40 per treatment, 10 per replicate) were used to initiate the study with exposure continuing for a total of 21 days.

Table 9.2.5.-1: Percent survival of *Daphnia magna* exposed to RH-117,281 technical

Mean measured RH-117,281 Technical concentration (mg a.s./L)	Rep.	Initial number neonates	Adult surv.	Percent surv.	Mean ± SD
Control	A	10	10	100	97.5 ± 5.0
	B	10	9	90	
	C	10	10	100	
	D	10	10	100	
Acetone vehicle blank	A	10	9	90	95.0 ± 5.8
	B	10	9	90	
	C	10	10	100	
	D	10	10	100	
Pooled control	---	80	77	---	96.3 ± 5.2
Level 1 (0.0090)	A	10	10	100	97.5 ± 5.0
	B	10	10	100	
	C	10	10	100	
	D	10	9	90	
Level 2 (0.020)	A	10	10	100	90.0 ± 14
	B	10	7	70	
	C	10	9	90	
	D	10	10	100	
Level 3 (0.039)	A	10	9	90	90.0 ± 10
	B	10	5	50	
	C	10	8	80	
	D	10	10	100	
Level 4 (0.074)	A	10	6	60	57.5* ± 9.6
	B	10	5	50	
	C	10	5	50	
	D	10	7	70	
Level 5 (0.16)	A	10	0	0.0	22.2* ± 15
	B	10	3	30	
	C	10	3	30	
	D	10	3	30	

Table 9.2.5.-2: Individual mortality and behaviour observations during the chronic toxicity study with *Daphnia magna* exposed to RH-117,281 Technical

Mean measured RH-117,281 Technical concentration (mg a.s./L)	Rep.	Initial number	Day 0		Day 7		Day 14		Day 21	
			Cum. mort.	Obs.	Cum. mort.	Obs.	Cum. mort.	Obs.	Cum. mort.	Obs.
Control	A	10	0	10 N	0	10 N	0	10 N	0	10 N
	B	10	0	10 N	0	10 N	0	10 N	1	9 N
	C	10	0	10 N	0	10 N	0	10 N	0	10 N
	D	10	0	10 N	0	10 N	0	10 N	0	10 N
Acetone vehicle blank	A	10	0	10 N	1	9 N	1	9 N	1	9 N
	B	10	0	10 N	1	9 N	1	9 N	1	9 N
	C	10	0	10 N	0	10 N	0	10 N	0	10 N
	D	10	0	10 N	0	10 N	0	10 N	0	10 N
Level 1 (0.0090)	A	10	0	10 N	0	10 N	0	10 N	0	10 N
	B	10	0	10 N	0	10 N	0	10 N	0	10 N
	C	10	0	10 N	0	10 N	0	10 N	0	10 N
	D	10	0	10 N	0	10 N	0	10 N	1	9 N
Level 2 (0.020)	A	10	0	10 N	0	10 N	0	10 N	0	10 N
	B	10	0	10 N	1	9 N	1	9 N	3	7 N
	C	10	0	10 N	1	9 N	1	9 N	1	9 N
	D	10	0	10 N	0	10 N	0	10 N	0	10 N
Level 3 (0.039)	A	10	0	10 N	1	9 N	1	9 N	1	9 N
	B	10	0	10 N	2	8 N	5	5 N	5	4 N, 1 LC
	C	10	0	10 N	1	9 N	2	8 N	2	8 N
	D	10	0	10 N	0	10 N	0	10 N	0	10 N
Level 4 (0.074)	A	10	0	10 N	3	7 N	3	7 N	4	6 N
	B	10	0	10 N	5	5 N	5	5 N	5	5 N
	C	10	0	10 N	5	5 N	5	5 N	5	5 N
	D	10	0	10 N	3	7 N	3	7 N	3	7 N
Level 5 (0.16)	A	10	0	10 N	9	1 N	10	---	10	---
	B	10	0	10 N	7	3 N	7	3 N	7	3 N
	C	10	0	10 N	7	3 N	7	3 N	7	3 N
	D	10	0	10 N	5	5 N	7	3 N	7	3 N

Key to observations: N = normal, LC = light discoloration

Table 9.2.5.-3: Reproductive activity exhibited by *Daphnia magna* exposed to RH-117,281 Technical

Mean measured RH-117,281 Technical concentration (mg a.s./L)	Rep.	Total young	Adult reprod. days	Young/adult reprod. day	Mean ± SD	Time to first brood days	Mean ± SD
Control	A	1210	140	8.64	7.73 ± 1.23	8	8.25 ± 0.50
	B	736	115	6.40		9	
	C	1246	140	8.90		8	
	D	978	140	6.99		8	
Acetone vehicle blank	A	1171	126	9.29	9.12 ± 0.804	8	8.00 ± 0.00
	B	1018	126	8.08		8	
	C	1270	140	9.07		8	
	D	1404	104	10.0		8	
Pooled control	---	---	---	---	8.43 ± 1.21	---	8.13 ± 0.35
Level 1 (0.0090)	A	1291	140	9.22	9.41 ±0.854	8	8.00 ± 0.00
	B	1190	140	8.50		8	
	C	1479	140	10.6		8	
	D	1303	139	9.37		8	
Level 2 (0.020)	A	1456	140	10.4	9.90 ± 0.600	8	8.00 ± 0.00
	B	1078	119	9.06		8	
	C	1244	126	8.87		8	
	D	1435	140	10.3		8	
Level 3 (0.039)	A	1144	126	9.08	9.05 ± 0.282	8	7.50 ± 0.58
	B	690	79	8.73		8	
	C	1158	123	9.41		7	
	D	1347	150	8.98		7	
Level 4[a] (0.074)	A	918	92	9.98	9.11 ± 0.816	8	8.00 ± 0.00
	B	634	70	9.06		8	
	C	562	70	8.03		8	
	D	919	98	9.38		8	
Level 5[a] (0.16)	A	12	2	6.00	6.37 ± 1.10	8	8.00[b] ± 0.82
	B	217	42	5.17		8	
	C	304	39	7.79		9	
	D	346	53	6.53		7	

***** No statistically significant ($p \leq 0\ 05$) reductions when compared to the vehicle blank

[a] Excluded from analysis because of significant survival effects, although similar to control reproductive performance

[b] Replicates C & D were excluded from analysis because of statistically significant outliers

Table 9.2.5.-4: Summary of statistical endpoints for the chronic toxicity study with *Daphnia magna* exposed to RH-117,281 Technical

Mean measured RH-117,281 Technical concentration (mg a.s./L)	Mean survival (%)	Mean YAD[a]	Time to first brood (days)	Mean length (mm)	Mean weight (mg)
Control	97.5	7.73	8.25	4.21	0.65
Acetone vehicle blank	95.0	9.12	8.00	4.28	0.76
Pooled control	96.3	8.41	8.13	4.24	0.70
0.0090	97.5	9.41	8.00	4.32	0.77
0.020	90.0	9.90	8.00	4.22	0.82
0.039	910.0	9.05	7.50	4.29	0.88
0.074	57.5*	9.11[b]	8.00[b]	4.26[b]	0.93[b]
0.16	22.5*	6.37[b]	8.00[b]	4.02[b]	0.87[b]

* Denotes values significantly less ($p \leq 0.05$) han the pooled control for survival, time to first brood, length, weight, and YAD when compared with the vehicle blank.

[a] YAD = young/adult reproduction day.

[b] Excluded from the analysis because of significant survival effects.

The mean measured concentrations of RH-117,281, determined by HPLC, were < minimum quantifiable limit (dilution water control and acetone vehicle blank 0.10 ml/l), 0.0090, 0.020, 0.039, 0.074, and 0.16 mg a.s./l. These values ranged from 82 to 89% of the nominal test concentrations of 0.0 (control), 0.0 (acetone vehicle blank), 0.011, 0.023, 0.045, 0.090, and 0.18 mg a.s./l. The minimum quantifiable limit was 0.00522 mg a.s./l.

Survival was significantly reduced at mean measured concentrations of 0.074 and 0.16 mg a.s./l after 21 days exposure (40% and 77% reductions respectively). Mean length and dry weight were not affected at concentrations of 0.039 mg a.s./l or below. Higher concentrations were excluded from the growth analyses because of the significant survival effects, but the data did not indicate a growth effect. Reproductive success, as measured by the time to first brood and young/adult/reproduction/day, was not affected at concentrations of 0.039 mg a.s./l or below. Higher concentrations were excluded from the reproduction analyses because of the significant survival effects, but the data did not indicate a reproductive effect.

Based on the absence of significant survival, growth or reproductive effects, the NOEC was identified as 0.039 mg a.s./l. Based on significant survival effects, the LOEC was identified as 0.074 mg a.s./l.

21-day EC_{50} = 0.085 mg a.i./L (mean measured).

21-day NOEC = 0.039 mg a.i./L (mean measured).

RMS comments:

EC_{10} value is not given in the study, no argumentation about absence of value, results considered not sufficient. The study is considered acceptable.
21-day EC_{50} = 0.085 mg a.i./L (mean measured).
21-day NOEC = 0.039 mg a.i./L (mean measured).

Report:	**CA, 8.2.5.3/01 van der Kolk, J. (1998a)** **RH-117,281: Chronic effects on midge larvae (*Chironomus riparius*) in a water/sediment system.**

Guidelines: "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in water sediment system" (proposal for BBA-guideline, 1995)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: midge larvae *Chironomus riparius*
Number of organisms, age: 3 days old first instar larvae
Applied and measured concentrations:
Nominal concentrations: control, solvent control, 0.45, 0.84, 1.5, 2.6, 4.7, and 8.5 mg a.i./L
Test conditions:
temperature: 20.4 to 22.2 °C
pH: 7.61
total hardness: 228 mg/L $CaCO_3$
light intensity: 624 – 1608 lux (average 1282 lux)

Results:

The chronic toxicity of RH-117,281 (purity 92.3%) to *Chironomus riparius* (3 day old, 1st instar larvae) was assessed in a 28 day water/sediment system under static conditions. Nominal test concentrations were 0.45, 0.81, 1.5, 2.6, 4.7 and 8.5 mg a.s./l. The test was performed in 2-litre glass beakers, each containing 25 midge larvae and approximately 2 cm of sediment (artificial OECD soil) and 16.5 cm of overlying water. There were four replicates for each test concentration, for the solvent control and untreated control. Two additional replicates were set up for the lowest and highest test concentrations to determine the distribution of the test substance between the overlying water and the sediment. Midge larvae were added to the test system one day prior to the addition of test substance. Test substance dilutions, in Elendt M4 medium, were sonicated for 5 minutes to obtain an even concentration of test substance as each dilution had concentrations above the water solubility of RH-117,281 (0.67 ppm). The concentrations of the test substance in the sediment and overlying water were monitored by adding a known amount of [^{14}C] RH-117,281. The ratio for the radiolabelled and non-radiolabelled test substance was 1:426. The concentration of [^{14}C] RH-117,281, based on total radioactivity, was determined on day 0 (at 1 hour) and on days 7 and 28. [^{14}C] RH-117,281 - based measured concentrations in the overlying water on day 0 (at 1 hour) were 0.39, 0.72, 1.08, 2.00, 1.89, and 2.79 mg a.s./l, representing 33 to 87 % of nominal. On day 28, measured concentrations of RH-117,281 in the overlying water were 0.14, 0.24, 0.44, 0.62, 0.96, and 1.13 mg a.s./l, representing 13 to 31% of nominal. Statistical endpoints were calculated using the nominal concentrations in the overlying water. The test vessels were monitored daily for the emergence of midges.

Table 9.2.5.-5: Cumulative emergence and development rate of midge (*Chironomus riparius*) during the 28-day static chronic study with RH-177,281

Nominal test concentration (mg a.s./L)	Repl.	Cumulative emergence (%)	mean ± std.	Development rate (day $^{-1}$)	mean ± std.
Control	A	88	77 ± 10	0.082	0.082 ± 0.001
	B	64		0.081	
	C	76		0.084	
	D	80		0.083	
Solvent control	A	80	82 ± 4	0.092	0.088 ± 0.003
	B	80		0.088	
	C	88		0.087	
	D	80		0.085	
0.45	A	76	82 ± 7	0.076	0.077 ± 0.006
	B	92		0.069	
	C	80		0.081	
	D	80		0.081	
0.81	A	76	67 ± 8[a*]	0.079	0.080 ± 0.001
	B	60		0.080	
	C	72		0.079	
	D	60		0.080	
1.5	A	36	49 ± 18[a*]	0.079	0.080 ± 0.002
	B	40		0.082	
	C	44		0.082	
	D	76		0.079	
2.6	A	24	32 ± 10[a*]	0.074	0.077 ± 0.002
	B	44		0.079	
	C	24		0.079	
	D	36		0.074	
4.7	A	4	6 ± 2[a*]	0.080	0.072 ± 0.010
	B	8		0.077	
	C	8		0.074	
	D	4		0.057	

8.5	A	8	15 ± 8[a*]	0.065	0.072 ± 0.005[b*]
	B	8		0.074	
	C	24		0.073	
	D	20		0.075	

[a*] Significantly less midges emerged when compared with the combined control and solvent control (Williams test, $p \leq 0.05$).

[b*] Significant difference when compared to the solvent control development rate (Kruskal-Wallis, $p \leq 0.05$).

The pH, and temperature were monitored weekly in all four replicates of the control, solvent control, and each test concentration. The water quality parameters remained within acceptable levels for the survival of midge larvae. The midge testing area was regulated with a photoperiod of 16 hours of light and 8 hours of darkness.

Table 9.2.5.-6: Emergence rate (ER) and development rate (DR) of midge (*Chironomus riparius*) during the 28-day static chronic study with RH-117,281.

Nominal test concentration (mg a.s./L)	Emergence rate (ER)[a] mean ± std.	Development rate (DR)[b] mean ± std.
Control	0.77 ± 0.10	0.082 ± 0.01[c]
Solvent control	0.82 ± 0.04	0.088 ± 0.003[c]
0.45	0.82 ± 0.07	0.077 ± 0.006
0.81	0.67 ± 0.08[d*]	0.080 ± 0.001
1.5	0.49 ± 0.18[d*]	0.080 ± 0.002
2.6	0.32 ± 0.10[d*]	0.077 ± 0.002
4.7	0.06 ± 0.02[d*]	0.0072 ± 0.010
8.5	0.15 ± 0.08[d*]	0.072 ± 0.005[e*]

[a] $ER = \frac{ne}{ni}$, where n_e is the sum of midges emerged per vessel and n_i is the number of larvae introduced (If $n_e > n_i$ then n_i is replaced by $n_{e)}$ (BBA, 1995).

[b] The mean development rate presents the mean time span (in day^{-1}) between the application of the test substance and the emergence of the experimental cohort of midges (normally between 0.05 and 0.1 day^{-1}).

[c] A statistically significant difference in the development rate of emerging midges was observed between the control and solvent (t-test, $p < 0.05$). The results of the solvent control were used for further statistical analysis.

[d*] Significantly less midges emerged when compared with the combined control and solvent control (Williams test, $p \leq 0.05$).

[e*] Significant difference when compared to the solvent control development rate (Kruskal-Wallis, $p \leq 0.05$). Although the mean value for the development rate was the same for the 4.7 and 8.5 mg/L test concentrations, only the effect in the highest test concentration was significant since in all four replicates the development rate was relatively low, whereas for the 4.7 mg/L test concentration only in one replicate a very low development rate was found.

Table 9.2.5.-7: Mean measured concentrations of RH-117,281 in water phase of the 28-day static chronic midge study in a water/sediment system.

Nominal test concentration (mg a.s./L)	Replicate A sample number	Meas. test conc. (mg a.s./L) day 0 (% of nominal)		Meas. test conc. (mg a.s./L) day 7		Meas. test conc. (mg a.s./L) day 28	
		a	b	a	b	a	b
0.45	1	0.45	0.39	0.20	0.21	0.16	0.14
	2	0.32	(87)	0.22	(47)	0.15	(31)
	3	0.41		0.20		0.11	
0.81	1	0.78	0.72	0.31	0.32	0.24	0.24
	2	0.77	(89)	0.32	(39)	0.25	(30)
	3	0.61		0.33		0.23	
1.5	1	1.15	1.08	0.39	0.41	0.42	0.44
	2	0.99	(72)	0.41	(28)	0.45	(29)

	3	1.10		0.44		0.45	
2.6	1	1.28	2.00	0.45	0.45	0.64	0.62
	2	1.98	(77)	0.45	(17)	0.61	(24)
	3	2.75		0.45		0.63	
4.7	1	2.01	1.89	0.51	0.58	1.01	0.96
	2	1.77	(40)	0.61	(12)	0.83	(20)
	3	1.89		0.61		1.05	
8.5	1	2.72	2.79	0.82	0.77	1.10	1.13
	2	2.82	(33)	0.77	(9)	1.08	(13)
	3	2.82		0.73		1.21	

a Individual values.

b mean values and % of nominal.

c The radioactivity is the sample was too low for an accurate determination of the test concentration.

Table 9.2.5.-8: Mean measured concentration of RH-117,281 in water phase, sediment phase and pore water of the 28-day static chronic midge study in a water/sediment system.

