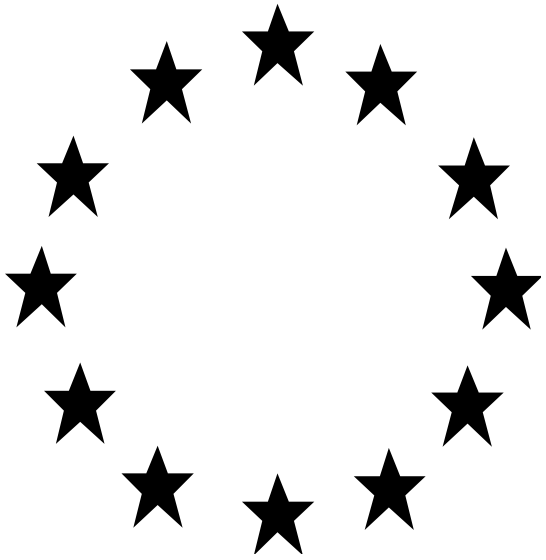


Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State The Netherlands for the existing active substance

ETRIDIAZOLE

of the third stage (part B) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, part 5, B.9

August 2007

B.9 Ecotoxicology

Background information

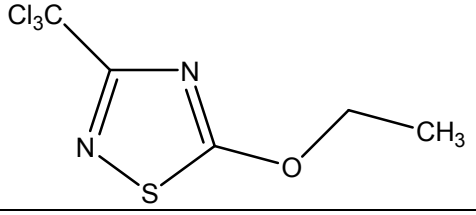
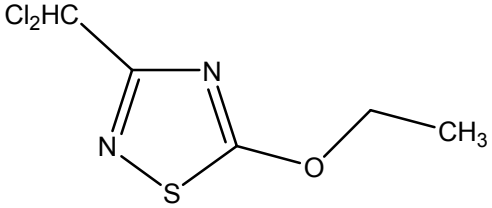
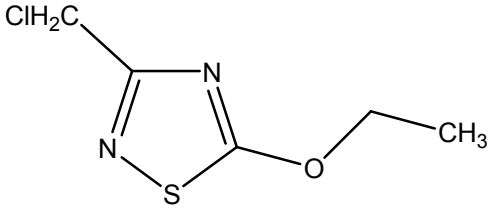
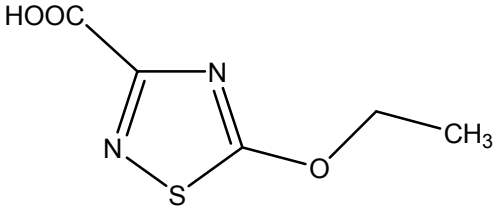
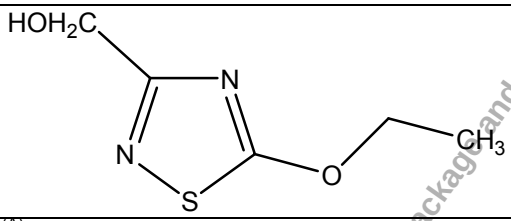
Etridiazole is an existing fungicide proposed for use in non-soil bound glasshouse ornamental crops (two applications with 14 day interval at 7 kg a.s./ha), substrate-grown tomatoes and peppers (two applications with 14 day interval at 0.28-0.56 kg a.s./ha) and substrate-grown cucumbers (two applications with 14 days interval at 0.28 kg a.s./ha). The proposed formulated product, Aaterra, also referred to as Aaterra ME, is a micro-emulsion formulation containing nominally 700 g/L etridiazole. Etridiazole was also referred to as Terrazole.

Data were presented on the technical active substance (a.s.) and on the proposed Aaterra formulation, as well as on major metabolites.

Table B.9.1 below gives an overview of the major metabolites of etridiazole in the matrices water/sediment. The maximum formation rates in the different matrices, as well as the occurrence of the metabolites in metabolism of rat, are included.

Studies summarised below under point B.9.1 to B.9.10 are acceptable unless noted.

Table B.9.1 Overview of metabolites of etridiazole

Structure	Name	Metabolite in	MW	LogPow[A]
	Etridiazole		247.53	Experimental: 3.4 Estimated: 3.6
	Dichloro-etridiazole[B]	Water: 9.5% AR Sediment: 1.4% AR	213.08	Experimental: 2.8[C] Estimated: 2.68
	Monochloro-etridiazole[D]	Water: ≤4.0% AR Sediment: ≤4.0% AR	178.64	Estimated: 2.50
	Etridiazole acid[E]	Water: 13% AR Sediment: 8.3% AR Rat: 20-36% AR	174.18	Experimental: 0.7 Estimated: 1.58
	3-hydroxymethyl etridiazole	Water: ≤3.4% AR Sediment: ≤3.4% AR	160.19	Estimated: 0.78

[A] Experimental values were taken from section B.2.1; estimated values were calculated by RMS (using EPA EPI Suite Software: 4.21)

[B] In this dissier, dichloro-etridiazole was also referred to as 3-dichloromethyl-terrazole and 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03)

[C] The experimental logPow value was considered not reliable; therefore, the estimated value is used throughout this section.