Nominal test concentration (mg a.s./L) replicate	sample number	Meas. test conc. (mg a.s./L) water phase (% of nominal)		Meas. test conc. (mg a.s./kg) sediment[c]		Meas. test conc. (mg a.s./L) pore water (% of nominal)	
		a	b	a	b	a	b
0.45 E	1	0.30	0.37	0.32	0.32	d	d
Day 0	2	0.47	(87)	0.29	(72)	d	
	3	0.35		0.37		d	
0.45 F	1	0.17	0.16	0.45	0.44	d	d
Day 7	2	0.16	(35)	0.39	(98)	d	
	3	0.15		0.48		d	
0.45 A	1	0.16	0.14	1.79	2.27	0.001	0.01
day 28	2	0.15	(31)	2.42	(505)	0.01	(2)
	3	0.11		2.61		0.02	
8.5 E	1	4.95	4.54	24.1	24.8	0.21	0.17
day 0	2	3.91	(53)	25.3	(291)	0.16	(2)
	3	4.76		24.9		0.14	
8.5 F	1	0.70	0.67	8.66	8.40	0.07	0.08
day 7	2	0.65	(8)	7.97	(99)	0.08	(1)
	3	0.68		8.59		0.09	
8.5 A	1	1.10	1.13	30.0	25.6	0.019	0.17
day 28	2	1.08	(13)	18..1	(301)	0.17	(2)
	3	1.21		28.6		0.17	

a Individual values.

b Mean values.

c Based on wet weight of the sediment.

d The radioactivity is the sapmle was too low for an accurate determination of the test concentration.

Note: The recoveries of the QC sample, which verified the combustion efficiency of RH-117,281 in the oxidizer were 97, 99 and 99% for days 0, 7 and 28.

A statistically significant difference was seen in the development rate of emerging midges between control and solvent control (t-test, $p<0.05$). The results of the solvent control were therefore used as the

basis for further statistical analysis. The ErC50 was >8.5 mg a.s./l (nominal) and the NOErC was 4.7 mg a.s./l (nominal)
No statistically significant difference in the total number of emerged adult midges was seen between control and solvent control (t-test, $p<0.05$). The results of both controls were therefore combined for further statistical analysis. The EC_{50} (emergence) was 2.0 mg a.s./l (nominal) and the NOEC was 0.45 mg a.s./l (nominal).

The EC_{50} (emergence) = 2.0 mg a.s./L (nominal)
NOEC = 0.45 mg a.s./L (nominal)

RMS comments:

The study was in accordance with GLP and using the BBA method. There were no significant deviations from the protocol. EC_{10} value is not given in the study, no argumentation about absence of value, results considered not sufficient. The study is considered acceptable.

The EC_{50} = 2.0 mg a.s./L (nominal)
NOEC = 0.45 mg a.s./L (nominal)

Report:	**8.2.5.2/01 Drottar, K.R., and Krueger, H.O., (1998) RH-117,281 Technical: A flow-through life-cycle toxicity test with the saltwater mysid (*Mysidopsis bahia*), Report number: 97RC-0077**
Guidelines:	OPPTS 850.1500; FIFRA Subdivision E Series 72-4
GLP:	Yes
Previous evaluation:	No, submitted for the purpose of renewal of a.s. approval

Executive Summary

In a lifecycle toxicity study saltwater mysids (*Mysidopsis bahia*) were exposed to RH-117,281 Technical. The test was conducted under flow-through conditions over a period of 27 days with the following nominal test item concentrations: 0, (control and solvent control), 1.6, 3.1, 6.3, 13, and 25 µg a.s./L (corresponding to 0, 1.7, 3.4, 7.2, 14 and 19 µg a.s./L mean measured concentrations). Groups of 60 mysids (< 24 hours old, 15 mysids in each of four replicate test chambers) were exposed to each test concentration and the controls.

Observations of mortality, clinical signs of toxicity and reproduction were made daily. At test termination, the lengths and dry weights of all surviving first-generation mysids were measured. Concentrations of RH-117,281 Technical were measured at pre-test, Days 0, 7 (before and after aeration), 14, 21 and 27 by LSC.

Reproduction (the number of young per reproductive day), length and mean dry weight were the most sensitive biological parameters. Mysids in the 14 µg a.s./L treatment group produced statistically fewer young and had decreased lengths and dry weights when compared to the pooled control group. Survival to pairing on Day 14 was also significantly reduced in the 19 µg a.s./L treatment group when compared to the pooled controls.

For this study, the NOEC was determined to be 7.2 µg a.s./L. The LOEC based on reproduction, length and mean dry weight, was 14 µg a.s./L.

Materials and methods
Materials

1.	**Test material:**	RH-117,281 Technical
	Lot number:	DSR-9510, TD No. 95-161
	Purity:	92.3%
	Description:	White powder
2.	**Radiolabelled:**	(phenyl ring-UL-14C)-RH-117,281
	Radioisotope	
	Inventory Lot No.:	94.0102
	Specific activity:	35.46 mCi/g
	Radiopurity:	96.3%
3.	**Dilution water:**	Natural seawater from Indian River Inlet, Delaware (filtered and diluted with well water to a salinity of ~20‰)
	Vehicle:	Acetone
4.	**Test organism**	
	Species:	Saltwater mysid *Mysidopsis bahia*
	Age at test initiation:	< 24 hours old
	Source:	In-house culture
	Diet:	Live brine shrimp (*Artemia* sp.) nauplii *ad libitum* three times a day during the test.
	Test vessels:	Prior to sexual maturity mysids were held in 12-cm glass culture dishes with sides of nylon mesh. After sexual maturity was attained, the reproductive pairs were placed in 5 cm glass petri dishes with sides of nylon mess screen attached. All test compartments were placed in 9-L aquaria test chambers containing approximately 5 L of test solution.

Study design and methods

1. Environmental conditions

Temperature:	26.4 – 27.1°C
pH:	8.1 – 8.3
Dissolved oxygen:	4.2 – 6.4 mg/L (mild aeration was initiated in all test chambers on Day 7, after dissolved oxygen had dropped as low as 4.2 mg/L)
Salinity:	20‰ (in control chambers)
Photoperiod:	16 hours light: 8 hours darkness (849 lux at test initiation)

2. Animal assignment and treatment:

To begin the test, neonate mysids were impartially distributed in groups of one, two or three among glass beakers until each beaker contained 15 mysids. The mysids were then transferred to the test compartments. A total of 60 mysids were exposed in each treatment (nominal concentration: 1.6, 3.1, 6.3, 13, and 25 µg a.s./L) and control (dilution water and solvent) group. On Day 14 of the test, female and male adults were paired (when possible 5 male/female pairs were made for each replicate test chamber) and the reproduction of the paired mysids was monitored through Day 27. All test compartments were placed in 9-L aquaria test chambers containing approximately 5 L of test solution.

3. Dose preparation:

The test substance was a mixture of radiolabelled RH-117,281 and non-radiolabelled RH-117,281. Primary stocks of ^{14}C-RH-117,281 and of RH-117,281 Technical were prepared in ethyl acetate and acetone, respectively. Secondary working stocks were prepared at concentrations of 31.3, 62.5, 125, 250 and 500 mg a.s./L by adding 10.54 mL (6.35 mg) of the ^{14}C-RH-117,281 primary stock to a 500-mL volumetric flask (the ethyl acetate was evaporated using a nitrogen stream) and appropriate amounts of RH-117,281 primary stock. All working stocks were brought to a final volume of 500 mL with acetone. The nominal radioactivity in each working stock was 1,000,000 dpm/mL. The five working stocks were injected into the diluter mixing chamber (at a rate of 0.0075 mL/minute) where they were mixed with dilution water (at a rate of 150 mL/minute) to achieve the desired test concentrations. Acetone only was injected into the mixing chamber for the solvent control group. The concentration of acetone in the solvent control and all RH-l 17,281 treatment groups was 0.050 mL/L.

4. Measurements/observations:

Observations of the survival and behaviour of each first-generation mysid were made daily throughout the test. After mysids were paired, the number of second-generation mysids were counted and recorded daily until test termination. Second-generation mysids were also observed for abnormal development and aberrant behaviour. After each observation, second-generation mysids were discarded. At test termination, the sex of each surviving first-generation mysid was confirmed and the length of each mysid was measured. The dry weight of each surviving first generation mysid was also measured.

Water samples were collected from one replicate test chamber of each treatment and control group prior to test initiation (pre-test). Water samples were also collected from alternating replicates on Days 0, 7 (before and after aeration), 14, 21 and 27. Samples were analysed by LSC as soon as possible without storage. On Day 0 of the test, an additional sample was collected from the 25 µg a.s./L treatment group for confirmation by HPLC.

5. Statistics:

Control and solvent control data where compared using 2 x 2 contingency tables and Chi-square test or Student's t-test and the data were pooled when no statistical differences were found for comparison to treatments. Survival data were analysed sing 2 x 2 contingency tables and Chi-square test to identify statistically differences of treatment groups compared to the control group. The reproduction and growth data were assessed by Shapiro-Wilk's Test and Bartlett's Test for normality and homogeneity, respectively. When data were deemed normal and homogenous an analysis of variance test was used to determine statistically significant differences among groups, and a Bonferroni t-test was used to identify statistical differences of treatment groups compared to the control group. If data were not normal or homogenous then transformations were performed and in the cases they failed to correct the non-normality or heterogeneity of variances, a nonparametric test (Wilcoxon's rank sum test) was used to evaluate the data.

All statistical tests were performed by SPSS/PC Version 2.0 or TOXSTAT Version 3.5 statistical software.

Results and discussion

A. Biological effects

A summary of mortality is summarised in Table 8.2.5.2-1. Mortality in the RH-117,281 Technical treatment groups ≤ 14 µg a.s./L were not statistically different from the pooled control group. The Bonferroni t-test showed that reproduction was significantly reduced in the 14 µg a.s./L treatment group in comparison to the pooled controls ($p \leq 0.05$). Summaries of the lengths and dry weight of the surviving adult mysids are also presented in Table 9.2.5-9. The Bonferroni t-test and the Wilcoxon;s rank sum test showed that mean length and mean dry weight, respectively, were significantly reduced in the 14 µg a.s./L treatment group when compared to the pooled controls ($p \leq 0.05$).

Table 9.2.5-9: Summary of effects on adult survival, reproduction and growth

Mean measured concentration (µg a.s./L)	Mean % mortality		Overall mean number of young per reproductive day	Mean body length of mysids (mm)	Mean dry weight of mysids (mg)
	Day 0-Day 14	Day 14 – Day 27			
Control	10	6.5	0.525 ± 0.076	6.98 ± 0.048	0.79 ± 0.029
Solvent control	13	14	0.527 ± 0.015	6.95 ± 0.046	0.77 ± 0.036
Pooled control	12	10	0.526 ± 0.051	6.97 ± 0.048	0.78 ± 0.033
1.7	17	9.8	0.514 ± 0.080	6.98 ± 0.043	0.75 ± 0.003
3.4	12	4.5	0.482 ± 0.053	6.89 ± 0.054	0.73 ± 0.037
7.2	17	14	0.457 ± 0.066	6.88 ± 0.110	0.72 ± 0.074
14	15	8.3	0.0889 ± 0.030*	6.65 ± 0.082*	0.59 ± 0.026**
19	100	--	--	--	--

*Indicates a significant difference from the pooled controls using the Bonferroni t-test ($p \leq 0.05$)
**Indicates a significant difference from the pooled controls using the Wilcoxon's rank sum test ($p \leq 0.05$)

B. Toxicity endpoint

The NOEC and LOEC values based on reproduction, mean length and mean dry weight are 7.2 µg a.s./L and 14 µg a.s./L, respectively.

C. Analytical verification

The results of the analysis of the exposure solutions for RH-117,281 Technical concentration during the in-life portion of the test are presented in Table 9.2.5-10. Analysis of the test solutions demonstrated that the expected concentration-gradient was generally maintained during the 28-day exposure.

Table 9.2.5-10: Measured concentrations of RH-117,281 Technical in the exposure solutions

Nominal concentration	Measured concentration (µg a.s./L)[a]							% Nominal
	Day 0	Day 7[a]	Day 7[b]	Day 14	Day 21	Day 27	Mean	
Control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	--	--
Solvent control	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	--	--
1.6	1.43	1.72	1.72	1.69	1.74	1.70	1.7	106
3.1	3.16	3.85	3.25	3.42	3.52	3.46	3.4	110
6.3	6.35	7.05	7.24	8.51	7.14	6.95	7.2	114
13	12.0	13.7	13.8	13.2	15.2	14.6	14	108
25	22.3	20.1	16.2	18.5	--[c]	--[c]	19	76

LOQ: Limit of quantification was 0.135 µg a.s./L
[a] Prior to aeration
[b] After aeration
[c] Samples were not collected due to 100% mortality

Conclusion:

LOEC (*Mysidopsis bahia*) = 14 µg a.s./L
NOEC (*Mysidopsis bahia*) = 7.2 µg a.s./L (mean measured).

RMS comments:
EC_{10} value is not given in the study, no argumentation about absence of value, results considered not sufficient. The study is considered acceptable.

NOEC = 7.2 µg a.s./L (mean measured).

B.9.2.6 Effects on algal growth

Report:	**CA, 8.2.6.1/01 Ziegler, T.A., Stewart, S. (1996)** **Acute toxicity of RH-117,281 Technical to S*elenastrum capricornutum* Printz.**

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. LG 3517), chemical purity: 92.9% a.s.
Test species: Freshwater green algae S*elenastrum capricornutum*
Number of organisms: 3 replicates/concentration each 1.0 mL of algal inoculum containing 3×10^5 cells/mL
Type of test: 120-hour acute toxicity test
Applied and measured concentrations:
nominal test concentrations: Control, vehicle Blank (acetone), 5.0, 10, 20, 40 and 80 µg/L
The measured test concentrations were in the range 86-103% of the nominal concentrations.
Test conditions:
temperature: 22 °C
pH: 7.5 to 8.3
lighting: light intensity was maintained at 400 ± 10% footcandles (approximately 4300 lux).
Analytical methods: HPLC analysis with UV detection

Results:
Analytical determination of RH-117,281 Technical in the exposure system during the 120-hour static acute toxicity test was conducted at 0-, 72-, and 120-hours. The 0-hour measured concentrations for RH-117,281 Technical in test levels 1 – 5 were 4.86, 11.6, 19.2, 38.3 and 79.1 µg a.i./L. The 0- to 72-hour mean measured concentrations were 4.57, 9.75, 20.6, 34.8 and 69.1 µg a.i./L for levels 1 – 5, which were 91, 98, 103, 87 and 86% of nominal concentrations. The 0- to 120-hour mean measured concentrations for the same levels were 4.13, 8.58, 17.8, 31.9 and 65.6 µg a.i./L, which were 83, 86, 89, 80 and 82% of the nominal concentrations. The72- and 120-hour mean measured concentrations for abiotic replicate were 67.9 and 64.9 µg a.i./L.
Water quality parameters of temperature and pH were measured at 0 and 120 hours of the study. The test temperature was 22 ^{0}C. The pH values of the solutions ranged from 7.5 to 8.3. Temperature and pH values were in acceptable limits.
Results are based on mean measured concentrations. The 72-hour mean was calculated using the 0- and 72-hour measured results. The 120-hour mean was calculated using the 0-, 72-, and 120-hours measured results.
The E_bC_{50} (0-72 hours), (0-96 hours), and (0-120 hours) for RH-117,281 Technical were 36 µg a.i./L (95% confidence limits = 33 and 38 µg a.i./L), 29 µg a.i./L (95% confidence limits = 26 and 33 µg a.i./L), and 23 µg a.i./L (95% confidence limits = 19 and 27 µg a.i./L).

Table B.9.2.6-1: Measured concentration of RH-117,281 Technical during the 120-hour acute toxicity study of RH-117,281 technical to *Selenastrum capricornutum* Printz

Sample	Nominal concentration	Measured concentration (µg/L)									
		0-hour	% of nominal	72-hour	% of nominal	120-hour	% of nominal	0-72 hours mean	% of nominal	0-120 hour mean	% of nominal
Control	---	< MQL[a]	NA	< MQL[a]	NA	< MQL[a]	NA	NA	NA	NA	NA
Vehicle Blank	---	< MQL[a]	NA	< MQL[a]	NA	< MQL[a]	NA	NA	NA	NA	NA
Level 1	5.0	4.86	97	4.28	86	3.26	65	4.57	91	4.13	83
Level 2	10	11.6	116	7.90	79	6.25	63	9.75	98	8.58	86
Level 3	20	19.2	96	21.9	110	12.4	62	20.6	103	17.8	89
Level 4	40	38.3	96	31.3	78	26.0	65	34.8	87	31.9	80
Level 5	80	79.1	99	59.0	74	58.7	73	69.1	86	65.6	82
Level 5 (abiotic)	80	NA	NA	67.9	85	61.8	77	67.9	85	64.9	81

[a] MQL = 0.403 µg/L for 0-hour, 0.809 µg/L for 72-hour, and 0.554 µg/L for 120-hour

Table B.9.2.6-2: Fortifications and recoveries of RH-117,281 Technical during the 120-hour acute toxicity study of RH-117,281 technical to *Selenastrum capricornutum* Printz

	0-hour		72-hour		120-hour	
Fortification level (µg/L)	Measured concentration (µg/L)	Percent recovery	Measured concentration (µg/L)	Percent recovery	Measured concentration (µg/L)	Percent recovery
Low spike (4.79)	3.25	67.8	3.77	78.7	3.64	76.0
Mid spike (25.2)	17.2	68.3	11.8	46.8[a]	18.5	73.4
High spike (80.8)	67.7	83.8	59.3	73.4	60.0	74.3
Avg. % recovery		73.3		76.1		74.6

[a] Due to a suspected sample preparation error, the Mid spike was not included in the mean.