[D] In this dossier, monochloro-etridiazole was also referred to as 3-monochloromethyl-terrazole

[E] In this dossier, etridiazole acid was also referred to as etridiazole carboxylic acid and 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02)

B.9.1 Effects on birds (IIA8.1; IIIA 10.1)

B.9.1.1 Acute oral toxicity (IIA 8.1.1; IIIA 10.1.1)

B.9.1.1.1 Acute oral toxicity of the active substance

Study 1

Characteristics

reference	:	Fletcher D. (1972a)	duration of observations	:	21 days
type of study	:	Acute oral toxicity study			
year of execution	:	Not reported	nominal doses	:	0, 215, 1470, 4640, 6810 and 10000 mg/kg bw
GLP statement	:	No			
guideline	:	Not reported	dosing method	:	Undiluted by gavage
test substance	:	Terrazole	acceptability	:	**Less reliable** (see text)
purity	:	Not reported	LD50	:	1640 mg/kg bw
species	:	Mallard duck			

Methods

In a study on the acute oral toxicity of Terrazole (etridiazole) to young adult mallard duck (*Anas platyrhynchos*), five male and five females per dose level (fasted overnight, body weight at test initiation about 1100 g) were orally dosed with etridazole as received (no vehicle) at dose levels of 0, 215, 1470, 4640, 6810 and 10000 mg/kg bw, followed by a twenty-one day observation period.

Results

No birds died in the control group. Three, four, five, six and ten birds died at 215, 1470, 4640, 6810 and 10000 mg/kg bw, respectively. Anorexia and dark green-coloured faeces were observed at all doses. Food consumption and body weight were significantly reduced at all doses during the first week. Discoloration of liver and lungs and slightly enlarged gall bladders were observed at necropsy at all doses.

Table B.9.2 Summary of acute oral toxicity of Terrazole (etridiazole) to birds

Purity	Species	Acute oral LD50 (mg/kg bw)
Not reported	Mallard duck (*Anas platyrhynchos)*	1640 (95% CI 540-4930)

Conclusion

The acute oral LD50 for mallard duck is 1640 mg/kg bw.

Guidelines and limitations

Although generally test procedures complied with EPA 71-1, the report of this non-GLP study lacked documentation on: the time of conduct of the study; test substance (batch, purity); individual body weights; methods of statistical analysis and test conditions (temperature, relative humidity, light regime). Proper characterisation of the test substance is an important condition for acceptance of a study. Therefore, the notifier was asked to provide information (including an analytical certificate) to confirm the purity of the test substance. Notifier replied that since no further batch reference was given in the report, it was not possible, after so many years, to retrieve the exact analytical data for the batch used for this study. Notifier also stated that since the sample was taken from a normal production batch, it complied with the product specification on purity, which implies that the purity of the test substance was at least 95%.

RMS concludes that the study can be accepted as 'less reliable' (instead of 'not acceptable'), in view of the low exposure of birds that is expected for the proposed uses, and to prevent unnecessary animal testing. This will also be clearly indicated in the List of endpoints. See further the text of the risk assessment under B.9.1.5.

Study 2

Characteristics

reference	:	Fletcher D. (1972b)	duration of observations	:	21 days
type of study	:	Acute oral toxicity study			
year of execution	:	Not reported	nominal doses	:	0, 464, 681, 1470, 2150 and 3160 mg/kg bw
GLP statement	:	No			
guideline	:	Not reported	dosing method	:	Undiluted by gavage
test substance	:	Terrazole	acceptability	:	**Less reliable** (see text)
purity	:	Not reported	LD50	:	560 mg/kg bw
species	:	Bobwhite quail			

Methods

In a study on the acute oral toxicity of Terrazole (etridiazole) to young adult bobwhite quail (*Colinus virginianus*), five male and five females per dose level (fasted overnight, body weight at test initiation about 200 g) were orally dosed with etridazole as received (no vehicle) at dose levels of 0, 464, 681, 1470, 2150 and 3160 mg/kg bw, followed by a twenty-one day observation period.

Results

No birds died in the control group. Four, six, ten, ten and ten birds died at 464, 681, 1470, 2150 and 3160 mg/kg bw, respectively. Lethargy, anorexia and hypoesthesia were observed at all doses. Food consumption was significantly reduced at 464, 681 and 1470 mg/kg bw during the first week and at 2150 mg/kg bw during the first two weeks. Body weight was significantly decreased at all dose levels during the first two weeks. Malnutrition was observed at necropsy at all doses.

Table B.9.3 Summary of acute oral toxicity of Terrazole (etridiazole) to birds

Purity	Species	Acute oral LD50 (mg/kg bw)
Not reported	Bobwhite quail (*Colinus virginianus)*	560 (95% CI 350-890)

Conclusion

The acute oral LD50 for bobwhite quail is 560 mg/kg bw.

Guidelines and limitations

Although generally test procedures complied with EPA 71-1, the report of this non-GLP study lacked documentation on: the time of conduct of the study; test substance (batch, purity); individual body weights; methods of statistical analysis and test conditions (temperature, relative humidity, light regime). Proper characterisation of the test substance is an important condition for acceptance of a study. Therefore, the notifier was asked to provide information (including an analytical certificate) to confirm the purity of the test substance. Notifier replied that since no further batch reference was given in the report, it was not possible, after so many years, to retrieve the exact analytical data for the batch used for this study. Notifier also stated that since the sample was taken from a normal production batch, it complied with the product specification on purity, which implies that the purity of the test substance was at least 95%.

RMS concludes that the study can be accepted as 'less reliable' (instead of 'not acceptable'), in view of the low exposure of birds that is expected for the proposed uses, and to prevent unnecessary animal testing. This will also be clearly indicated in the List of endpoints. See further the text of the risk assessment under B.9.1.5.

B.9.1.1.2 Acute oral toxicity of metabolites

No data were submitted.

B.9.1.1.3 Acute oral toxicity of the plant protection product

No data were submitted.

B.9.1.2 Dietary toxicity (IIA 8.1.2)

B.9.1.2.1 Dietary toxicity of the active substance

Study 1

Characteristics

reference	:	Fletcher D.W. (1981a)	exposure duration	:	5 days
type of study	:	Dietary LC50 study	nominal	:	0, 313, 625, 1250, 2500 and 5000
year of execution	:	1980-1981	concentrations		mg/kg diet
GLP statement	:	No	dosing method	:	Diet mixed with Terrazole in corn oil
guideline	:	Not reported	acceptability	:	**Less reliable** (see text)
test substance	:	Terrazole^{R} Technical	LC50	:	

purity	:	99%			>5000 mg/kg diet ⇔ >636 mg/kg bw/day
species	:	Bobwhite quail			

Methods

In a study on the dietary toxicity of Terrazole^{R} (etridiazole, purity 99%), five groups of 14-day old Bobwhite quails of indeterminate sex (10/group) were fed Terrazole in the diet at analytically confirmed concentrations (see comment 4 below) of 313, 625, 1250, 2500 and 5000 mg/kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. Five control groups of ten 14-day old Bobwhite quails received feed treated with the vehicle (corn oil).

Results

One bird died in one of the control groups, and one bird died at 2500 mg/kg. No further birds died during the study. No clinical signs were noted up to 1250 mg/kg, but birds were lethargic after four days at 2500 and 5000 mg/kg. Body weight, body weight gain and feed consumption were significantly reduced at 2500 and 5000 mg/kg. Food conversion efficiency was reduced at all doses. No abnormalities were noted at necropsy.

The reported 5-day dietary LC50 and NOEC were >5000 and 1250 mg/kg diet, which is acceptable. For estimation of these values, expressed in mg/kg bw/day, the oral doses (in mg/kg bw/day) were calculated by the RMS from the mean body weight over the 8-day period (day 5 value for body weight was not determined) and the day 0-5 feed consumption data. According to this calculation, test diet concentrations of 1250 and 5000 mg/kg diet were equivalent to 179 and 636 mg/kg bw/day.

Conclusion

The 5-day dietary LC50 was >5000 mg/kg diet, equivalent to >636 mg/kg bw/day. The 5-day NOEC (based on clinical signs, body weight and feed consumption) was 1250 mg/kg diet, equivalent to 179 mg/kg bw/day.

Guidelines and limitations

(1) Guideline compliance was not claimed for this non-GLP study, conducted prior to coming into force of GLP-regulations. Generally however test procedures complied with OECD 205.

(2) The 5-day dietary LC50 and NOEC were expressed by the reviewer in mg/kg bw/day based on the reported feed consumption and the mean body weight (day 0-5).

(3) Analytically determined concentrations of etridiazole in untreated control diet from day 0 and 5 were 32-78 mg/kg (average 54 mg/kg diet). This does not compromise the study, in the absence of mortality to the highest test concentration.

(4) Analytically determined concentrations in the test diet from day 0 and 5 ranged between 83% and 132% of nominal. Information on the analytical method was limited to: extraction with hexane, filtration, GC-EC analysis. No concurrent validation was provided to demonstrate the suitability of the method

for the diet matrix. Therefore, the notifier was requested to provide this information. In response, notifier has indicated that the raw data could not be retrieved, since they were no longer available.
Since etridiazole has a high solubility in organic solvents (heptane), GC-EC analysis is a common method and the proposed post-registration method for etridiazole is extraction with hexane and GC-analysis, RMS sees no real reason to assume that the analytical method was not suitable. However, validation of the analytical method is a requisite for study acceptability. Therefore, RMS concludes that the study can be accepted as 'less reliable' (instead of 'not acceptable'), with the low exposure of birds that is expected for the proposed uses in mind, and to prevent unnecessary animal testing. This will also be clearly indicated in the List of endpoints. See further the text of the risk assessment under B.9.1.5.

Study 2

Characteristics

reference	:	Fletcher D.W. (1981b)	exposure duration	:	5 days
type of study	:	Dietary LC50 study	nominal	:	0, 313, 625, 1250, 2500 and 5000
year of execution	:	1980-1981	concentrations		mg/kg diet
GLP statement	:	No	dosing method	:	Diet mixed with Terrazole in corn oil
guideline	:	Not reported	acceptability	:	**Less reliable** (see text)
test substance	:	Terrazole^{R} Technical	LC50	:	1650 mg/kg diet ⇔ 286 mg/kg bw/day
purity	:	99%			
species	:	Mallard duck			

Methods

In a study on the dietary toxicity of Terrazole^{R} (etridiazole, purity 99%), five groups of 12-day old Mallard ducks of indeterminate sex (10/group) were fed Terrazole in the diet at analytically confirmed concentrations (see comment 3 below) of 313, 625, 1250, 2500 and 5000 mg/kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. Five control groups of ten 12-day old Mallard ducks received feed treated with the vehicle (corn oil).

Results

No birds died in the control groups and at 313 and 625 mg/kg diet, while 2, 9 and 10 birds died at 1250, 2500 and 5000 mg/kg, respectively. No clinical signs were noted up to 625 mg a.s/kg, but birds were lethargic after two and four days at and above 1250 mg/kg. Body weight and body weight gain were significantly reduced at all doses. Feed consumption was significantly reduced at and above 1250 mg/kg. Food conversion efficiency was reduced at 625 and 1250 mg/kg. At necropsy, liver lobes showed diffuse discoloration with a friable texture at and above 1250 mg/kg. Black diffuse discoloration of the intestinal tract, distention of the gall bladder and a reduction in the diameter of the intestinal tract were observed at 2500 and 5000 mg/kg.