Table B.9.2.6-3: Cell counts for *Selenastrum capricornutum* Printz during the exposure to RH-117,281 Technical

Nominal test conc. (µg/L)	Mean cell counts (Three flasks) 10^4 cells/mL					
	0-hour	24-hour	48-hour	72-hour	96-hour	120-hour
Control	0.29	0.82	2.1	19	30	100
Vehicle Blank	0.26	0.74	1.9	12	30	100
5.0		0.74	2.0	17	29	100
10		0.85	2.0	16	22[a]	78[a]
20		0.74	2.0	14	17[a]	49[a]
40		0.52[a]	1.2[a]	8.6[a]	14[a]	42[a]
80		0.26[a]	0.33[a]	0.037[a]	0.18[a]	0.22[a]

[a] Denotes a significant ($p \leq 0.05$) inhibition effect from the pooled control population as calculated using cell counts and Dunnett`s test

The 72-, 96-, and 120-hour EC_{50} values were 36 µg a.i./L (95% confidence limits = 32 and 40 µg a.i./L), 21 µg a.i./L (95% confidence limits = 17 and 26 µg a.i./L) and 19 µg a.i./L (95% confidence limits = 15 and 24 µg a.i./L).

The E_rC_{50} (48-72 hours), (72-96 hours), and (96-120 hours) for RH-117,281 Technical were 40 µg a.i./L (95% confidence limits = 26 and 55 µg a.i./L), 17 µg a.i./L (95% confidence limits = 0 and 48 µg a.i./L), and 48 µg a.i./L (95% confidence limits = 29 and 67 µg a.i./L).

The 72-, 96-, and 120-hour no-observed-effect-concentrations (NOEC) were calculated to be 21, 4.1, and 4.1 µg a.i./L, based on mean measured concentrations.

72-hour EC_{50} = 36 µg a.i./L (95% confidence limits = 32 and 40 µg a.i./L)

96-hour EC_{50} = 21 µg a.i./L (95% confidence limits = 17 and 26 µg a.i./L)

120-hour EC_{50} = 19 µg a.i./L (95% confidence limits = 15 and 24 µg a.i./L)

0-72-hour E_bC_{50} = 36 µg a.i./L (95% confidence limits = 33 and 38 µg a.i./L)

0-96-hour E_bC_{50} = 29 µg a.i./L (95% confidence limits = 26 and 33 µg a.i./L)

0-120-hour E_bC_{50} = 23 µg a.i./L (95% confidence limits = 19 and 27 µg a.i./L)

24-48-hour E_rC_{50} = 53 µg a.i./L (95% confidence limits = 35 and 71 µg a.i./L)

48-72-hour E_rC_{50} = 40 µg a.i./L (95% confidence limits = 26 and 55 µg a.i./L)

72-96-hour E_rC_{50} = 17 µg a.i./L (95% confidence limits = 0 and 48 µg a.i./L)

96-120-hour E_rC_{50} = 48 µg a.i./L (95% confidence limits = 29 and 67 µg a.i./L).

72-hour NOEC = 21 µg a.i./L

96-hour NOEC = 4.1 µg a.i./L

120-hour NOEC = 4.1 µg a.i./L

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

120-hour E_rC_{50} = 48 µg a.i./L

120-hour E_bC_{50} = 23 µg a.i./L

Report:	**CA, 8.2.6.1/02 Drottar, K.R., Sutherland, C.A., Krueger, H.O. (1998e) RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*).**

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: freshwater bluegreen algae *Anabaena flos-aquae*
Number of organisms: 3 replicates/concentration, 10 000 cells/mL at initiation
Type of test: 96-hour static toxicity test
Applied and measured concentrations:
nominal test concentrations: Negative control, solvent control (0.1 mL DMF/L), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.i./L
The 0 day measured test concentrations: negative control, solvent control, 0.050, 0.10, 0.20, 0.42 and 0.86 mg a.i./L.
Test conditions:
temperature: 24 ± 2 °C
pH: 7.4 to 7.9
lighting: 2150 ± 320 lux

Results:

Table B.9.2.6-4: Mean cell density and percent inhibition in study with freshwater alga *Anabaena flos-aquae*

Day 0 measured concentration (mg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Cell density[1]	Percent Inhibition[2]	Cell density	Percent Inhibition	Cell density	Percent Inhibition	Cell density	Percent Inhibition
Negative control	17,000	--	74,667	--	308,333	--	1,020,000	--
Solvent control	14,000	--	54,667	--	290,000	--	965,000	--
Pooled controls	15,500	--	64,667	--	299,167	--	992,500	--
0.050	14,000	9.7	61,667	4.6	241,667	19	916,667	7.06
0.10	18,000	-16	74,000	-14	218,333	27	1,061,667	-7.0
0.20	15,333	1.1	60,333	6.7	233,333	22	1,116,667	-13
0.42	15,333	1.1	74,333	-15	341,667	-14	1,076,667	-8.5
0.86	13,667	12	68,333	-5.7	210,000	30	1,050,000	-5.8

[1] The initial cell density (cells/mL) of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 10 000 cells/mL at test initiation (Day 0).

[2] Percent inhibition was calculated relative to the pooled control replicates.

Table B.9.2.6-5: Mean growth rate and percent inhibition values in study with freshwater alga *Anabaena flos-aquae*

Day 0 measured concentration (mg a.s./L)	Day 1		Day 2		Day 3		Day 4	
	Mean growth rate	Percent inhibition[1]	Mean growth rate	Percent inhibition	Mean growth rate	Percent inhibition	Mean growth rate	Percent inhibition
Negative control	0.0205	--	0.0415	--	0.0471	--	0.0482	--
Solvent control	0.0135	--	0.0353	--	0.0467	--	0.0476	--
Pooled controls	0.0170	--	0.0384	--	0.0469	--	0.0479	--
0.050	0.0137	19	0.0376	2.0	0.0442	5.7	0.0471	1.7
0.10	0.0212	-24	0.0417	-8.6	0.0428	8.7	0.0486	-1.5
0.20	0.0173	-1.5	0.0367	4.4	0.0436	7.0	0.0491	-2.6
0.42	0.0177	-3.9	0.0418	-8.8	0.0487	-3.9	0.0487	-1.8
0.86	0.0123	27	0.0400	-4.2	0.0422	10	0.0484	-1.2

[1] Percent inhibition was calculated relative to the pooled control replicates.

Table B.9.2.6-6: Mean area under the growth curve and percent inhibition values in study with freshwater alga *Anabaena flos-aquae*

Day 0 measured concentration (mg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Mean area	Percent inhibition[1]	Mean area	Percent inhibition	Mean area	Percent inhibition	Mean area	Percent inhibition
Negative control	84,000	--	944,000	--	5,300,000	--	21,000,000	--
Solvent control	48,000	--	632,000	--	4,528,000	--	19,348,000	--
Pooled controls	66,000	--	788,000	--	4,914,000	--	20,174,000	--
0.050	48,000	27	716,000	9.1	4,116,000	16	17,776,000	12
0.10	96,000	-45	960,000	-22	4,228,000	14	19,348,000	4.1
0.20	64,000	3.0	732,000	7.1	4,016,000	18	19,976,000	1.0
0.42	64,000	3.0	900,000	-14	5,652,000	-15	22,432,000	-11
0.86	44,000	33	788,000	0.0	3,888,000	21	18,768,000	7.0

[1] Percent inhibition was calculated relative to the pooled control replicates.

The response of the algae was measured in terms of biomass, expressed as cell density, area under the growth curve, and growth rate. EC_{50} values (i.e., the theoretical test concentration that produced a 50% reduction in the measured parameter) for these parameters were estimated to be greater than 0.86 mg a.i./L (at or above the functional water solubility) for each 24-hour interval.

The no observed effect concentration (NOEC), which is the highest test concentration that has no inhibitory effect on algal growth, was determined for biomass, area under the growth curve and growth rate to be 0.86 mg a.i./L, the highest concentration tested. Visible growth was observed in all RH-117,281 Technical treatment groups at test termination, including that the compound is algistatic, recovery phase was not performed.

96-hour EC_{50} (cell density): >0.86 mg a.i./L (confidence limits not calculable) (mean measured).

96-hour E_rC_{50} (growth rate): >0.86 mg a.i./L (confidence limits not calculable) (mean measured).

96-hour E_bC_{50} (area under the growth curve): >0.86 mg a.i./L (confidence limits not calculable)

NOEC: 0.86 mg a.i./L

RMS comments:

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

96-hour E_rC_{50} (growth rate): >0.86 mg a.i./L

96-hour E_bC_{50} (area under the growth curve): >0.86 mg a.i./L

Report:	**CA, 8.2.6.1/03 Drottar, K.R., Sutherland, C.A., Krueger, H.O. (1998f) RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Scenedesmus subspicatus*).**

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: freshwater alga *Scenedesmus subspicatus*
Number of organisms: 10 000 cells/mL in each test chamber
Type of test: 96-hour static toxicity test
Applied and measured concentrations: Nominal test concentrations: Negative control, solvent control (0.1 mL DMF/L), 1.7, 3.4, 6.8, 14, and 27 µg a.i./L. The mean measured test concentrations: Negative control, solvent control, 1.6, 3.5, 7.0, 14 and 28 µg a.i./L
Test conditions:
temperature: 24 ± 2 °C
pH: 7.4 to 8.8
lighting: 4300 ± 430 lux

Results:
The freshwater alga, *Scenedesmus subspicatus*, was exposed to a geometric series of five test concentrations, and a negative (culture medium) control and solvent control (0.1 mL/ DMF/L) under static conditions for96-hour. Concentrations of RH-117,281 Technical were measured in samples of the test solutions collected at the beginning of the test, at 72-hours, and at the end of the test.

Temperature measurements were within the acceptable limits (24 ± 2 0C) throughout the 96-hour exposure period. Light intensity measurements at the test initiation were within the range established for the test (4300 ± 430 lux). The pH measurements from the test solutions on Day 0 and 4 ranged from 7.4 to 8.8, and were typical of pH values obtained in tests conducted with *Scenedesmus subspicatus.*

The response of the algae was measured in terms of cell density, growth rate, and biomass, expressed as area under the growth curve. EC50 values (i.e., the theoretical test concentration that produced a 50% reduction in the measured parameter) for these parameters were calculated at 72 and 96 hours and ranged from 11 to 18 µg a.i./L. The no observed effect concentration (NOEC), which is the highest test concentration that has no inhibitory effect on algal growth, was determined to be 7.0 µg a.i./L for cell density, growth rate and biomass. Visible growth was observed in all RH-117,281 Technical treatment groups at test termination, indicating that the compound is algistatic, recovery phase was not performed.

96-hour EC_{50} (cell density): 11 µg a.i./L (95% CI – 9.4 and 12 µg a.i./L) (mean measured).

96-hour E_rC_{50} (growth rate): 18 µg a.i./L (95% CI – 15 and 22 µg a.i./L) (mean measured).

96-hour E_bC_{50} (area under the growth curve): 11 µg a.i./L (95% CI – 9.4 and 12 µg a.i./L)

NOEC: 7.0 µg a.i./L

Table B.9.2.6-7: Mean cell density and percent inhibition in study with freshwater alga *Scenedesmus subspicatus*

Day 0 measured concentration (µg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Cell density[1]	Percent Inhibition[2]	Cell density	Percent Inhibition[2]	Cell density	Percent Inhibition[2]	Cell density	Percent Inhibition[2]
Negative control	32,969	--	77,615	--	410,910	--	1,634,226	--
Solvent control	33,009	--	68,690	--	392,767	--	1.504,860	--
Pooled controls	32,989	--	73,153	--	401,838	--	1,596,543	--
1.6	33,270	-1	84,761	-16	348,959	13	1,391,235	11
3.5	26,886	19	96,182	-31	330,156	18	1,378,293	12
7.0	30,835	7	89,625	-23	320,878*	20	1,336,026	15
14	18,026*	45	47,575*	35	108,259*	73	320,362*	80
28	8,927*	73	10,342*	86	9,222*	98	30,618*	98

[1] The initial cell density (cells/mL) of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 10 000 cells/mL at test initiation (Day 0).

[2] Percent inhibition was calculated relative to the pooled control replicates. Calculations were performed using SAS 6.12. manual calculations may differ slightly due to rounding.

* Statistically different ($p \leq 0.05$) compared to the pooled control replicates.

Table B.9.2.6-8: Mean growth rate and percent inhibition values in study with freshwater alga *Scenedesmus subspicatus*

Day 0 measured concentration (µg a.s./L)	Day 1		Day 2		Day 3		Day 4	
	Mean growth rate	Percent inhibition[1]	Mean growth rate	Percent inhibition	Mean growth rate	Percent inhibition	Mean growth rate	Percent inhibition
Negative control	0.0493	--	0.0427	--	0.0515	--	0.0529	--
Solvent control	0.0496	--	0.0398	--	0.0509	--	0.0522	--
Pooled controls	0.0494	--	0.0412	--	0.0512	--	0.0526	--
1.6	0.0496	-0.37	0.0438	-6.2	0.0493	3.8	0.0513	2.3
3.5	0.0407	18	0.0468	-14	0.0484	5.5	0.0512	2.6
7.0	0.0469	5.1	0.0457	-11	0.0481	6.2	0.0509	3.1
14	0.0240*	51	0.0322*	22	0.0327*	36	0.0348*	34
28	0.0024*	95	0.0031*	92	0.0007*	99	0.0071*	87

[1] Percent inhibition was calculated relative to the pooled control replicates.

* Statistically different ($p \leq 0.05$) compared to the pooled control replicates.

Table B.9.2.6-9: Mean area under the growth curve and percent inhibition values in study with freshwater alga *Scenedesmus subspicatus*

Day 0 measured concentration (µg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Mean area[1]	Percent inhibition[1]	Mean area	Percent inhibition	Mean area	Percent inhibition	Mean area	Percent inhibition
Negative control	275,634	--	1,362,647	--	6,984,941	--	31,268,567	--
Solvent control	276,112	--	1.256,508	--	6,553,994	--	29,085,519	--
Pooled controls	275,873	--	1,309,578	--	6,769,468	--	30,186,043	--
1.6	279,243	-1.2	1,455,615	-11	6,420,247	5.2	27,062,568	10
3.5	202,628	27	1,439,444	-10	6,315,506	6.7	26,576,896	12
7.0	250,017	9.4	1,455,538	-11	6,141,574	9.3	25,784,426	15
14	96,308*	65	643,513*	51	2,273,524*	66	7,176,987*	76
28	7,577*	97	37,662*	97	66,363*	99	317,303*	99

[1] Calculations were performed using SAS 6.12. Manual calculations may differ slightly due to rounding. Percent inhibition was calculated relative to the pooled control replicates.

* Statistically different ($p \leq 0.05$) compared to the pooled control replicates.

RMS comments:

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

96-hour E_rC_{50} (growth rate): 18 µg a.i./L.

96-hour E_bC_{50} (area under the growth curve): 11 µg a.i./L

Report:	**IIA, 8.2.6.1/04 Drottar, K.R., Sutherland, C.A., Krueger, H.O. (1998g) RH-117,281 Technical: A 96-hour toxicity test with the freshwater diatom (*Navicula pelliculosa*).**

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: freshwater diatom *Navicula pelliculos; Number of organisms:* 10 000 cells/mL in each test chamber; *Type of test:* 96-hour static toxicity test
Applied and measured concentrations: nominal test concentrations - Negative control, solvent control (0.1 mL/L dimethylformamide (DMF)), 0.058, 0.11, 0.21, 0.41 and 0.93 mg a.i./L. The mean measured test concentrations: Negative control, solvent control, 0.058, 0.11, 0.21, 0.41 and 0.93 mg a.i./L
Test conditions:
temperature: 24 ± 2 °C; pH: 7.4 to 8.3; lighting: 4300 ± 430 lux

Results:

The freshwater diatom, *Navicula pelliculosa*, was exposed to a geometric series of five test concentrations, a negative and solvent control under static conditions for 96-hours. Test concentrations were adjusted for purity of the active ingredient in the test substance. All test substances appeared clear and colorless.

The response of algae was measured in terms of biomass, expressed as cell density, area under the growth curve, and growth rate. EC_{50} values (i.e., the theoretical test concentration that produced a 50% reduction in the measured parameter) for these parameters were estimated to be greater than 0.93 mg a.i./L (at or above the functional water solubility) for each 24-hour interval. The no observed effect concentration (NOEC), which is the highest test concentration that has no inhibitory effect on algal growth, was determined for biomass, area under the growth rate to be 0.21 mg a.i./L.

96-hour EC_{50} (cell density): > 0.93 mg a.i./L (mean measured).

96-hour E_rC_{50} (growth rate) : >0.93 mg a.i./L (mean measured).