The reported 5-day dietary LC50 was 1650 mg/kg diet, which is acceptable. For estimation of the 5-day dietary LC50, expressed in mg/kg bw/day, the oral doses (in mg/kg bw/day) were calculated by

the RMS from the mean body weight over the 8-day period (day 5 value for body weight was not determined) and the day 0-5 feed consumption data. According to this calculation, test diet concentrations of 313, 625, 1250, 2500 and 5000 mg/kg diet were equivalent to 87, 180, 267, 350 and 522 mg/kg bw/day. From the latter dose levels, and the mortality data, the LC50 value was estimated by the RMS using probit analysis (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition) to be 286 mg/kg bw/day (95% CI 262-331 mg/kg bw/day).
Based on significant reductions in body weight and body weight gain, the 5-day NOEC was <312 mg/kg diet, equivalent to <87 mg/kg bw/day.

Conclusion

The 5-day dietary LC50 was 1650 mg/kg diet, equivalent to 286 mg/kg bw/day; the 5-day NOEC was <313 mg/kg diet, equivalent to <87 bw/day.

Guidelines and limitations

(1) Guideline compliance was not claimed for this non-GLP study, conducted prior to coming into force of GLP-regulations. Generally however test procedures complied with OECD 205. The reported temperature during the study varied between 51 and 64°F, corresponding to 11 and 18°C. This is lower than the range specified by OECD 205 (28-32°C and 22-28°C for birds of 8-14 days and >14 days of age, respectively). Since this deviation may have resulted in an overestimation of the toxicity, the study result could be acceptable as a worst-case value.
(2) Analytically determined concentrations of etridiazole in untreated control diet from day 0 and 5 were 32-78 mg/kg (average 54 mg/kg diet). This does not compromise the study, in the absence of treatment related mortality up up to 625 mg/kg diet.
(3) Analytically determined concentrations in the test diet from day 0 and 5 ranged between 83% and 132% of nominal. Information on the analytical method was limited to: extraction with hexane, filtration, GC-EC analysis. No concurrent validation was provided to demonstrate the suitability of the method for the diet matrix.Therefore, the notifier was requested to provide this information. In response, notifier has indicated that the raw data could not be retrieved, since they were no longer available.
Since etridiazole has a high solubility in organic solvents (heptane), GC-EC analysis is a common method and the proposed post-registration method for etridiazole is extraction with hexane and GC-analysis, RMS sees no real reason to assume that the analytical method was not suitable. However, validation of the analytical method is a requisite for study acceptability. Therefore, RMS concludes that the study can be accepted as ‘less reliable’ (instead of ‘not acceptable’), with the low exposure of birds that is expected for the proposed uses in mind, and to prevent unnecessary animal testing. This will also be clearly indicated in the List of endpoints. See further the text of the risk assessment under B.9.1.5.

B.9.1.2.2 Dietary toxicity of the plant protection product

No data were submitted.

B.9.1.3 Long term/Reproductive toxicity (IIA 8.1.3)

Study 1

Characteristics

reference	:	Pedersen C.A. & Solatycki A.M. (1995a)	exposure duration	:	20 weeks
			nominal	:	First week:
type of study	:	Dietary reproductive toxicity study	concentrations		0, 75, 350 and 500 mg/kg diet Remainder of the study:
year of execution	:	1994			0, 50, 350 and 700 mg/kg diet
GLP statement	:	Yes	dosing method	:	Diet mixed with Terrazole
guideline	:	EPA 71-4	acceptability	:	Acceptable
test substance	:	Terrazole® Technical, Lot No. 203-S-026	NOEC reproduction	:	42 mg/kg diet ⇔ 4.1 and 4.2 mg a.s./kg bw/day for males and females, respectively
purity	:	97.9%			
species	:	Mallard duck			

Methods

In a reproductive toxicity study, 22-weeks-old Mallard ducks were exposed to Terrazole (etridiazole, purity 97.9%) in the diet for 20 weeks. During the first week, nominal diet concentrations were 0 (control), 75, 350 and 500 mg/kg diet. During the remainder of the exposure period, nominal diet concentrations were 0, 50, 350 and 700 mg/kg diet. After exposure, 75% of the birds were fed untreated diet for three more weeks. There were 16 pairs per dose level, one pair per cage. The birds were maintained in a controlled environment test room. In week 5, L-S 50 Water Soluble® Powder, an antibacterial and antimycoplasmal agent was administered in the drinking water at a rate of 0.53 grams/L for 4 days.

Eggs were collected daily from the onset of egg production (week 8). Hatchlings were leg banded and maintained for 14 days in cages according to pen. Reproduction endpoints were determined with eggs produced during the last 13 weeks of the study, and in addition with eggs produced during the 3 weeks of recovery following exposure. Reproduction endpoints were: number of eggs laid, number of eggs cracked, egg size, eggshell thickness, number of fertile eggs, number of live 21-day-old embryos, number of hatchlings, hatchling weight, number of 14-day-old survivors, and chick weights at day 14 of age. For eggshell thickness determination, eggs were collected on the first day of weeks 1, 3, 5, 7, 9, 11 and 13 throughout the egg laying period, and on the first day of week 2 during the recovery period.

Results

Three birds died before and one after onset of egg production during the exposure period of the study. The distribution of deaths among the treatment groups was two (control), one (50 mg/kg diet) and one (700 mg/kg diet). During the recovery period, one bird died at 50 and 350 mg/kg diet. Body weights were significantly reduced as compared to the control at 700 mg/kg diet during weeks 4, 6 and 8 (males) and 6, 8 and 20 (females), but not during the entire test period. Mean body weight change was reduced for females at 700 mg/kg diet (not statistically tested). There were no treatment-related effects on feed consumption, survival and behavioural endpoints. No substance related abnormalities were noted at necropsy. The table below summarises the study results.

Table B.9.4 Results of a reproductive study on Mallard duck (20-week exposure period)

Nominal concentration (mg/kg diet)(A)	0	50	350	700
Mean measured concentration (mg a.s./kg diet)	-(B)	42	312	623
Mean parental food consumption (g/bird/day)	117	117	111	111
Mean body weight change males (%)	+7.7	+5.0	+6.5	+4.6
Mean body weight change females (%)	+23	+17	+15	+7.3
Mean egg production (eggs/cage)	61	56	44*	39*
Total number of eggs set	777	831	648	547
Mean eggshell thickness (mm)	0.38	0.38	0.38	0.39
Percent of eggs laid that were cracked (%)	1	1	0	1
Number of eggs reduced in size of eggs per cage (%)	1	1	7	16
Number of normal eggs of eggs laid (%)	97	98	93	82*
Percent of eggs set that were fertile (%)	75	89	62	64
21-day embryos as percentage of fertile eggs (%)	78	81	78	77
21-day embryos as percentage of eggs set (%)	68	77	53	54
Number hatchlings as percent of 21-day embryos (%)	73	61	66	49*
Number hatchlings as percent of fertile eggs (%)	58	51	52	37
Number hatchlings as percent of eggs set (%)	50	48	36	25*
Number 14-day chicks as percent of hatchlings (%)	98	100	99	99
Number 14-day chicks as percent of fertile eggs (%)	67	58	62	42
Number 14-day chicks as percent of eggs set (%)	50	48	35	24*
Number 14-day chicks per hen	28	26	15*	10*
Mean body weight (g) of hatchlings	40	37*	34*	31*
Mean body weight (g) of 14-day chicks	295	286	263*	254*

(A) 0, 75, 350 and 500 mg/kg diet during the first week
(B) Not measured
* significantly different from control at 5% level

At 700 mg/kg diet, the number of smaller eggs was increased compared to the control (not statistically significant), resulting in a significant decrease in the number of normal eggs. At 700 mg/kg diet, the number of hatchlings as percentage of eggs set, fertile eggs and 21-day embryos was reduced, resulting in a decrease in the number of 14-day chicks as percentage of eggs set and fertile eggs. At 350 mg/kg diet, the number of hatchlings as percentage of eggs set was also reduced, although this did not reach statistical significance. At 350 and 700 mg/kg diet, the number of 14-day chicks per hen and the mean body weight of hatchlings and of 14-day chicks was significantly reduced. The author of the report was of the opinion that the significant decrease in mean body weight of hatchlings at 50 mg/kg diet was not related to treatment, since no statistical significance was noted in the body weights of 14-day chicks, since no other impairment of reproduction was noted at this level, since historical data for control hatchling body weights in seven out of twenty reproduction studies were lower than the value of 37.1 g at 50 mg/kg diet, and since the value of 37.1 g was within one standard

deviation of the control group's average of 39.9 g. Although no detailed historical control data were presented in the report for verification of this part of the author's statement, the NOEC of 50 mg/kg diet is accepted, since at the end of the 3-week recovery period a comparable difference in hatchling body weight existed between the birds of the 50 mg/kg group and the control (mean body weights were 40.0 and 37.1 g respectively), which was clearly due to biological variation in his case, since at 350 mg/kg diet a mean body weight of 38.5 g per hatchling was recorded.

The results at the end of the 3-week recovery period are shown in the table below.

Table B.9.5 Results of a reproductive study on Mallard duck (3-week recovery period)

Nominal concentration (mg/kg diet) (A)	0	50	350	700
Mean parental food consumption (g/bird/day)	120	124	122	132
Mean body weight change males (%)	+1.4	-0.5	+6.2	+4.4
Mean body weight change females (%)	-3.7	-1.4	-1.5	+2.7
Mean egg production (eggs/cage)	13	13	8	9
Total number of eggs set	119	133	89	93
Mean eggshell thickness (mm)	0.39	0.38	0.36	0.38
Percent of eggs laid that were cracked (%)	2	0	2	0
Number of eggs reduced in size of eggs per cage (%)	0	0	0	9
Number of normal eggs of eggs laid (%)	98	100	98	91
Percent of eggs set that were fertile (%)	74	95	47	43
21-day embryos as percentage of fertile eggs (%)	95	89	94	66
21-day embryos as percentage of eggs set (%)	70	85	45	36
Number hatchlings as percent of 21-day embryos (%)	94	83	95	63*
Number hatchlings as percent of fertile eggs (%)	88	77	90	47*
Number hatchlings as percent of eggs set (%)	66	74	43	27
Number 14-day chicks as percent of hatchlings (%)	99	100	100	100
Number 14-day chicks as percent of fertile eggs (%)	88	76	88	79
Number 14-day chicks as percent of eggs set (%)	65	74	43	27
Number 14-day chicks per hen	8	9	2*	2*
Mean body weight (g) of hatchlings	40	37	39	35*
Mean body weight (g) of 14-day chicks	307	292	304	265*

(A) 0, 75, 350 and 500 mg/kg diet during the first week
* significantly different from control at 5% level

During the recovery period, the reproductive effects were only shown to be partly reversible. The number of normal eggs was still reduced at the highest dose, although not statistically significant. The number of hatchlings as percentage of 21-day embryos, fertile eggs and eggs set was still reduced at at 700 mg/kg diet, and at 350 mg/kg diet the number of hatchlings as percentage of eggs set was still reduced (not statistically significant). The body weights of hatchlings and 14-day chicks at 700 mg/kg diet were still reduced, and so was the number of 14-day old chicks per hen at 350 and 700 mg/kg diet. The only effect that was entirely reversible was the reduction of hatchling and 14-day chick body weight at 350 mg/kg diet.