96-hour E_bC_{50} (Area under the growth curve): >0.93 mg a.i./L

NOEC: 0.21 mg a.i./L

Table B.9.2.6-10: Mean cell density and percent inhibition in study with freshwater diatom *Navicula pelliculosa*

Day 0 measured concentration (mg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Cell density[1]	Percent Inhibition[2]	Cell density	Percent Inhibition[2]	Cell density	Percent Inhibition[2]	Cell density	Percent Inhibition[2]
Negative control	18,707	--	132,840	--	483,894	--	1,718,580	--
Solvent control	33,521	--	135,783	--	478,535	--	1,805,466	--
Pooled controls	26,114	--	134,311	--	481,214	--	1,762,023	--
0.058	21,353	18	122,443	8.8	464,071	3.6	1,677,642	4.8
0.11	16,148	38	82,205	39	273,643**	43	930,363**	47
0.21	7,474*	71	116,173	14	364,116	24	1,693,813	3.9
0.41	10,620	59	85,843	36	219,125*	55	1,107,210	37
0.93	9,323*	64	99,402	26	284,325*	41	980,194*	44

[1] The initial cell density (cells/mL) of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 10 000 cells/mL at test initiation (Day 0).

[2] Percent inhibition was calculated relative to the pooled control replicates.

* Statistically different ($p \leq 0.05$) compared to the pooled control replicates.

** Not considered to be dose-responsive due to the cell density observed in the 0.21 mg a.s./L treatment group.

Table B.9.2.6-11: Mean growth rate and percent inhibition values in study with freshwater diatom *Navicula pelliculosa*

Day 0 measured concentration (mg a.s./L)	Day 1		Day 2		Day 3		Day 4	
	Mean growth rate	Percent inhibition[1]	Mean growth rate	Percent inhibition[1]	Mean growth rate	Percent inhibition[1]	Mean growth rate	Percent inhibition[1]
Negative control	0.0240	--	0.0509	--	0.0535	--	0.0535	--
Solvent control	0.0503	--	0.0543	--	0.0536	--	0.0540	--
Pooled controls	0.0371	--	0.0526	--	0.0535	--	0.0537	--
0.058	0.0295	21	0.0517	1.7	0.0532	1.0	0.0532	1.0
0.11	0.0161	57	0.0435	17	0.0451**	16	0.0470**	13
0.21	0.0045*	88	0.0507	3.6	0.0493	7.9	0.0532	1.0
0.41	0.069	81	0.0441	16	0.0425*	21	0.0486*	9.5
0.93	0.0036*	90	0.0477	9.3	0.0455*	15	0.0475*	12

[1] Percent inhibition was calculated relative to the pooled control replicates.

* Statistically different ($p \leq 0.05$) compared to the pooled control replicates.

** Not considered to be dose-responsive due to the growth rate observed in in the 0.21 mg a.s./L treatment group.

Table B.9.2.6-12: Mean area under the growth curve and percent inhibition values in study with freshwater diatom *Navicula pelliculosa*

Day 0 measured concentration (mg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Mean area	Percent inhibition[1]	Mean area	Percent inhibition[1]	Mean area	Percent inhibition[1]	Mean area	Percent inhibition[1]
Negative control	110,000	--	1,688,562	--	8,849,362	--	35,039,047	--
Solvent control	282,257	--	2,073,905	--	9,205,714	--	36,373,728	--
Pooled controls	196,129	--	1,881,233	--	9,027,538	--	35,706,388	--
0.058	151,543	23	1,637,095	13	8,435,267	6.6	33,895,830	5.1
0.11	81,848	58	1,022,086	46	5,052,257**	44	19,260,327**	46
0.21	15,190*	92	1,258,952	33	6,782,419	25	31,237,563	13
0.41	23,448*	88	941,000	50	4,360,619*	52	20,036,646*	44
0.93	11,895*	94	1,076,590	43	5,441,314*	40	20,357,541*	43

[1] Percent inhibition was calculated relative to the pooled control replicates.

* Statistically different ($p \leq 0.05$) compared to the pooled control replicates.

** Not considered to be dose-responsive due to the area observed in the 0.21 mg a.s./L treatment group.

RMS comments:

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

96-hour E_rC_{50} (growth rate) : >0.93 mg a.i./L

96-hour E_bC_{50} (Area under the growth curve): >0.93 mg a.i./L

Report:	**CA, 8.2.6.1/05 Drottar, K.R.,Krueger, H.O. (1998c)** **RH-117,281 Technical: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*)**

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical (Lot No. DSR-9510), chemical purity: 92.3% a.s.
Test species: marine diatom *Skeletonema costatum*
Number of organisms: 77 000 cells/mL
Type of test: 96-hour static toxicity test
Applied and measured concentrations:
Nominal test concentrations: Negative control, solvent control (0.1 mL/L DMF), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.i./L
Day 0 measured test concentrations: Negative control, solvent control, 0.052, 0.11, 0.24, 0.49 and 0.91 mg a.i./L
Test conditions:
temperature: 20 ± 2 °C
pH: 7.9 to 8.8
lighting: 4300 ± 430 lux

Results:
The biomass, growth rate and area under growth curve 24, 48, 72 and 96-hour EC_{50} values for *Skeletonema costatum* exposed to RH-117,281 were > 0.91 mg a.s./L (at or above the functional water solubility), which was the highest concentration tested. The 96-hour no observed effect concentration (NOEC), defined as the highest test concentration which had no inhibitory effects upon algal growth, was determined to be 0.49 mg a.s./L.

96-hour EC_{50} (cell density): >0.91 mg a.i./L (mean measured)
96-hour E_rC_{50} (growth rate): >0.91 mg a.i./L (mean measured)
96-hour E_bC_{50} (area under the growth curve): >0.91 mg a.i./L (mean measured)
NOEC: 0.49 mg a.i./L

Table B.9.2.6-13: Mean cell density and percent inhibition in study with marine diatom *Skeletonema costatum*

Day 0 measured concentration (mg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Cell density[1]	Percent Inhibition[2]	Cell density	Percent Inhibition[2]	Cell density	Percent Inhibition[2]	Cell density	Percent Inhibition[2]
Negative control	371,611	--	1,267,259	--	2,215,008	--	1,933,869	--
Solvent control	293,647	--	1,102,337	--	1,631,856	--	1,852,974	--
Pooled controls	332,629	--	1,184,798	--	1,923,432	--	1,893,422	--
0.052	295,517	-0.64	959,257	13	1,603,401	1.7	1,662,996	10
0.11	304,996	-3.9	1,173,541	-6.5	1,495,541	8.4	1,792,821	3.2
0.24	314,344	-7.0	1,066,952	3.2	1,763,250	-8.1	1,863,345	-0.56
0.49	263,773	10	907,880	18	1,433,687	12	1,785,641	3.6
0.91	190,769*	35	779,049*	29	1,184,725	27	1,550,349*	16

[1] The initial cell density (cells/mL) of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 77 000 cells/mL at test initiation (Day 0).

[2] Percent inhibition was calculated relative to the solvent control group mean.

* Statistically different ($p \leq 0.05$) compared to the solvent control group mean.

Table B.9.2.6-14: Mean growth rate and percent inhibition values in study with marine diatom *Skeletonema costatum*

Day 0 measured concentration (mg a.s./L)	Day 1		Day 2		Day 3		Day 4	
	Mean growth rate[1]	Percent inhibition[2]	Mean growth rate[1]	Percent inhibition[2]	Mean growth rate[1]	Percent inhibition[2]	Mean growth rate[1]	Percent inhibition[2]
Negative control	0.0655	--	0.0582	--	0.0466	--	0.0336	--
Solvent control	0.0557	--	0.0553	--	0.0424	--	0.0331	--
Pooled controls	0.0606	--	0.0568	--	0.0445	--	0.0333	--
0.052	0.0557	-0.0050	0.0525	5.1	0.0421	0.74	0.0320	3.4
0.11	0.0571	-2.5	0.0567	-2.6	0.0409	3.3	0.0328	1.0
0.24	0.0585	-5.0	0.0548	0.97	0.0434	-2.5	0.0332	-0.20
0.49	0.0512	8.1	0.0514	7.1	0.0406	4.1	0.0327	1.1
0.91	0.0395*	36	0.0481*	13	0.0378*	11	0.0312	5.9

[1] Calculations were performed using SAS 6.12. Manual calculations may differ slightly due to rounding.

[2] Percent inhibition was calculated relative to the solvent control group mean.

* Statistically different ($p \leq 0.05$) compared to the solvent control group mean.

Table B.9.2.6-15: Mean area under the growth curve and percent inhibition values in study with marine diatom *Skeletonema costatum*

Day 0 measured concentration (mg a.s./L	Day 1		Day 2		Day 3		Day 4	
	Mean area[1]	Percent inhibition[2]	Mean area[1]	Percent inhibition[2]	Mean area[1]	Percent inhibition[2]	Mean area[1]	Percent inhibition[2]
Negative control	3,535,326	--	21,353,766	--	61,292,973	--	109,231,501	--
Solvent control	2,599,767	--	17,503,574	--	48,465,881	--	88,435,839	--
Pooled controls	3,067,547	--	19,428,670	--	54,879,427	--	98,833,371	--
0.052	2,622,201	-0.86	15,831,486	10	44,735,387	7.7	82,084,153	7.2
0.11	2,735,949	-5.2	18,630,393	-6.4	48,811,384	-0.71	86,423,737	2.3
0.24	2,848,133	-10	17,575,693	-0.41	49,690,125	-2.5	91,361,275	-3.3
0.49	2,241,276	14	14,453,110	17	40,703,907	16	77,487,843	12
0.91	1,365,23*	47	11,155,047*	36	32,872,335*	32	63,845,225*	28

[1] Calculations were performed using SAS 6.12. Manual calculations may differ slightly due to rounding.

[2] Percent inhibition was calculated relative to the solvent control group mean.

* Statistically different ($p \leq 0.05$) compared to the solvent control group mean.

RMS comments:

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

96-hour E_rC_{50} (growth rate): >0.91 mg a.i./L
96-hour E_bC_{50} (area under the growth curve): >0.91 mg a.i./L

Report: **CA, 8.2.6.1/07 Rhodes, J.E., Williams, S. (1998c)**
Acute toxicity of RH-127,450 to the green alga, S*elenastrum capricornutum* Printz

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-127,450 (Lot No. BM3933), chemical purity: 99.27% a.s.
Test species: freshwater green algae S*elenastrum capricornutum*
Number of organisms, age: 1.0 x 10^4 cells/mL for each flask, three days old
Type of test: 96-hour static toxicity test
Applied and measured concentrations:
Nominal concentrations: 0 (control), 0 (0.10 mL/acetone control), 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.i./L
Mean measured concentrations: <MQL (control), <MQL (acetone control), 0.27, 0.52, 1.1, 2.4 and 4.1 mg a.i./L
Test conditions:
temperature: range 24.8 °C
pH: 7.5
lighting: 4313 ± 18 lux

Results:

Table B.9.2.6-16: Cell number for green alga *Selenastrum capricornutum* during a 96-hour exposure to RH-127,450

Mean measured concentration (mg a.s./L)	Rep.	Cell numbers (x 10^4 cells/mL)				
		24-hour	48-hour	72-hour	96-hour	% inhibition[a]
Control	A	2.4	11	80	149	---
	B	2.0	12	82	225	
	C	2.1	10	84	143	
	Mean	**2.2**	**11**	**82**	**172**	
Acetone control	A	3.8	17	96	189	---
	B	2.7	9.8	58	141	
	C	2.3	12	79	166	
	Mean	**2.9**	**13**	**78**	**165**	
Pooled control	**Mean**	**2.6**	**12**	**80**	**169**	---
0.27	A	2.6	24	85	213	**0**
	B	2.4	2.	91	234	
	C	2.0	16	93	115	
	Mean	**2.3**	**20**	**90**	**187**	

0.52	A	2.3	24	70	163	**0**
	B	2.3	24	81	173	
	C	3.3	23	78	207	
	Mean	**2.6**	**24**	**76**	**181**	
1.1	A	2.2	24	76	168	**0**
	B	3.3	11	66	202	
	C	2.9	6.7	58	159	
	Mean	**2.8**	**14**	**67**	**176**	
2.4	A	2.1	6.6	36	179	**1**
	B	2.7	14	22	169	
	C	2.4	13	38	154	
	Mean	**2.4**	**11**	**32**	**167**	
4.1	A	1.3	3.1	17	16	**92**
	B	0.89	1.6	12	12	
	C	1.0	2.0	12	11	
	Mean	**1.1**	**2.2**	**14**	**13***	

Mean cell numbers for the control and vehicle blank at 0-hour were 0.85 x 10^4 and 0.92 x 10^4 cells/mL.

[a] Percent inhibition compared to the pooled control value at 96 hours.

[*] Statistically significant reduction ($p \leq 0.05$) when compared to the pooled control value at 96 hours.

Table B.9.2.6-17: Area under the growth curve values for green alga *Selenastrum capricornutum* during a 96-hour exposure to RH-127,450

Mean measured concentration (mg a.s./L)	Rep.	Area under the growth curve values			
		0-24 hours[a]	0-48 hours[a]	0-72 hours[a]	0-96 hours[a]
Control	A	17	155	1224	3948
	B	15	159	1264	4929
	C	16	142	1252	3957
	Mean	**16**	**152**	**1247**	**4278**
Acetone control	A	36	271	1612	5013
	B	22	150	943	3309
	C	14	163	1232	4146
	Mean	**24**	**195**	**1262**	**4156**
Pooled control	**Mean**	**20**	**173**	**1255**	**4217**
0.27	A	21	318	1605	5160
	B	18	266	1576	5455
	C	13	208	1495	3969
	Mean	**17**	**264**	**1559**	**4861**
0.52	A	17	311	1418	4193
	B	17	311	1550	4577
	C	29	323	1514	4913
	Mean	**21**	**315**	**1494**	**4561**
1.1	A	16	309	1488	4394
	B	29	179	1082	4277
	C	24	118	873	3456
	Mean	**23**	**202**	**1148**	**4042**
2.4	A	15	98	588	3146
	B	22	201	612	2882
	C	18	182	772	3055
	Mean	**18**	**160**	**667***	**3028***
4.1	A	5	36	256	631
	B	0	9	150	417
	C	1	16	163	417
	Mean	**2**	**20**	**190***	**488***

[a] values rounded to the nearest whole number.

* Statistically significant inhibition ($p \leq 0.05$) as compared to the pooled control values at 72 and 96 hours.

Table B.9.2.6-18: Growth rate values for green alga *Selenastrum capricornutum* during a 96-hour exposure to RH-127,450

Mean measured concentration (mg a.s./L)	Rep.	Growth rate values			
		0-24 hours[a]	0-48 hours[a]	0-72 hours[a]	0-96 hours[a]
Control	A	0.036	0.050	0.061	0.052
	B	0.039	0.056	0.065	0.059
	C	0.041	0.053	0.065	0.054
	Mean	**0.039**	**0.053**	**0.064**	**0.055**
Acetone control	A	0.066	0.065	0.067	0.057
	B	0.046	0.050	0.058	0.053
	C	0.031	0.050	0.059	0.052
	Mean	**0.048**	**0.055**	**0.061**	**0.054**
Pooled control	**Mean**	**0.043**	**0.054**	**0.063**	**0.055**
0.27	A	0.045	0.069	0.063	0.057
	B	0.041	0.065	0.064	0.058
	C	0.034	0.060	0.065	0.051
	Mean	**0.040**	**0.065**	**0.064**	**0.055**
0.52	A	0.040	0.069	0.061	0.054
	B	0.040	0.069	0.063	0.055
	C	0.055	0.068	0.062	0.057
	Mean	**0.045**	**0.069**	**0.062**	**0.055**
1.1	A	0.038	0.069	0.062	0.055
	B	0.055	0.052	0.060	0.057
	C	0.049	0.042	0.058	0.054
	Mean	**0.047**	**0.054**	**0.060**	**0.055**
2.4	A	0.036	0.042	0.051	0.055
	B	0.046	0.057	0.045	0.055
	C	0.041	0.056	0.052	0.054
	Mean	**0.041**	**0.052**	**0.049***	**0.055**
4.1	A	0.016	0.026	0.041	0.030
	B	0.000078	0.012	0.036	0.027
	C	0.0049	0.017	0.036	0.026
	Mean	**0.0070**	**0.018**	**0.038***	**0.028***

[a] Values rounded to two significant figures.

* Statistically significant inhibition ($p \leq 0.05$) as compared to the pooled control values at 72 and 96 hours.