Conclusion

The NOEC in Mallard duck exposed to Terrazole in the diet was 50 mg/kg diet (mean measured concentration 42 mg a.s./kg diet, equivalent to 4.1 and 4.2 mg a.s./kg bw/day for males and females, respectively).

Guidelines and limitations

(1) The following results were calculated from the reported raw data by the reviewer:

- Mean measured concentration
- Percentage body weight change for adult birds
- Number of 14-day chicks as percentage of fertile eggs
- NOEC at 50 mg/kg diet nominal (42 mg a.s./kg diet mean measured), expressed as mg a.s./kg bw/day, based on mean measured Terrazole concentration, mean feed consumption for the whole group and mean body weights of males and females.

(2) The study was conducted according to EPA 71-4 (1982), in compliance with GLP, and is acceptable.

(3) The number of fertile eggs as percentage of eggs set for the control group was below the normal range described in the OECD 206 guideline (85-98%). Since the value for the lowest treatment was 87%, and there was no indication for any treatment related effect on reproduction at this dose, the slightly lower value for the control is accepted.

Study 2

Characteristics

reference	:	Pedersen C.A. & Solatycki A.M. (1995b)	exposure duration	:	22 weeks
			nominal	:	First week:
type of study	:	Dietary reproductive toxicity study	concentrations		0, 75, 350 and 500 mg/kg diet Remainder of the study:
year of execution	:	1994-1995			0, 50, 350 and 700 mg/kg diet
GLP statement	:	Yes	dosing method	:	Diet mixed with Terrazole
guideline	:	EPA 71-4	acceptability	:	Acceptable
test substance	:	Terrazole® Technical; Lot No. 203-S-026	NOEC reproduction	:	42 mg/kg diet ⇔ 4.1 and 3.7 mg/kg bw/day for males and females, respectively
purity	:	97.9%			
species	:	Bobwhite quail			

Methods

In a reproductive toxicity study, 22-weeks-old Bobwhite quail were exposed to Terrazole (etridiazole, purity 97.9%) in the diet for 22 weeks. During the first week, nominal diet concentrations were 0 (control), 75, 350 and 500 mg/kg diet. During the remainder of the exposure period, nominal diet concentrations were 0, 50, 350 and 700 mg/kg diet. After exposure, 75% of the birds were fed untreated diet for three more weeks. There were 16 pairs per dose level, one pair per cage. The birds were maintained in a controlled environment test room. Eggs were collected daily from the onset of egg production (week 12). Hatchlings were leg banded and maintained for 14 days in cages according to pen. Reproduction endpoints were determined with eggs produced during the last 11 weeks of the study, and in addition with eggs produced during the 3 weeks of recovery following exposure. Reproduction endpoints were: number of eggs laid, number of eggs cracked, egg size, eggshell

thickness, number of fertile eggs, number of live 18-day-old embryos, number of hatchlings, hatchling weight, number of 14-day-old survivors, and chick weights at day 14 of age. For eggshell thickness determination, eggs were collected on the first day of weeks 1, 3, 5, 7, 9 and 11 throughout the egg laying period, and on the first day of week 2 during the recovery period.

Results

Two birds died before and no bird died after onset of egg production during the exposure period of the study. The distribution of deaths among the treatment groups was one (50 mg/kg diet) and one (350 mg/kg diet). During the recovery period, one bird died at 350 mg/kg diet. Body weights were significantly reduced as compared to the control at 700 mg/kg diet during weeks 22 (males) and 2, 10 and 22 (females), but not during the entire test period. Mean body weight change was reduced for males and females at 700 mg/kg diet (not statistically tested). At 700 mg/kg diet, feed consumption was significantly reduced during weeks 2, 16, 18 and 22, but not for the entire test period. There were no treatment-related effects on survival and behavioural endpoints. No substance related abnormalities were noted at necropsy. The table below summarises the study results.

Table B.9.6 Results of a reproductive study on Bobwhite quail (22-week exposure period)

Nominal concentration (mg/kg diet)[A]	0	50	350	700
Mean measured concentration (mg a.s./kg diet)	-[B]	42	312	623
Mean parental food consumption (g/bird/day)	20	20	20	20
Mean body weight change males (%)	+6.1	+3.1	+4.5	-0.92
Mean body weight change females (%)	+25	+23	+22	+14
Mean egg production (eggs/cage)	54	53	55	53
Total number of eggs set	803	780	826	788
Mean eggshell thickness (mm)	0.22	0.22	0.23	0.22
Percent of eggs laid that were cracked (%)	0	2	0	0
Number of eggs reduced in size of eggs per cage (%)	0	0	5	55
Number of normal eggs of eggs laid (%)	99	96	94*	44*
Percent of eggs set that were fertile (%)	93	92	85	22*
18-day embryos as percentage of fertile eggs (%)	99	94	93*	72*
18-day embryos as percentage of eggs set (%)	91	88	80	16*
Number hatchlings as percent of 18-day embryos (%)	93	97	78*	32*
Number hatchlings as percent of fertile eggs (%)	92	90	71*	23*
Number hatchlings as percent of eggs set (%)	85	85	61*	4*
Number 14-day chicks as percent of hatchlings (%)	92	95	70*	0*
Number 14-day chicks as percent of fertile eggs (%)	85	88	52	0
Number 14-day chicks as percent of eggs set (%)	78	81	44*	0*
Number 14-day chicks per hen	39	40	23*	0*
Mean body weight (g) of hatchlings	7.1	7.1	5.6*	4.5*
Mean body weight (g) of 14-day chicks	31	31	26*	-

(A) 0, 75, 350 and 500 mg a.s./kg diet during the first week
(B) Not measured
* significantly different from control at 5% level

There was an increase in the number of smaller eggs, resulting in a statistically significant decrease in the number of normal eggs at 350 and 700 mg/kg diet compared to the control. All other reproductive parameters (egg fertility, embryo hatchability, hatchling survival and hatchling and 14-day chick body weight) were also reduced at the two highest concentrations. At 700 mg/kg diet, no chicks at all survived until 14 days.