24-hour E_C50 = 3.9 mg a.i./L
24-hour E_bC_{50} = 3.2 mg a.i./L
24-hour E_rC_{50} = 3.4 mg a.i./L
48-hour E_C50 = 3.4 mg a.i./L
48-hour E_bC_{50} = 3.2 mg a.i./L
48-hour E_rC_{50} = 3.7 mg a.i./L
72-hour E_C50 = 2.1 mg a.i./L
72-hour E_bC_{50} = 2.5 mg a.i./L
72-hour E_rC_{50} = >4.1mg a.i./L
72-hour NOEC = 1.1 mg a.i./L (based on both area under the growth curve and growth rate)
96-hour E_c50 = 3.2 mg a.i./L (mean measured)
96-hour E_bC_{50} = 2.8 mg a.i./L (mean measured)

96-hour E_rC_{50} = 4.1 mg a.i./L (mean measured)
96-hour NOEC = 1.1 mg a.i./L (based on area under growth curve)
96-hour NOEC = 2.4 mg a.i./L (based on growth rate)

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

96-hour E_c50 = 3.2 mg a.i./L (mean measured)
96-hour E_bC_{50} = 2.8 mg a.i./L (mean measured)
96-hour E_rC_{50} = 4.1 mg a.i./L (mean measured)

Report:	**CA, 8.2.6.1/08 Rhodes, J.E., Williams, S. (1999)** **Acute toxicity of RH-163,353 to S*elenastrum capricornutum* Printz in a range-finding test under static conditions.**

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical, (Lot No. LG 3517), chemical purity: 92.9% a.s.
Test species: freshwater green algae S*elenastrum capricornutum*
Number of organisms, age: 1.0 x 10^4 cells/mL for each flask, three days old
Type of test: 96-hour static toxicity test
Applied and measured concentrations:
Nominal concentrations: 0 (control), 0 (500 µL/L acetone control), 1.3, 2.5, 5.0, 10 and 20 mg a.i./L
Mean measured concentrations: <MQL (control), <MQL (acetone control), 1.3, 2.9, 6.1, 12 and 23 mg a.i./L
Test conditions:
temperature: range 24 ± 2 °C
pH: 7.5 ± 0.1
lighting: 4401 ± 137 lux

Results:
50% inhibition was not reached at the highest concentration tested, the 24-, 48-, 72- and 96-hour EC_{50} based on cell density, area under the growth curve, and growth rate, are estimated to be > 23 mg a.i./L (the functional water solubility limit). The 72-hour no-observed-effect concentration (NOEC) is 12 mg a.i./L based on both area under the growth curve and growth rate. The 96-hour NOEC is 12 mg a.i./L based on area under the growth curve and 6.1 mg a.i./L based on growth rate.

Table B.9.2.6-19: Cell number for green alga *Selenastrum capricornutum* during a 96-hour exposure to RH-163,353 technical

Mean measured concentration (mg a.s./L)	Rep.	Cell numbers (x 10^4 cells/mL)				
		24-hour	48-hour	72-hour	96-hour	% inhibition[a]
Control	A	2.1	11	46	80	---

	B	1.3	14	23	82	
	C	2.6	12	25	104	
	Mean	**2.0**	**12**	**31**	**89**	
Acetone control	A	2.0	10	42	73	---
	B	2.8	12	39	97	
	C	2.3	11	33	98	
	Mean	**2.4**	**11**	**38**	**89**	
Pooled control	**Mean**	**2.2**	**12**	**35**	**89**	---
1.3	A	1.7	14	45	113	**0**
	B	2.6	11	45	106	
	C	2.9	10	44	103	
	Mean	**2.4**	**12**	**45**	**107**	
2.9	A	3.4	14	35	89	**1**
	B	2.4	14	29	76	
	C	3.1	15	28	100	
	Mean	**3.0**	**14**	**31**	**88**	
6.1	A	2.7	16	28	70	**29**
	B	3.4	12	44	53	
	C	4.3	11	27	66	
	Mean	**3.5**	**13**	**33**	**63***	
12	A	2.1	9	18	85	**10**
	B	2.4	11	31	80	
	C	3.6	9	33	75	
	Mean	**2.7**	**10**	**27**	**80**	
23	A	2.0	7	15	57	**26**
	B	1.2	8	24	71	
	C	3.1	7	22	70	
	Mean	**2.1**	**7**	**20**	**66***	

Mean cell numbers for the control and vehicle blank at 0-hour were 0.82 x 10^4 and 0.85 x 10^4 cells/mL.

[a] Percent inhibition compared to the pooled control value at 96 hours.

[*] Statistically significant reduction ($p \leq 0.05$) when compared to the pooled control value at 96 hours.

Table B.9.2.6-20: Area under the growth curve values for green alga *Selenastrum capricornutum* during a 96-hour exposure to RH-163,353 technical

Mean measured concentration (mg a.s./L)	Rep.	Area under the growth curve values			
		0-24 hours[a]	0-48 hours[a]	0-72 hours[a]	0-96 hours[a]
Control	A	16	154	820	2313
	B	6	171	596	1838
	C	21	174	597	2124
	Mean	**14**	**167**	**671**	**2091**
Acetone control	A	12	132	732	2088
	B	24	183	776	2390
	C	18	159	668	2222
	Mean	**18**	**158**	**726**	**2223**
Pooled control	**Mean**	**16**	**162**	**698**	**2162**
1.3	A	10	179	867	2743
	B	21	164	816	2608
	C	25	160	788	2531
	Mean	**19**	**168**	**824**	**2627**
2.9	A	31	220	788	2255
	B	19	196	692	1931
	C	27	224	720	2236
	Mean	**26**	**213**	**733**	**2141**
6.1	A	22	227	735	1891

	B	31	196	848	1991
	C	42	205	641	1737
	Mean	**32**	**209**	**741**	**1873**
12	A	15	128	432	1648
	B	19	160	644	1955
	C	33	164	648	1924
	Mean	**22**	**151**	**575**	**1843***
23	A	14	102	346	1190
	B	4	95	459	1579
	C	27	128	456	1540
	Mean	**15**	**108**	**420***	**1436***

[a] values rounded to the nearest whole number.

[*] Statistically significant inhibition ($p \leq 0.05$) as compared to the pooled control values at 72 and 96 hours.

Table B.9.2.6-21: Growth rate values for green alga *Selenastrum capricornutum* during a 96-hour exposure to RH-163,353 technical

Mean measured concentration (mg a.s./L)	Rep.	Growth rate values			
		0-24 hours[a]	0-48 hours[a]	0-72 hours[a]	0-96 hours[a]
Control	A	0.041	0.055	0.057	0.048
	B	0.021	0.060	0.047	0.048
	C	0.045	0.054	0.046	0.050
	Mean	**0.036**	**0.056**	**0.050**	**0.049**
Acetone control	A	0.029	0.048	0.052	0.045[b]
	B	0.053	0.057	0.054	0.050
	C	0.045	0.055	0.052	0.050
	Mean	**0.042**	**0.053**	**0.053**	**0.050**
Pooled control	**Mean**	**0.039**	**0.055**	**0.051**	**0.049**
1.3	A	0.030	0.059	0.055	0.051
	B	0.047	0.054	0.055	0.050
	C	0.052	0.052	0.055	0.050
	Mean	**0.043**	**0.055**	**0.055**	**0.051**
2.9	A	0.059	0.059	0.052	0.049
	B	0.044	0.059	0.049	0.047
	C	0.055	0.060	0.049	0.050
	Mean	**0.052**	**0.059**	**0.050**	**0.048**
6.1	A	0.049	0.062	0.049	0.046
	B	0.059	0.056	0.055	0.043
	C	0.068	0.054	0.048	0.046
	Mean	**0.059**	**0.057**	**0.051**	**0.045***
12	A	0.038	0.050	0.043	0.048
	B	0.044	0.054	0.050	0.048
	C	0.061	0.050	0.051	0.047
	Mean	**0.048**	**0.051**	**0.048**	**0.048**
23	A	0.036	0.044	0.040	0.044
	B	0.015	0.047	0.047	0.046
	C	0.055	0.044	0.045	0.046
	Mean	**0.035**	**0.045**	**0.044***	**0.045***

[a] Values rounded to two significant figures.

[b] Statistical outlier (Rstudent value > 4.0) excluded from data set.

[*] Statistically significant inhibition ($p \leq 0.05$) as compared to the pooled control values at 72 and 96 hours.

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

E_bC_{50}: > 23 mg a.i./L
E_rC_{50}: > 23 mg a.i./L

Report: **CA, 8.2.6.1/09 Hoberg, J.R. (2002)**
Zoxamide metabolite RH-139,432 – toxicity to freshwater green alga *Scenedesmus subspicatus*

Guidelines: OECD 201, US EPA OPPTS 850.5400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: zoxamide metabolite RH-139-432, Lot No. JG7-39A, identification No. TSN 103194, chemical purity: 99.8% a.s.
Test species: freshwater green algae *Scenedesmus subspicatus*
Number of organisms, age: 181 x 10^4 cells/mL for each flask, three days old
Type of test: 96-hour static toxicity test
Applied and measured concentrations:
Nominal concentrations: 1.6, 3.1, 6.3, 13, 25 and 50 mg a.i./L
Mean measured concentrations: 1.1, 1.9, 4.2, 8.1, 16 and 30 mg a.i./L
Test conditions:
temperature: range 23 to 24 °C
pH: 7.3 to 9.5
lighting: 4600 to 5900 lux (425 to 550 footcandles)

Results:
The 96-hour EC_{50} (corresponding 95% confidence limits) was calculated to be 21 mg a.s./L (19 to 22 mg a.s./L). The 96-hour NOEC was determined to be 8.1 mg a.s./L based on Williams` test.

The 72-hour E_bC_{50} (corresponding 95% confidence limits) was calculated to be 26 mg a.s./L (23 to 27 mg a.s./L). Based on the results of Williams` test, the 72-hour NOEC was determined to be 16 mg a.s./L.

The 72-hour E_rC_{50} (corresponding 95% confidence limits) was empirically estimated to be >30 mg a.s./L, the highest mean measured concentration tested. Based on the results of Williams` test, the 72-hour NOEC was determined to be 16 mg a.s./L.

Table B.9.2.6-22: Cell density of *Scenedesmus subspicatus* after 24, 48, 72 and 96 hours of exposure to zoxamide metabolite RH-139,432

Mean measured concentration (mg a.s./L)	Cell density (x10^4 cells/ml)					
	Observation interval (hours)					
		24	48	72	96	Percent inhibition[a]
Control	A	3.75	5.75	25.3	80	
	B	3.25	7.00	28.0	93	
	C	4.25	8.00	35.0	70	
	Mean (SD)[b]	3.75 (0.5)	6.92 (1.13)	29.4 (5.03)	81 (11.79)	NA[c]

Solvent control	A	2.50	5.25	32.3	54	
	B	6.00	3.75	33.8	75	
	C	2.00	8.50	26.3	65	
	Mean (SD)	3.5 (2.18)	5.83 (2.43)	30.8 (3.97)	65 (10.26)	NA
Pooled control		4.0 (1.42)	6.0 (1.79)	30 (4.12)	73 (13.27)	NA
1.1	A	5.25	6.00	26.0	83	
	B	4.25	7.00	28.3	107	
	C	5.00	4.00	27.3	101	
	Mean (SD)	4.83 (0.52)	5.67 (1.53)	27.2 (1.13)	97 (12.41)	-33
1.9	A	4.25	5.50	24.0	79	
	B	3.50	10.25	24.5	89	
	C	2.25	5.75	27.0	72	
	Mean (SD)	3.33 (1.01)	7.17 (2.67)	25.2 (1.61)	80 (8.39)	-10
4.2	A	5.25	5.25	28.5	76	
	B	4.25	4.75	24.8	86	
	C	4.00	7.00	34.3	68	
	Mean (SD)	4.5 (0.66)	5.67 (1.18)	29.2 (4.78)	77 (9.27)	-5
8.1	A	2.45	6.75	29.8	85	
	B	6.00	5.75	23.8	54	
	C	3.75	8.25	19.0	71	
	Mean (SD)	4.08 (1.77)	6.92 (1.26)	24.2 (5.39)	70 (15.52)	4
16	A	4.00	7.25	32.2	54	
	B	4.00	4.50	19.5	50	
	C	4.75	7.75	26.0	56	
	Mean (SD)	4.25 (0.43)	6.5 (1.75)	25.9 (6.38)	53 (3.12)[d]	27
30	A	3.25	2.75	8.5	16	
	B	2.75	4.50	6.0	14	
	C	2.50	4.25	4.0	12	
	Mean (SD)	2.83 (0.38)	3.83 (0.95)	6.2 (2.25)	14 (1.64)[d]	81

[a] Percent inhibition relative to the pooled control.

[b] Mean, standard deviation (SD) and percent inhibition are calculated from original raw data, not from the rounded values presented in this table.

[c] NA=Not applicable

[d] Significant reduced compared to pooled control, based on Williams` test.

Table B.9.2.6-23: Calculated biomass (area under the growth curve) of *Scenedesmus subspicatus* after 24, 48 and 72 hours of exposure to zoxamide metabolite RH-139,432

Mean measured concentration (mg a.s./L)		Biomass ($x10^4$ cells days/ mL)				
		Observation interval (hours)				
		0-24	24-48	48-72	Total biomass	Percent inhibition[a]
Control	A	1.37	3.26	15.8	20.4	
	B	1.12	3.58	17.9	22.6	
	C	1.62	4.45	22.3	28.3	
	Mean (SD)[b]	1.37 (0.25)	3.76 (0.62)	18.7 (3.32)	23.8 (4.11)	NA[c]
Solvent control	A	0.75	2.50	19.3	22.5	
	B	2.49	3.36	19.3	25.1	
	C	0.50	3.69	17.8	22.0	
	Mean (SD)	1.25 (1.09)	3.18 (0.62)	18.8 (0.86)	23.2 (1.69)	NA
Pooled control					23.5 (2.82)	NA
1.1	A	2.12	4.01	16.3	22.4	

	B	1.62	4.01	18.1	23.7	
	C	1.99	3.04	15.9	20.9	
	Mean (SD)	1.91 (0.26)	3.69 (0.56)	16.8 (1.16)	22.4 (1.39)	5
1.9	A	1.62	3.36	14.9	19.9	
	B	1.25	5.10	17.8	24.1	
	C	0.62	2.60	16.7	19.9	
	Mean (SD)	1.16 (0.5)	3.69 (1.28)	16.5 (1.44)	21.3 (2.43)	9
4.2	A	2.12	3.69	17.3	23.1	
	B	1.62	3.04	14.9	19.6	
	C	1.49	3.91	21.3	26.7	
	Mean (SD)	1.74 (0.33)	3.54 (0.45)	17.8 (3.23)	23.1 (3.56)	2
8.1	A	0.75	3.15	18.7	22.6	
	B	2.49	4.23	14.9	21.7	
	C	1.37	4.34	13.7	19.4	
	Mean (SD)	1.54 (0.88)	3.91 (0.66)	15.8 (2.62)	21.2 (1.65)	10
16	A	1.49	4.01	20.4	25.9	
	B	1.49	2.82	12.0	16.3	
	C	1.87	4.56	17.3	23.7	
	Mean (SD)	1.62 (0.22)	3.8 (0.89)	16.5 (4.26)	21.99 (5.04)	7
30	A	1.12	1.74	5.0	7.9	
	B	0.87	2.28	4.6	7.8	
	C	0.75	2.06	3.4	6.2	
	Mean (SD)	0.91 (0.19)	2.03 (0.27)	4.3 (0.85)	7.3 (0.94)[d]	69

[a] Percent inhibition relative to the pooled control.

[b] Mean, standard deviation (SD) and percent inhibition are calculated from original raw data, not from the rounded values presented in this table.

[c] NA=Not applicable

[d] Significant reduced compared to pooled control, based on Williams` test.

Table B.9.2.6-24: Calculated growth rates of *Scenedesmus subspicatus* after 24, 48 and 72 hours of exposure to zoxamide metabolite RH-139,432

Mean measured concentration (mg a.s./L)	Growth rate (days $^{-1}$)				
	Observation interval (hours)				
		0-24	0-48	0-72	Percent inhibition[a]
Control	A	1.33	0.94	1.09	
	B	1.18	1.04	1.13	
	C	1.45	1.12	1.20	
	Mean (SD)[b]	1.32 (0.13)	1.03 (0.09)	1.14 (0.06)	NA[c]
Solvent control	A	0.92	0.89	1.18	
	B	1.80	0.71	1.19	
	C	0.70	1.15	1.11	
	Mean (SD)	1.14 (0.58)	0.92 (0.22)	1.16 (0.05)	NA
Pooled control				1.15 (0.05)	NA
1.1	A	1.66	0.96	1.10	
	B	1.45	1.04	1.13	
	C	1.62	0.74	1.12	
	Mean (SD)	1.58 (0.11)	0.92 (0.16)	1.12 (0.01)	3
1.9	A	1.45	0.91	1.08	
	B	1.26	1.25	1.08	
	C	0.81	0.94	1.12	
	Mean (SD)	1.17 (0.33)	1.03 (0.19)	1.09 (0.02)	5
4.2	A	1.66	0.89	1.14	

	B	1.45	0.84	1.09	
	C	1.39	1.04	1.20	
	Mean (SD)	1.5 (0.14)	0.92 (0.11)	1.14 (0.06)	1
8.1	A	0.92	1.02	1.15	
	B	1.80	0.94	1.07	
	C	1.33	1.13	1.00	
	Mean (SD)	1.35 (0.44)	1.03 (0.1)	1.07 (0.08)	7
16	A	1.39	1.06	1.18	
	B	1.39	0.81	1.01	
	C	1.56	1.10	1.10	
	Mean (SD)	1.45 (0.1)	0.99 (0.16)	1.10 (0.09)	4
30	A	1.18	0.54	0.73	
	B	1.02	0.81	0.61	
	C	0.92	0.78	0.47	
	Mean (SD)	1.04 (0.13)	0.71 (0.14)	0.60 (0.13)[d]	48

[a] Percent inhibition relative to the pooled control.

[b] Mean, standard deviation (SD) and percent inhibition are calculated from original raw data, not from the rounded values presented in this table.

[c] NA=Not applicable

[d] Significant reduced compared to pooled control, based on Williams` test.