The results at the end of the 3-week recovery period are shown in the table below.

Table B.9.7 Results of a reproductive study on Bobwhite quail (3-week recovery period)

Nominal concentration (mg/kg diet) (A)	0	50	350	700
Mean parental food consumption (g/bird/day)	27	28	26	26
Mean body weight change males (%)	+1.3	-0.58	+2.8	+3.9
Mean body weight change females (%)	-4.7	-1.0	+0.63	-1.0
Mean egg production (eggs/cage)	19	20	18	20
Total number of eggs set	220	208	187	230
Mean eggshell thickness (mm)	0.22	0.22	0.22	0.22
Percent of eggs laid that were cracked (%)	0	2	1	0
Number of eggs reduced in size of eggs per cage (%)	0	0	0	95
Number of normal eggs of eggs laid (%)	99	97	98	4*
Percent of eggs set that were fertile (%)	90	99	80	28*
18-day embryos as percentage of fertile eggs (%)	96	95	89	57*
18-day embryos as percentage of eggs set (%)	86	94	73	21*
Number hatchlings as percent of 18-day embryos (%)	91	93	89	78*
Number hatchlings as percent of fertile eggs (%)	88	89	79	43*
Number hatchlings as percent of eggs set (%)	79	88	65	15*
Number 14-day chicks as percent of hatchlings (%)	92	94	77	55*
Number 14-day chicks as percent of fertile eggs (%)	80	85	61	35
Number 14-day chicks as percent of eggs set (%)	73	83	48*	10*
Number 14-day chicks per hen	13	16	9*	2*
Mean body weight (g) of hatchlings	7.5	7.5	5.9*	5.1*
Mean body weight (g) of 14-day chicks	31	31	26*	24*

(A) 0, 75, 350 and 500 mg a.s./kg diet during the first week
* significantly different from control at 5% level

During the recovery period, the effects at 700 mg a.s./kg diet were not reversible. The reproductive effects at 350 mg a.s./kg diet were only partly reversible: the number of 14-day chicks, and the body weights of hatchlings and 14-day chicks were still significantly reduced, whilst some other parameters (e.g. egg fertility) were still reduced without statistical significance.

Conclusion

The NOEC in Bobwhite quail exposed to Terrazole in the diet was 50 mg a.s./kg diet (mean measured concentration 42 mg a.s./kg diet, equivalent to 4.1 and 3.7 mg a.s./kg bw/day for males and females, respectively).

Guidelines and limitations

(1) The following results were calculated from the reported raw data by the reviewer:

- Mean measured concentration
- Percentage body weight change for adult birds
- Number of 14-day chicks as percentage of fertile eggs
- NOEC at 50 mg/kg diet nominal (42 mg a.s./kg diet mean measured), expressed as mg a.s./kg bw/day, based on mean measured Terrazole concentration, mean feed consumption for the whole group and mean body weights of males and females.

(2) The study was conducted according to EPA 71-4 (1982), in compliance with GLP, and is acceptable.

B.9.1.4 Summary

Less reliable acute oral toxicity studies with etridiazole were submitted, from which the lowest LD50 was 560 mg a.s./kg bw (bobwhite quail).

Less reliable short-term dietary toxicity studies with etridiazole were supplied, from which the lowest LC50 was 286 mg a.s./kg bw/d (mallard duck).

Reproductive toxicity studies with etridiazole in mallard duck and bobwhite quail were submitted, in which there were no effects on reproductive parameters at 50 mg/kg diet, equivalent to 4.1 mg a.s./kg bw/day (males) and 4.2 and 3.7 mg a.s./kg bw/day (females), respectively.

B.9.1.5 Risk assessment for birds

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC (Working Document Sanco/4145/2000, Final 25 September 2002).

B.9.1.5.1 Risk of active substance for birds

Routes of exposure

Exposure of soil is not relevant for the intended uses in glasshouses with application through (drip-) irrigation on substrate. Therefore exposure of birds via contaminated soil is considered to be irrelevant for these uses. An accepted EU scenario for emission from greenhouses to surface water is not available. Exposure of surface water for greenhouse applications was based on an emission rate to surface water of 0.1% of the applied dose, which is the emission rate percentage used by The Netherlands,

For the greenhouse applications in ornamental crops, tomato, pepper and cucumber, the routes of exposure to birds are therefore considered to be:

- consumption of surface water containing residues of etridiazole (acute/short-term);
- consumption of fish contaminated with residues of etridiazole (long-term).

Acute/short term risk assessment (drinking water)

The acute/short term risk for birds as a result of consumption of water containing residues is performed based on the available less reliable acute and short-term toxicity data. The lowest endpoint,

the LC50 of 286 mg a.s./kg bw/d, is used for risk assessment. RMS considers the use of the less reliable endpoint acceptable, since there is no real reason to assume that the analytical method was not suitable (see B.9.1.2.1) and the expected exposure of birds for the proposed uses is very low, resulting in a very large margin of safety.