RMS comments:

Based on OECD 201 validity criteria pH in control cultures shall not not increase more than 1.5 unit, in this study pH control culture has increased from 7.1 to 9.5. No information about solubility of metabolite RH-139,432 and used solvent in the study. RMS considers study acceptable.

The 96-hour EC_{50}: 21 mg a.s./L

The 72-hour E_bC_{50} : 26 mg a.s./L

The 72-hour E_rC_{50}: >30 mg a.s./L

B.9.2.7 Effects on aquatic macrophytes

Report:	**CA, 8.2.7/01 Drottar, K.R., Krueger, H.O. (1998b)** **RH-117,281 Technical: A 14-day static-renewal toxicity test with duckweed (*Lemna gibba* G3).**

Guidelines: US EPA OPPTS 850.4400/FIFRA Guideline 123-2
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical, (Lot No. DSR 9510), chemical purity: 92.3% a.s.
Test species: duckweed *Lemna gibba*
Type of test: 14-day static toxicity test
Applied and measured concentrations:
Nominal concentrations: negative control, solvent control (0.1 mL acetone/L), 1.3, 2.5, 5.0, 10 and 20 µa.i./L

Mean measured concentrations: Negative control, Solvent control, 1.1, 2.2, 4.4, 9.0 and 18 µg a.i./L
Test conditions:
temperature: range 25 ± 2 °C
lighting: 5000 ± 750 lux

Results:

Table 9.2.7.-1: Day 7 frond numbers, mean frond numbers, and percent inhibition values

Mean measured test concentration (µg a.s./L)	Replicate	Day 7 Frond number	Mean frond number	Percent inhibition[1,2]
Negative control	A B C	111 116 110	112	--
solvent control	A B C	119 122 106	116	--
1.1	A B C	116 123 118	119	-4
2.2	A B C	115 114 124	118	-4
4.4	A B C	126 110 132	123	-8
9.0	A B C	96 106 108	103	10
18	A B C	85 83 72	80*	30

[1] Percent inhibition was calculated relative to mean frond numbers in the pooled control replicates. Pooled control mean = 114.

[2] The 7-day IC50 value (i.e., the theoretical test concentration that would produce 50% inhibition of frond production relative the controls) was > 18 µg a.s./L.

* Indicates a significant difference from pooled controls using the Bonferroni t-test ($p \leq 0.05$).

Table 9.2.7.-2: Day 14 plant and frond numbers, mean frond numbers, and percent inhibition values

Mean measured test concentration (µg a.s./L)	Replicate	Day 14 plant number	Day 14 frond number	Mean frond number	Percent inhibition[1,2]
Negative control	A B C	147 160 165	697 689 780	722	--
solvent control	A B C	154 194 208	735 661 649	682	--
1.1	A B C	117 200 169	806 873 803	827	-18
2.2	A	184	881	808	-15

	B	181	767		
	C	189	775		
4.4	A	189	729	796	-13
	B	181	790		
	C	194	869		
9.0	A	133	414	569	19
	B	144	591		
	C	173	703		
18	A	101	301	359*	49
	B	108	341		
	C	143	436		

[1] Percent inhibition was calculated relative to mean frond numbers in the pooled control replicates. Pooled control mean = 702.

[2] The 14-day IC50 value (i.e., the theoretical test concentration that would produce 50% inhibition of frond production relative the controls) calculated using linear interpolation was 17 µg a.s. equivalents/L. The 95% confidence limits were 13 and 18 µg a.s. equivalents/L.

* Indicates a significant difference from pooled controls using the Bonferroni t-test ($p \leq 0.05$).

7-day IC50 = >18 µg a.i./L
NOEC = 9.0 µg a.i./L
14-day IC50 = 17 µg a.i./L

RMS comments:
The study is considered acceptable.
7-day IC50 = >18 µg a.i./L
14-day IC50 = 17 µg a.i./L

B.9.2.8 Further testing on aquatic organisms

Further higher tier testing with RH-117,281 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.9.3 EFFECTS ON ARTHROPODS

B.9.3.1 Effects on bees

Report:	**CA, 8.3.1.1.1/01 Kirkland, R.L. (1993)** **Acute contact toxicity of RH-117,281 Technical to honey bees**

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 Technical (Lot No. WJZ 3561/ELM 1092), 95% a.i.
Test species: honeybees (*Apis mellifera L.*)
Number of organisms: 100 honey bees, 25 bees/replicates

Type of test: acute contact toxicity test (48 hours)
Applied concentrations: 0.0, 0.25, 2.5, 25 and 100 µg/bee a.i.
Exposure route: RH-117,281 Technical was topically applied to the thorax of honey bees using micropipette delivering a 1 µg/µL droplet
Test conditions: temperature of approximately 17.5 – 28 ^{0}C
relative humidity above approximately 38%

Results:

Table B.9.3.1-1: Bee mortality caused by test substance RH-117,281 Technical at 24 hours post-treatment (May 19, 1993)

Rate (µg/bee)	No. dead/ 25 bees				Total dead	% effect
	Rep: 1	2	3	4		
0.0	1	0	0	0	1	--
0.25	2	0	0	0	2	1.0
2.5	0	0	0	0	0	0.0
25.0	0	0	0	0	0	0.0
100.0	0	0	0	0	0	0.0

No adverse behaviour was observed in living bees.

Table B.9.3.1-2: Bee mortality caused by test substance RH-117,281 Technical at 48 hours post-treatment (May 20, 1993)

Rate (µg/bee)	No. dead/ 25 bees				Total dead	% effect
	Rep: 1	2	3	4		
0.0	1	0[3]	1	0	2	--
0.25	2[3]	0	0	1	3	1.0
2.5	0	0[3]	0	1	1	-1.0
25.0	0	1[1,2]	1	0	2	0.0
100.0	1	1	1	0[4]	3	1.0

Adverse behaviour observed in asingle living bee per cage:

[1] Regurgitation

[2] Paralysis

[3] Inability to right self

[4] Uncordinated movement

Acute contact, 48-hr LD_{50}: >100 µg as/bee

RMS comments:
Study is considered acceptable.
Acute contact, 48-hr LD_{50}: >100 µg as/bee

B.9.3.2 Effects on non-target arthropods other than bees

Report: Nicòtina, M; Capone, G C (2008) Comparison between downy mildew fungicides in a vineyard of the Avellino province (Campania South Italy) and their influence on the population of Phytoseid mites (Parasitiformes, Phytoseiidae).

The following scientific and peer-reviewed open literature articles, published within the last 10 years before the date of submission of the dossier, were considered as potentially relevant and reliable for the ecotoxicology evaluation of the active substance and its formulations.

Pesticides for downy mildew control disturb and influence the population of useful arthropods. In fact, phytoseiid mites, internationally recognized as the most efficient deterrent against various families of both phytophagus mites and some insects, are also valuable biological indicators. Indeed, their existence in agro ecosystems guarantees the use of less toxic pesticides for downy mildew control.

Matherials and methods:

Research has been carried out in the province of Avellino in the Vadiaperti farm of the Montefredane vineyards. Vineyards are located in typical areas of high quality wines produced in traditional areas of Irpinia region (VQPRD).The vineyard covers an area of 2 ha on a medium clayey soil. It is situated in a hilly location at an average altitude of 350 m above sea level with south-eastern exposition.

For three consecutive years (2005-2007) tests for some downy mildew control were carried out in the company vineyards with the aim of testing and selectivity of the population of useful mites of the Phytoseiidae Family and assess their ability in controlling certain cryptogams.

For experiments, on the basis of the calendar treatment to be effected, a trial field of about 2000 sq m of vineyard was chosen; each plot underwent a specific calendar treatment. Applications were effected with a motor pump sprayer manually. A mixture, previously calibrated, equal to about 1000 liters/ha was applied to the plots and after each treatment the quality disturbed was carefully checked.

To avoid drifting effect treatment was only effected on the central rrows of the plots.Spraying was carried out by wetting both sides of the vine rows up to drip level. Each application was monitored by a technican who controlled the application methods with theobjective of reducing the danger of the water drifting to adjacent rows. To control the Phytoseiids, leaf samples were taken immediately before each application with the exception of the last sampling when spraying was not effected.

The experimental field consisted of 14 rows with an average of 20 vine plants, the rows were divided into two sampling stations (repetition) of 10 plants each, therefore for each of the 7 plots there were 4 repetitions.

In this test, new commercialized products were also used as well as products authorized in biological agriculture made up of a mixture of seaweed and cooper and cooper “gluconate” (see Table B.9.3.2-1)

Table B.9.3.2-1: Programme of application of downy mildew products from 2005-2007

The qua

Results:

On year 2005 the data clearly shows the trend of the population of Phytoseiid mites, population was somewhat low at preliminary control no 24th May. However, right from the first application on 31st May there is a reduction in the population of phytoseiids in all the plots but this is particulary noticeable in Plot 6 (Azinphos methyl + Copper oxychloride) in which the variation is more apparent. On the other hand, it must be considered that decrease of Phytoseiis is also connected to environmental factors. During experimental period there were abundant rainfalls which presumably contributed to the reduction of the population.

The statistical elaboration indicates significant differences among the plots sampled in terms of selectivity ($F=6.45$; $p<0.0001$).

Table B.9.3.2-2: Trend of Phytoseiid population during downy mildew control on year 2005

On year 2006 the initial population of Phytoseiids found in the vineyard was very low. On average not more tah 6 phytoseiids per sampling were collected, however, towards the last decade of May a variation was recored in the population of these mites. Olny untreated plot, which had initially presented a steady trend not having undergone any interferences, showed an increase in the population from second week (Table B.9.3.2-3).

The statistical elaboration showed significant diferences between the various plots (F=15.48, P <0.0001). Considering this the experiment continued in order to determinē the most sensitive plot amongst those tested. The results obtained at the Duncan test reveal that the polts all show discreet selectivity, plot 6 (Azinphos methyl + Copper oxychloride) confirmed the expected toxicity.

Table B.9.3.2-3: Trend of the Phytoseiid population during the test on 2006

On year 2007 during the period of applications the population of phytoseiids underwent two variations, the first was recored at the begging of June and the second after about a month around the 1st July. After having statistically analysed to vertify the absence of significant differencesamong the repetitions of the same plot in terms of selectivity, the tests continued to determine whether there were significant differences among the various sampled tests. The results obtained show highly significant results among the plots tested (F=16.48, p<0.00001) (Table B.9.3.2-4).

Comapring the results at the Duncan test, plot 3 (Copper gluconate) recored a very low selectivity to phytoseiids, in fact just a little less than plot 7 wich had been trated with water (untreated).

Table B.9.3.2-4: Trend of the Phytoseiid population during the test on year 2007

The research results not only give indications connected to the selectivity of useful mites but also favourable indications on the efficacy of the active ingredients tested for downy mildew control. Copper based products are still today valid tool in the fight for downy mildew control and have secondary effect against different pathologies in viticulture.

A fundamental argument in favour of limiting the use of cupric products is that this metal accumulates in the soil causing devastating effects. In the meantime, it is indispensable to alternate the use of copper based products with other non cupric pesticides; there are numerous synthetic molecules which can be used as an alternative to copper based products but there is very little experience relative to "biological"goods used as an alternative to copper based products.

The need is felt reduce the presence of copper and to have a mixture that is efficient against downy mildew but at the same time selective for useful arthropods.

In conclusion research results show that he plots with the greatest efficacy in 2005 and 2007 are Zoxamide + Copper oxychloride and Folpet. In 2006, when there were more frequent rainfalls, the best outcome was recorded for the mixture Zoxamide + Oxychloride.

RMS comments:

The study is considered acceptable. Article presents monitoring information on efficacy and effects on Phyoseiid mites. For zoxamide, effects on mites were acceptable. No implications for risk assessment.

Report: Miles, M; Green, E (2004) Effects of the fungicide zoxamide, alone and in combination with mancozeb, to beneficial arthropods under laboratory and field conditions.

Matherials and methods:

Test substance: zoxamide formulated as 240 g/L SC formulation and Electis (83 g zoxamide and 667 g macozeb/kg WG formulation)

Table B.9.3.2-5: Study type and beneficial species tested in side-effects studies with zoxamide formulations

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Laboratory (tier I) studies

Test substance and application rate:

Zoxamide (240 g/L SC formulation) with application rate of 150 and 300 g a.s./ha

Electis with application rate of 1800 and 3600 g a.s./ha (150 g zoxamide + 1200 g mancozeb and 300 g zoxamide + 2400 g mancozeb/ha)

Sensitive life history stages were exposed to artificial substrates (glass plate, quartz sand) according to internationally recognized guidelines.

Extended laboratory studies

These tests are characterised by the inclusion of a natural substrate in the test system. The effect of Electis on predatory mite *T. pyri* was investigated using the laboratory tier I design of Blümel *et al* (2000a), test was concuted to estimate the rate corresponding to 50% mortality.

To investigate the effect of repeated applications aged residue extended laboratory tests were on *A. rhopalosiphi* and *C. carnea* using a multiple application factor (MAF) to calculate the dose. Rates equivalent to 6400 and 9200 g product/ha were tested to simulate extreme worst case exposure arising from season long sray programmes.

Field studies

Electis was applied to field populations of *T. pyri* in vines at a site located in a vine growing area of Germany in 2001 according to BBA guideline VI, 23-2.3.4. (BBA, 1991).

Test concentration, application rates: 0.18% and two regimes – 1) four applications (two pre- and two post-flowering), 2) six applications (two pre- and four post-flowering.

Water volume: 600, 800, 1000, 1400, 1600 and 1600 L water/ha for applications 1, 2, 3, 4, 5 and 6

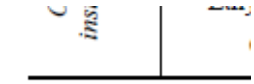
Nominal appication rate: 1080, 1440, 1800, 2520, 2880 and 2880 g Electis/ ha for each of the six application timings.

Results:

Laboratory (tier I) study

For zoxamide all species tested resulted in either a harmless (class 1) or slightly harmful (calss 2). In terms of mortality zoxamide was always harmless. For *T. pyri* the 150 g zoxamide/ha rate indicated a 53.7% reduction in fecundity, however it was based on a low number of eggs per female in all treatments and no effect was seen at the x2 rate (22.8%). Findings indicate a low risk to beneficial arthropods due to applications of zoxamide (Table B.9.3.2-6). When zoxamide was tested in combination with macozeb, with the excepti on of the predatory mites, all tests returned either a harmless of slightly harmful classification. For both *T. pyri* and *A. andersoni*, Electis was classified as harmful (calss 4).

Table B.9.3.2-6: Effects of zoxamide (240 g/L SC) to a range of beneficial arthropods species in tier I laboratory tests.



Notes: * Indicates signi
mortality, P=parasitism
No. prey/individual/day.

Extended laboratory studies

All test rates of 0.1 g Electis/ha and above exceeded the 25% threshold for harmlenessness. In terms of a non-statistical evaluation effects above 50% were seen at rates equal or greater than 100 g Electis/ha indicating that the LR_{50} for product lies between 50 and 100 g Electis/ha.

Figure B.9.3.2-1: Effect of Electis on the mortality of the predatory mite *Typhlodromus pyri,* under extended laboratory conditions.

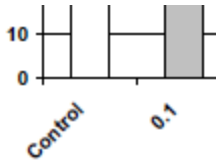
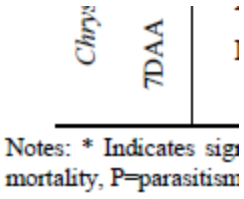


Table B.9.3.2-7: Effects of Electis (83 g zoxamide and 667 g mancozeb/kg WG formulation) to *Aphidus rhopalosiphi* and *Chrysoperla carnea* in tier II extended laboratory tests.



When Electis was applied twice per season maximum reductions of 41, 34 and 35% were observed for the early, mid and late season sprays. In four application regime, 22% was seen for the early season combination and 27% for the late season combination. By the end of the study (28 days after the final application) all Electis treatments were below 25% effect.

Conclusions:

It was concluded that zoxamide was highly selective to all beneficial insect, mite and spider species tested in tier I laboratory studies. When tested under identical conditions Electis (zoxamide + mancozeb) was safe to all species with the exception of the predatory mites *T. pyri* and *A. andersoni*. Further studies under extended laboratory conditions confirmed the potential toxicity to mites and revealed safety to parasitic wasps and lacewing larvae. The effect on mites was clearly due to the presence of macozeb in the formulation. Under field conditions multiple applications of Elects on predatory mites in vines was studied. When applied at field rate, four applications were shown to be suitably selective to *T. pyri* (less than 50% maximum effect over the season). Six applications at field rate caused significant reductions in mite number but recovery was observed the following season. Further studies under different climatic and growing conditions with a range of important predatory mite species in key locations in Europe confirmed that up to fpur applications per season of Electis was suitably selective. Furthermore Electis could be integrated into disease control programmes at a variety of spary timings giving the grower excellent flexibility, disease control and selectivity to predatory mites and other beneficial arthropods.

RMS comments:
the study is considered acceptable.

Article presents an overview of data previously submitted for the registration of zoxamide. It contains no new data on technical zoxamide or the formulation ‚Zoxium 240 SC'. No implications for risk assessment.

Refer to Volume 3, Section B.9 PPP for the formulation.

B.9.4 EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1 Earthworm – sub-lethal effects

Report:	**CA, 8.4.1/01 Downing, J., Leak , T. (1995)** **RH-117,281 Technical: toxicity to earthworm (*Eisenia fetida*).**

Guidelines: OECD 207
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical, (Lot No. DK 2011), chemical purity: 94.2% a.i.
Test species: earthworm *Eisenia fetida*
Number of organisms, weight: 4 replicates, 10 earthworms/each replicate; weight 300-600 mg
Type of test: artificial soil test (14 days)
Applied concentrations:
nominal test concentrations: Control, vehicle blank (acetone), 62.5, 125, 250, 500, and 1000 mg a.i./kg;
mean measured test concentrations: control (NA), vehicle blank (< MQL), 66.7, 134, 273, 520, and 1070 mg a.i./kg
Soil type and test conditions:
test substrate: 10% spaghnum peat, 20% kaolin clay, 70% industrial sand

temperature: 20 ± 1 °C
water content: 35% moisture

Results:
In an acute toxicity study, earthworms (*Eisenia fetida*) were exposed to technical RH-117,281 (purity 94.2%) for 14 days in artificial soil. The test was conducted in glass jars with screw caps containing 750 g of moist soil. Nominal soil concentrations of 62.5, 125, 250, and 1000 mg RH-117,281/kg dry soil were tested in 4 replicates of 10 worms each. Doses were measured at 104 to 109% of nominal. Jars were maintained in a constant-temperature water bath, arranged in a random design. Survival was reduced in all treatments. Results are summarised in Table B.9.6.1-1. Lethargy was observed in the 134, 273, 520, and 1070 mg/kg treatments, but was not observed in the control, vehicle control, or 66.7 mg a.s./kg concentration. All earthworm groups including the control lost weight during the study, as expected (range 22 to 28%) but no trend in average weight loss per worm was observed.

Table B.9.6.1-1: Results of an acute toxicity study on earthworms for technical RH-117,281.

Concentration (mg a.s./kg soil)		% Survival
Nominal	Mean Measured	at 14 days
Control	---	100
Vehicle Control	---	100
62.5	66.7	95
125	134	75
250	273	65
500	520	78
1000	1070	80

The LC50 for the earthworm (*Eisenia fetida*) was >1070 mg a.s./kg, the highest concentration tested. The NOEC was 66.7 mg a.s./kg based on less than 10% mortality. The 14-day LOEC was 134 mg a.s./kg based on reduced survival and lethargy.

RMS comments:
The study was conducted to OECD guideline 207 and GLP, and is considered to be acceptable.
14-day LC_{50}: >1070 mg a.s./kg soil dw
14-day NOEC (mortality): 66.7 mg a.s./kg soil dw

Report:	**CA, 8.4.1/02 Bryan, R.L., Porch, J.R., Krueger, H.O. (2000) RH-127,450 Technical: an acute toxicity study with the earthworm in an artificial soil substrate.**

Guidelines: OECD 207
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-127,450 (Lot No. WJZ 4302A), containing 100% RH-127,450
Test species: earthworm *Eisenia fetida*

Number of organisms, weight: 4 replicates, 10 earthworms/each replicate
Type of test: acute toxicity test (14 days)
Applied concentrations:
nominal test concentrations: negative control, solvent control, 62.5, 125, 250, 500, and 1000 mg/kg dry soil
Soil type and test conditions:
test substrate: 10% spaghnum peat, 20% kaolin clay, 70% sand
temperature: 20 ± 2 °C
water content: 33% moisture
light intensity: range from 400 to 800 lux.

Results:
In an acute toxicity study, earthworms (*Eisenia fetida*) were exposed to the metabolite RH-127,450 (100% pure) for 14 days. The test was performed in glass beakers containing 750 g of moist soil and RH-127,450 was added at nominal concentrations from 62 to 1000 mg/kg.
The 14 day LC50 was >1000 mg/kg dry soil and the NOEC was 1000 mg/kg dry soil based on the absence of statistically significant effects on survival or body weight change.

RMS comments:
The study was conducted to OECD 207 and GLP, and study is considered acceptable.
The 14 day LC50 : 1000 mg/kg soil dw
NOEC: 1000 mg/kg soil dw

Report:	**CA, 8.4.1/01 Nienstedt, K. (1999)** **A chronic toxicity and reproduction test exposing the earthworm *Eisenia fetida* to RH-117,281 Technical material in OECD artificial soil, based on the BBA-guideline VI, 2-2 (1994) and the ISO-draft (ISO/DIS 11268-2)**

Guidelines: BBA guideline VI, 202 (1994), ISO Draft (ISO/DIS 11268-2)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical, (Lot No. DK 2011), chemical purity: 94.2% a.i.
Test species: earthworm *Eisenia fetida*
Number of organisms, weight: 4 replicates, 10 earthworms/each replicate
Type of test: sublethal chronic toxicity and reproduction test (28 days)
Applied concentrations:
nominal test concentrations: control, a solvent control, 0.5, 1, 5, 10 and 20 mg a.i. per kg dry soil
Soil type and test conditions:
test substrate: artificial soil according to OECD Guideline # 207
temperature: 18.5 to 21.5 °C
water content: 25.75 and 27.25% moisture
light intensity: range from 450 to 665 lux.

Results:

A sublethal chronic toxicity and reproduction study was conducted as multiple applications of plant protection product will be made each year. Technical RH-117,281 (purity 92.3%) was administered to earthworms (*Eisenia fetida*) during an 8 week chronic toxicity and reproduction study. There were four replicates with 10 worms per replicate, using artificial soil in 3-litre chambers containing 550 g of soil (dry weight). Nominal soil concentrations were 0 (control), 0 (solvent control, 2 ml acetone/kg soil), 0.5, 1, 5, 10, and 20 ppm a.s. (mg a.s./kg soil). A positive reference substance (benomyl, 50% a.s.), was also tested to ensure that the study procedures could adequately detect sublethal toxicity. The test dilutions were applied to the soil surface and the acetone was allowed to evaporate for 1 hour at room temperature before the adult earthworms were placed on the surface of the soil. The mean biomass loading was 0.192 g biomass/20 g soil. It was not stated whether the test material was incorporated into the soil. After four weeks of exposure, mortality and growth were evaluated. Cocoons containing the next generation of worms were left in the test exposure beakers and were allowed to hatch over the next 28 days in order to assess effects on reproduction. On Day 56, reproduction (the number of juvenile worms produced) was assessed. Results are summarised in Table B.9.6.2.-1.

Table B.9.6.2-1: Chronic toxicity of technical RH-117,281 (purity 92.3%) to *Eisenia fetida*

Concentration (mg a.s./kg)	% Mortality at 28 days	% Growth (biomass increase)	Reproduction (mean offspring/adult earthworm) at 56 days
Control	0.0	44.6	7.8
Vehicle Control	0.0	36.7	14.6
0.5	0.0	31.2	13.9
1	5.0	36.4	11.0
5	2.5	38.0	0.7*
10	0.0	37.2	0.2*
20	0.0	35.6	0.3*

* Significantly different from solvent control, $p \leq 0.05$, Scheffé nonparametric test

There were no statistically significant differences from control or solvent control in mortality or growth of earthworms after 28 days of exposure at all concentrations. Mean percent biomass increased by 31 - 45 %. Statistically significant decreases in mean number of offspring per adult earthworm were observed in the test concentrations of 5 mg a.s./kg soil or greater. The reference test substance, benomyl, exhibited a 28 day LC50 of 13.4 mg a.s./kg soil and a reproduction and growth NOEC of 1 mg a.s./kg soil, demonstrating that the test protocol was adequate to detect sublethal toxicity.
The 28-Day LC50 for the earthworm (*Eisenia fetida*) was > 20 mg a.s./kg, the highest concentration tested. The 28 day NOEC for mortality and growth was 20 mg a.s./kg. The 56-day NOEC for reproduction was 1 mg a.s./kg based on reduced number of offspring per adult earthworm.
28-Day LC50 > 20 mg a.s./kg soil dw
28 day NOEC = 20 mg a.s./kg soil dw
56-day NOEC = 1 mg a.s./kg soil dw

RMS comments:
Study is performed on artificial soil. The study was conducted to BBA guideline VI 2-2 (1994) and GLP, the study is considered acceptable.
28-Day LC50 > 20 mg a.s./kg soil dw
28 day NOEC (mortality) = 20 mg a.s./kg soil dw
56-day NOEC (reproduction) = 1 mg a.s./kg soil dw

Report:	**CA, 8.4.1/01 Nienstedt, K. M. (2001)** **Effects of RH-117,281 Technical applied on natural soil on the cocoon and juvenile production of the earthworm *Eisenia fetida*.**

Guidelines: ISO11268-2 (1988), BBA-guideline VI, 2-2 (Kula *et al.*, 1994) OECD guideline (2000)
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical, (Lot DSR 9510), chemical purity: 93.1% a.i.
Test species: earthworm *Eisenia fetida*
Number of organisms, weight: 6 replicates for the control and solvent, 4 replicates per test item treatment, with 10 earthworms/each replicate, adults, weighting 307 to 598 mg each, 5 to 6 months old
Type of test: sublethal chronic toxicity and reproduction test (28 days)
Applied concentrations: control (deionized water), solvent control (acetone), 1, 2, 4, 5, 7 and 10 mg a.i. kg/dry soil
Soil type and test conditions:
test substrate: fresh natural soil (Lufa 2.2, Batch F 2.24700, loamy sand, 2.17% organic)
temperature: 19.0 to 21.5 °C
water content: 31.34% moisture
light intensity: range from 415 to 705 lux.

Results:
A second sublethal chronic toxicity and reproduction study was conducted in natural soil. Technical RH-117,281 (purity 93.1%) was administered to earthworms (*Eisenia fetida*) during an 8 week chronic toxicity and reproduction study in natural soil. Four replicates (10 worms per replicate, six replicates for control and solvent control, though two replicates of the control treatment were removed later due to unexpected mortality) were exposed to RH-117,281 Technical applied to the soil surface in 1.5 litre test beakers at an equivalent application rate of 600 l/ha at nominal soil concentrations of 0 (control), 0 (solvent control, 2 ml acetone/kg soil), 1, 2, 4, 5, 7 and 10 mg a.s./kg soil. A positive reference substance (benomyl, 50% a.s.), was also tested to ensure that the study procedures could adequately detect sublethal toxicity. The test dilutions were applied to the soil surface and the acetone was allowed to evaporate for 1 hour at room temperature before the adult earthworms were placed on the surface of the soil. Five days prior to the experimental start date, worms were transferred from standard culture medium (peat plus commercial garden soil) to the test soil for acclimatisation. The test soil was fresh natural LUFA 2.2 soil (batch F 2.24700, loamy sand) originating from Rheinland-Pfalz, Germany. The sampling site was a meadow for the last five years with no fertilisation or pesticide applications. The water holding capacity of the soil was 50%. The soil contained 2.17% organic carbon and exhibited a pH of 5.7. Photoperiod was 16 hours light: 8 hours darkness. Other environmental parameters including temperature (19.0 - 21.5 °C), pH (mean Day 0, 7.13-7.24; mean Day 56, 6.01-6.41) light intensity (415 - 705 lux) and soil moisture as a percent of water holding capacity (62.7-68.4%) were within acceptable limits. Worms were fed weekly on dried cattle manure and water. After four weeks of exposure, mortality, growth, and number of cocoons laid were evaluated. After counting, cocoons containing the next generation of worms were replaced in the test exposure beakers and were allowed to hatch over the next 28 days in order to assess effects on reproduction. On Day 56, reproduction (the number of juvenile worms produced per vessel) was assessed. Results are summarised in Table B.9.6.2.-2.

Table B.9.6.2-2: Chronic toxicity of technical RH-117,281 (purity 93.1%) to *Eisenia foetida*

Concentration (mg a.s./kg) Nominal	% Survival at 28 days	Growth (mg biomass increase)[a] after 28 days	Number of cocoons /vessel[b] at 28 days	Reproduction (juveniles/vessel) at 56 days
Control	90.0	147.3	89.5	294.3
Solvent Control	85.0	194.7	91.8	312.4
1	85.0[ns]	136	99.5	357.8
2	87.5[ns]	193	106.8	359.3
4	70.0[ns]	143	97.8	219.3
5	85.0[ns]	148	122.7	322.0
7	87.5[ns]	167	118.0	256.8
10	77.5[ns]	177	110.0	154.5*
Toxic Standard (Benomyl)	12.5	-155.3	0.25	0.0

(a) No significant differences between treatments and solvent control (ANOVA, p = 0.88).
(b) No significant differences between treatments and solvent control (ANOVA, p = 0.15).
(ns.) Not significantly different from solvent control (Fisher exact tests, p > 0.05)
(*) Significantly different from solvent control (ANOVA p = 0.009/ Dunnett α = 0.05).
Note: The sample size in the solvent control was n = 6, and in the test item, toxic standard and control treatments, n = 4.

There were no statistically significant differences from solvent control in mortality after 28 days of exposure to RH-117,281 Technical at all concentrations in natural soil. After the 28 day exposure phase, there were no statistically significant differences from solvent control (or control) in growth of earthworms and the number of cocoons laid per vessel at all RH-117,281 Technical concentrations in natural soil. Mean biomass increased in all treatments to the same extent except in the toxic standard in which a decrease was observed. There were no statistically significant differences from solvent control (or control) in mean number of juveniles per vessel at all RH-117,281 Technical concentrations in natural soil except at 10 mg a.s./kg. The reference test substance, benomyl, exhibited significant toxicity, growth and cocoon reduction after 28 days and significant effects on hatching and survival of juvenile earthworms after 56 days, demonstrating that the test protocol was adequate to detect sublethal toxicity in a natural soil.

The 28 day LC50 for the earthworm (*Eisenia fetida*) in natural soil was > 10 mg a.s./kg, the highest concentration tested. The 28 day NOEC for mortality, growth, and cocoons laid was 10 mg a.s./kg. The 56-day NOEC for reproduction in natural soil was 7 mg a.s./kg.
28 day LC50 > 10 mg a.s./kg soil dw
28 day NOEC = 10 mg a.s./kg soil dw
56-day NOEC = 7 mg a.s./kg. soil dw

RMS comments:
Study is performed on using natural soil.The study was conducted to BBA guideline VI 2-2 (1994) and GLP and study is considered acceptable.
28 day LC50 > 10 mg a.s./kg soil dw
28 day NOEC (mortality, growth, cocoons laid) = 10 mg a.s./kg soil dw
56-day NOEC (reproduction)= 7 mg a.s./kg. soil dw

B.9.4.2 Effects on non-target soil meso and macrofauna (other than earthworms)

For the first EU review for Annex I inclusion, additional testing on non-target soil meso- and macro-organisms was not required due to the fact that field and laboratory study DT_{90} values for zoxamide were <100 days (41.3 days) (see Volume 3, CP, Section B.8 for further details). In addition, data are available on both *Aphidius rhopalosiphi* and *Typhlodromus pyri* and these have been used in an initial risk assessment that demonstrates an acceptable risk (see Volume 3, CP, Section B.9). As no concern is raised with either species tested and as the product is not applied directly to soil data on *Folsomia candida* and *Hypoaspis aculeifer* are not required.

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 any of these metabolites on non-target soil meso- and macrofauna (other than earthworms).

All four metabolites lack the haloketone toxophore associated with the parent compound's mode of toxic action. In addition, a risk assessment conducted for earthworms based on the worst case assumption that the metabolites are 10 fold more toxic than the parent demonstrated that both acute and chronic risks to earthworms are acceptable (see Volume 3, CP, Section B.8).

Regarding their persistence in soil, the DT_{90} values of metabolite RH-125450 are in the range of 6.6 to 38.8 days and of metabolite RH-24549 - 10.15 to 53.9 days, all values are <100 days. For metabolite RH-163353 the DT_{90} values are in the range of 18.7 to 178 days but with a geomean value of 34.8 days (<100 days) and for metabolite RH-141455 DT_{90} values are in the range of 40 to 105.3 days but with a geomean value of 53.8 days (<100 days). In line with the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 of October 2002) as the soil DT_{90} are <100 days no further testing should be required.

Therefore, it is considered that testing on other non-target soil meso- or macrofauna such as *Folsomia candida* and *Hypoaspis aculeifer* is not required.

B.9.5 EFFECTS ON SOIL NITROGEN TRANSFORMATION

For the first EU review, adequate data were submitted to assess the potential impact on soil microbial activity. 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).

Report:	**CA, 8.5/01 van der Kolk, J. (1998b)** **RH-117,281 Technical: Determination of the effects on soil microflora activity.**

Guidelines: EPPO, Chapter 7, 1994
GLP: Yes
Previous evaluation: In DAR (May 2001); relevant for renewal application

Material and Methods:
Test substance: RH-117,281 Technical, (Lot DSR-9510), chemical purity: 92.3% a.i.
Type of test: soil respiration and nitrification tests (28 days)

Test concentration: 2.0 mg a.i./kg dry soil (equivalent to an application rate of 10 times 150 g a.i./ha)
Soil type and test conditions:
test substrate: loamy sand, BBA1 (Batch #030895)
pH: 7.0
organic C (%): 0.9
maximum water holding capacity: 22.1 g/100 g dry soil
pre-study microbial biomass: 36.0 mg/100 g dry soil
post-study microbial biomass: 96.7 mg/100 g dry soil
temperature: 19.0 to 20.5 °C

Results:
Data were submitted from a laboratory study of the effect of technical RH-117,281 (92.3% purity) on respiration and nitrification in a loamy sand soil which had been treated 10 times with 150 g a.s./ha (= 10 times the maximum field rate for potatoes).

The short term respiration results showed that RH-117,281 Technical treated soil sample consumed -9, 1 and 4% of the oxygen consumed by control samples within the 28 day incubation period (days 0, 14 and 28). It can be concluded that RH-117,281 Technical had no influence on the respiration of the soil.

On days 0 and 28 of the test the ammonium concentrations for RH-117,281 Technical trated samples deviated 17 and 16% when compared with the untreated samples. On days 14 and 42, the concentration of ammonium in all the soil samples was below the detection limit (0.02 mg NH_4^+-N/100 g dry soil).

The concentration of NO_2^-N was below the detection limit of 0.11 mg/100 g dry soil in the soil samples at all sampling intervals.

The deviations for the nitrate concentration in the treated samples when compared to the control samples were -18, 17, 33 and -4% on days 0, 14, 28 and 42 of the study.

The concentration of total mineralised N in treated soils deviated -6, 17, 30 and -4% from the concentration in the control soil on days 0, 14, 28 and 42 of the study.

The results showed that on days 14 and 28 of the study the N-mineralization process in the treated soils were higher than in the control, but this effect was transient, since the differences were below the critical value of 25% after 42 days of the study.

Based on the results of the respiration and nitrification, it can be concluded that RH-117,281 Technical can be categorized as low risk to soil microflora following the criteria given under Chapter 7 of the European Plan Protection Organization`s (EPPO) Guidelines.

Table B.9.5-1: Summary of data on the toxicity of zoxamide to soil micro-organisms

Test	Endpoint for zoxamide
Nitrogen mineralisation	<25% after 42 days at 2 mg a.s./kg soil
Carbon transformation	<25% after 28 days at 2 mg a.s./kg soil

RMS comments:
The study was conducted according to the EPPO Guidelines (1994) and to GLP, the study is considered 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.

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 the metabolites RH-24549, RH-127450 and RH-163353 would have 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.

Therefore, it is considered that the risk assessment of the parent also addresses the potential risk posed by the major soil metabolites (please see Volume 3, Section B.8 PPP).

No further data are considered necessary.

B.9.6 EFFECTS ON TERRESTRIAL NON-TARGET HIGHER TIER PLANTS

B.9.6.1 Summary of screening data

During the previous EU review screening data on terrestrial vascular plants were only tested. 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 Tier I studies examining the effects on seedling emergence and vegetative vigour are not required.

B.9.6.2 Testing on non-target plants

Tests on non-target plants have been performed with the current European representative formulation: RH-117,281 2f (240SC). Please refer to Volume 3 CP B.9.11.2. for further information.

B.9.7 EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Report:	**CA, 8.6/03 Sames, B.A. (1998)** **Insecticidal screening report - prescreen insecticidal activity with RH-117,281; Primary screening activity per RH-117,281.**

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

Material and Methods:
Test substance: RH-117,281 Technical Lot No. NSS1168Z, chemical purity: 95% a.i; Lot ELM-11-75, >99% a.i.
Type of test: insecticide test
Test concentrations: 38, 150 and 600 g/a.i./ha

Results:
A laboratory screening study was performed to assess the effects of technical RH-117,281 (98% purity) on a range of seven insect species. When applied at foliar rates of 38, 150 and 600 g a.s./ha (0.25 to 4 times the maximum application rate to potatoes), RH-117,281 was harmless to two species of Lepidoptera, one species of mite (two-spotted spider mite; *T. urticae*) and two species of Homoptera. On a third species of Homoptera, 40% mortality was seen at 600 g a.s./ha but no effect at 150 g a.s./ha. When applied to soil, RH-117,281 was harmless to one species of Coleoptera at 76 times the label recommended dose on potatoes.

RMS comments:

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

B.9.8 EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

During the previous EU review adequate data were submitted to assess the potential impact of zoxamide on activated sludge (ready biodegradability, CO_2 evolution test). Please refer to the point B.9.7. in the current document.

No further data are considered necessary.

B.9.9 MONITORING DATA

No available data to be reported.

B.9.10 BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

No available data to be reported. Information about metabolites in groundwater is seen in Vol. 3 Part B.8

B.9.12 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
KCA, 8.2.4.2/01	Roberts, C.A. and Swigert, J.P	1997	RH-117,281 Technical: A 96-Hour-Flow Through Acute Toxicity Test with the Saltwater Mysid (Mysidopsis bahia) Wildlife International Ltd 898 Commerce Drive, Eastern Maryland 21601, USA. Report Number: 95RC-0275 GLP, Not published	N	Y	Data were generated to support registration in the US and were not available or required for first Annex I inclusion	Gowan
KCA, 8.2.5.2/01	Drottar, K.R. and Krueger, H.O.	1998	RH-117,281 Technical: A flow-through life-cycle toxicity test with the saltwater mysid (Mysidopsis bahia) Wildlife International Ltd 898 Commerce Drive, Eastern Maryland 21601, USA. Report Number: 97RC-0077 GLP, Not published	N	Y	Data were generated to support registration in the US and were not available or required for first Annex I inclusion	Gowan
KCA, 8.3.1.2/01	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
KCA, 8.3.1.3/01	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
KCA, 8.3.1.4/01	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 (Old)	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
CA, 8.1.1.1/01	IIA, 8.1.1/01	[redacted]	1997a	RH-117,281 Technical: 14-day acute oral LD50 study in bobwhite quail. [redacted], Project No. RH117BWLD-595. ER Ref No: 2.6 US Ref No: 94RC-0240 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.1.1.2/01	IIA, 8.1.2/01	[redacted]	1997b	RH-117,281 Technical: 8-day acute dietary LC50 study in bobwhite quail. [redacted], Project No. RH117BWLC-395. ER Ref No: 2.4 US Ref No: 94RC-0242 GLP, Unpublished	Y	N	NA	Gowan

Data point	Annex point (Old)	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
CA, 8.1.1.2/02	IIA, 8.1.2/02	[redacted]	1997c	RH-117,281 Technical: 8-day acute dietary LC50 study in mallard ducklings. [redacted]., Project No. RH117MDLC-395. ER Ref No: 2.5 US Ref No: 94RC-0241 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.1.1.3/01	IIA, 8.1.3/01	[redacted]	1998	Avian reproduction study of RH-117,281 Technical with northern bobwhite. [redacted]. Project ID No. RH7281BW-97-2 ER Ref No: 28.2 US Ref No: 97RC-0081 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.1.1.3/02	IIA, 8.1.3/02	[redacted]	1999	RH-117,281 technical: A reproduction study with the mallard (*Anas platyrhynchos*). [redacted] Project No. 129-164 ER Ref No: 33.13 US Ref No: 98RC-0166 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.1/01	IIA, 8.2.1/01	[redacted]	1995a	Acute flow-through toxicity of RH-117,281 Technical to rainbow trout (*Oncorhynchus mykiss*). [redacted] Report No. 41681. ER Ref No: 2.1 US Ref No: 94RC-0078 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.1/02	IIA, 8.2.1/02	[redacted]	1995b	Acute flow-through toxicity of RH-117,281 Technical to bluegill (*Lepomis macrochirus*). [redacted] Report No. 41682. ER Ref No: 4.10 US Ref No: 94RC-0080 GLP, Unpublished	Y	N	NA	Gowan

Data point	Annex point (Old)	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
CA, 8.2.1/03 (8.2.2.3 /01)	IIA, 8.2.1/03 (8.2.2.3 /01)	[redacted]	1998d	RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*). [redacted] Project No. 129A-141 ER Ref No: 17.4 US Ref No: 97RC-0079 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.1/04	IIA, 8.2.1/04	[redacted]	1998a	RH-117,281 Technical: A 96-hour flow-through acute toxicity test with the zebra fish (*Brachydanio rerio*). [redacted] Project No. 129A-150 ER Ref No: 12.11 US Ref No: 97RC-0134 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.1/05	IIA, 8.2.1/05	[redacted]	1997	RH-117,281 Technical: a 96-hour flow-through acute toxicity test with the sheepshead minnow (*Cyprinodon variegatus*). [redacted] Project No. 129A-135. ER Ref No: 8.1 US Ref No: 95RC-0274 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.1/06	IIA, 8.2.1/06	[redacted]	1998a	Acute toxicity of RH-127,450 to the rainbow trout (*Oncorhynchus mykiss)* in a range-finding test under static conditions. [redacted] Report No. 44667 ER Ref No: 17.5 US Ref No: 98RC-0095 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.1/07	IIA, 8.2.1/07	[redacted]	2002	Zoxamide Metabolite RH-139,432 - Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) under static conditions [redacted] Study Number 12550.6290 **CA 1 ER Ref. 47.4** GLP, Not published	Y	N	NA	Gowan

Data point	Annex point (Old)	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
CA, 8.2.2.1/01	IIA, 8.2.2.2/01	[redacted]	1996	Early life-stage toxicity of RH-117,281 Technical to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. [redacted] Report No. 42400. ER Ref No: 7.1 US Ref No: 94RC-00239 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.2.2/01 (8.2.1/03)	IIA, 8.2.2.3/01 (8.2.1/03)	[redacted]	1998d	RH-117,281 Technical: A flow-through life-cycle toxicity test with the fathead minnow (*Pimephales promelas*). [redacted] Project No. 129A-141 ER Ref No: 17.4 US Ref No: 97RC-0079 GLP, Unpublished	Y	N	NA	Gowan
CA, 8.2.4.1/01	IIA, 8.2.4/01	Sword, M.C., Gardner, C.	1995c	Acute flow-through toxicity of RH-117,281 Technical to *Daphnia magna.* ABC Laboratories Report No. 41683. ER Ref No: 5.1 US Ref No: 94RC-0081 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.4.1/02	IIA, 8.2.4/02	[redacted]	1998b	Acute toxicity of RH-127,450 to *Daphnia magna* in a range-finding test under static conditions. [redacted] Study No. 44666 ER Ref No: 16.4 US Ref No: 98RC-0096 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.4.1/03	IIA, 8.2.4/03	Caferella, M.A	2002	Zoxamide Metabolite RH-139,432 - Acute Toxicity to Daphnids (Daphnia magna) under static conditions Springborn Smithers Laboratories, Study Number 12550.6289 ER Ref. 47.3 GLP, Not published	N	N	NA	Gowan

Data point	Annex point (Old)	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
CA, 8.2.5.1/01	IIA, 8.2.5/01	Murrell, H., Rhodes, J.E., Stewart, S.	1997	Chronic toxicity of RH-117,281 Technical to *Daphnia magna* under flow-through test conditions. ABC Laboratories Report No. 43209. ER Ref No: 6.11 US Ref No: 95RC-0273 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.5.3/01	IIA, 8.2.7/01	van der Kolk, J.	1998a	RH-117,281: Chronic effects on midge larvae (Chironomus riparius) in a water/sediment system. Springborn Laboratories (Europe)AG Report No. 97-063-1007 ER Ref No: 13.3 US Ref No: 97RC-0083 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.6.1/01	IIA, 8.2.6/01	Ziegler, T.A., Stewart, S.	1996	Acute toxicity of RH-117,281 Technical to S*elenastrum capricornutum* Printz. ABC Laboratories Report No. 42399. ER Ref No: 1.1 US Ref No: 94RC-0238 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.6.1/02	IIA, 8.2.6/02	Drottar, K.R., Sutherland, C.A., Krueger, H.O.	1998e	RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*). Wildlife International, Ltd. Project No. 129A-154 ER Ref No: 13.2 US Ref No: 97RC-0130 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.6.1/03	IIA, 8.2.6/03	Drottar, K.R., Sutherland, C.A., Krueger, H.O.	1998f	RH-117,281 Technical: A 96-hour toxicity test with the freshwater alga (*Scenedesmus subspicatus*). Wildlife International, Ltd. Project No. 129A-151 ER Ref No: 13.4 US Ref No: 97RC-0133 GLP, Unpublished	N	N	NA	Gowan

Data point	Annex point (Old)	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
IIA, 8.2.6.1/04	IIA, 8.2.6/04	Drottar, K.R., Sutherland, C.A., Krueger, H.O.	1998g	RH-117,281 Technical: A 96-hour toxicity test with the freshwater diatom (*Navicula pelliculosa*). Wildlife International, Ltd. Project No. 129A-153 ER Ref No: 13.5 US Ref No: 97RC-0131 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.6.1/05	IIA, 8.2.6/05	Drottar, K.R., Krueger, H.O.	1998c	RH-117,281 Technical: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*) Wildlife International, Ltd. Project No. 129A-152 ER Ref No: 12.10 US Ref No: 97RC-0132 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.6.1/07	IIA, 8.2.6/07	Rhodes, J.E., Williams, S.	1998c	Acute toxicity of RH-127,450 to the green alga, S*elenastrum capricornutum* Printz ABC Laboratories Report No. 44665 ER Ref No: 28.3 US Ref No: 98RC-0097 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.6.1/08	IIA, 8.2.6/08	Rhodes, J.E., Williams, S.	1999	Acute toxicity of RH-163,353 to S*elenastrum capricornutum* Printz in a range-finding test under static conditions. ABC Laboratories Report No. 45164 ER Ref No: 36.1 US Ref No: 99RC-0023 GLP, Unpublished	N	N	NA	Gowan
CA, 8.2.6.1/09		Hoberg, J.R	2002	Zoxamide Metabolite RH-139,432 - Toxicity to Freshwater Green Algae, Scenedesmus subspicatus Springborn Smithers Laboratories Study Number 12550.6288 **CA 2 ER Ref. 47.5** GLP, Not published	N	N	NA	Gowan

Data point	Annex point (Old)	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
CA, 8.2.7/01	IIA, 8.2.8/01	Drottar, K.R., Krueger, H.O.	1998b	RH-117,281 Technical: A 14-day static-renewal toxicity test with duckweed (*Lemna gibba* G3). Wildlife International, Ltd. Report No. 129A-147. ER Ref No: 12.7 US Ref No: 97RC-0080 GLP, Unpublished	N	N	NA	Gowan
CA, 8.3.1.1.1/01	IIA, 8.3.1.1/01	Kirkland, R.L.	1993	Acute contact toxicity of RH-117,281 Technical to honey bees. Bio Research Study No. 109-93. ER Ref No: 12.6 US Ref No: 92RC-0235 GLP, Unpublished	N	N	NA	Gowan
CA, 8.4.1/01	IIA, 8.4.1/01	Downing, J. Leak, T.	1995	RH-117,281 Technical: toxicity to earthworm (*Eisenia foetida*). ABC Laboratories Report No. 42398. ER Ref No: 5.2 US Ref No: 94RC-0237 GLP, Unpublished	N	N	NA	Gowan
CA, 8.4.1/02	IIA, 8.4.1/02 **See add. vol. April 2000**	Bryan, R.L., Porch, J.R., Krueger, H.O.	2000	RH-127,450 Technical: an acute toxicity study with the earthworm in an artificial soil substrate. Wildlife International, Ltd., Easton, MD, USA, Project No. 129-173, March 7, 2000 ER Ref No. 41.5 US Ref No. 99RC-0282 GLP, unpublished	N	N	NA	Gowan
CA, 8.4.1/01	IIA, 8.4.2/01	Nienstedt, K.	1999	A chronic toxicity and reproduction test exposing the earthworm *Eisenia fetida* to RH-117,281 Technical material in OECD artificial soil, based on the BBA-guideline VI, 2-2 (1994) and the ISO-draft (ISO/DIS 11268-2) Springborn Laboratories (Europe) AG Report No. 99-092-1007 ER Ref No: 34.1 US Ref No: 98RC-0181 GLP, Unpublished	N	N	NA	Gowan

Data point	Annex point (Old)	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
CA, 8.4.1/02	IIA 8.4.2/02 **Add. Vol. March 2001**	K.M Niesnstedt	2001	Effects of RH-117,281 Technical Applied On Natural Soil On The Cocoon And Juvenile Production Of The Earthworm *Eisenia fetida* Springborn Laboratories (Europe) AG - Switzerland, February 9, 2001. ER Ref No. 45.2 US Ref. No. 00RC-0209, GLP, unpublished	N	N	NA	Gowan
CA, 8.5/01	IIA, 8.5/01	van der Kolk, J.	1998b	RH-117,281 Technical: Determination of the effects on soil microflora activity. Springborn Laboratories (Europe) AG # 97-060-1007. ER Ref No: 14.1 US Ref No: 97RC-0084 GLP, Unpublished	N	N	NA	Gowan
CA, 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
CA, 8.6/02	IIA, 8.6/02	Nunez, M.V.	1998b	Greenhouse crop phytotoxicity tests with RH-117281 2F. Rohm and Haas Report No. 98R-1114 ER Ref No: 28.5 US Ref No: 98R-1114 GLP, Unpublished	N	N	NA	Gowan
CA, 8.6/03	IIA, 8.6/03	Sames, B.A.	1998	Insecticidal screening report - prescreen insecticidal activity with RH-117,281; Primary screening activity per RH-117281. Rohm and Haas Report No. 98R-1113 ER Ref No: 28.6 US Ref No: 98R-1113 GLP, Unpublished	N	N	NA	Gowan