The total water ingestion rate is based on a small bird of 10 g body weight. This leads to a total water ingestion rate of $0.059 \times 0.010^{0.67} = 2.7 \times 10^{-3}$ L water/d. The daily dose of the active substance etridiazole is based on the highest actual PECsw of 2.343 µg a.s./L (ornamentals, see fate section B.8.6.1) and is calculated to be $(2.343 \times 2.7 \times 10^{-3})/0.010 = 0.63$ µg a.s./kg bw/d. based on the LC50 of 286 mg a.s./kg bw/d the TER for a small bird drinking contaminated surface water amounts 286000/0.63 = 452095. This TER is far above the trigger value of 10, which addresses the possible uncertainty associated with the lesser reliability of the endpoint.

Based on the above, the acute and short term risk for birds as a result of drinking contaminated surface water is considered acceptable.

Long-term risk assessment (bioaccumulation and food chain behaviour)

Fish

Long-term risk assessment is based on a fish-eating bird weighing 1000 g with a daily food intake (DFI) of 206 g fish/day. The BCF of etridiazole for whole fish is 165 L/kg wet weight (section B.9.2.3). Residue levels in fish were calculated from the 21-day TWA PECsw (0.253, 0.020 and 0.010 µg/L, respectively, for the application in ornamentals, tomatoes/peppers and cucumbers, see section B.8.6.1.) as follows: PEC_{FEED} = PECfish = PECsw*BCFfish.

The NOEC used for risk assessment is 3.7 mg/kg bw/day. The ETE for the long-term time scale is calculated as PEC*DFI/BW. Residue levels in fish and ETE and TERlt values are presented in Table B.9.8.

Table B.9.8 Long-term Toxicity Exposure Ratios for exposure of birds to etridiazole due to consumption of contaminated fish

appln.	Dose (kg as/ha)	NOEC (mg/kg bw/d)	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt
Ornamentals	2 x 7.0	3.7	0.04	0.01	430
Tomatoes/peppers	2 x 0.56	3.7	3E-03	7E-04	5443
Cucumbers	2 x 0.28	3.7	2E-03	3E-04	10886

TERlt values for consumption of fish in Table B.9.8 are above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk to birds as a result of bio-accumulation in fish is considered to be acceptable.

Bio-magnification in terrestrial food chains

The bioaccumulation potential reported in the List of Endpoints of the Toxicology section is stated to be low. Hence, according to the guidance provided in SANCO/4145/2000 – final (25 September 2002), the risk for biomagnification in terrestrial food chains is low.

B.9.1.5.2 Risk of metabolites for birds

According to the the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), the risk of metabolites to birds should be addressed.

B.9.1.5.2.1 Major metabolites

Etridiazole acid

Etridiazole acid was the only major (>10%) metabolite in water (maximum in water 13%, see section B.8.4), which was also found in rat (20-36% AR in 0-24 h urine, section B.6.1), orally dosed with parent etridiazole. Etridiazole acid was also a major metabolite in soil (maximum 31%, section B.8.1). No data however are available on the metabolism of etridiazole, and hence on the occurrence of etridiazole acid, in birds, but it is likely to be formed also in birds dosed with parent etridiazole. Data on acute oral toxicity of etridiazole acid in birds was not submitted. This metabolite is of low acute toxicity to rat (acute oral LD50 >2000 mg/kg bw). The acute toxicity of this metabolite to aquatic organisms was a factor of about 100 lower than that of parent etridiazole (LC50 trout >100 mg/L *versus* 2.4 mg/L; EC50 daphnia 350 mg/L *versus* 3.1 mg/L; EbC50 algae 27 mg/L *versus* 0.30 mg/L). The acute toxicity of this metabolite to earthworms was also lower than that of etridiazole (LC50 >1000 mg/kg *versus* 198 mg a.s./kg for the plant protection product).

Based on the above and in view of the large margin of safety that was calculated for the parent in both the short- and long-term risk assessment, the risk for birds from etridiazole acid is considered as low. In addition, the experimental logPow of etridiazole acid is 0.7 (section B.2.1), and hence <3, therefore long-term risk to birds due to consumption of fish contaminated with etridiazole acid is considered to be acceptable.

B.9.1.5.2.2 Minor metabolites

According to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), minor (<10%) metabolites only have to be considered in exceptional cases (e.g. if containing the active moiety of the molecule), and a qualitative approach can be used.

Dichloro-etridiazole

Dichloro-etridiazole was a minor metabolite in a water-sediment study (max. 9.5% AR in water, see section B.8.4), which was not found in rat (section B.6.1) orally dosed with etridiazole. No data are available on its occurrence in birds dosed with etridiazole. Data on acute oral toxicity of dichloro-etridiazole in birds and mammals was not submitted. The acute toxicity of this metabolite to fish and daphnia was a factor of about 3 higher than that of parent etridiazole (LC50 trout 0.77 mg/L *versus* 2.4 mg/L; EC50 daphnia 1.1 mg/L *versus* 3.1 mg/L), whilst its toxicity to algae was a factor of about 2 lower (EbC50 algae 0.62 mg/L *versus* 0.30 mg/L). On the basis of this information dichloro-etridiazole still possesses the active moiety.

However, in the acute risk assessment for the parent, a TER-value of 452095 was calculated, which means that the metabolite dichloro-etridiazole should be over a factor 45000 more toxic than the parent to show a risk (TER below 10). This is considered unlikely. For the long term risk assessment this factor should be over 86, which is also considered unlikely, and in addition the estimated logPow of dichloro-etridiazole is 2.7 (Table B.9.1), indicating a low potential for bioaccumulation.

Therefore, the risk for birds as a result of exposure to dichloro-etridiazole is considered acceptable.

Monochloro-etridiazole

Monochloro-etridiazole was a minor (<10%) metabolite in a water-sediment study (≤4.0% AR in any compartment, see section B.8.4), which was not found in rat (section B.6.1), orally dosed with parent etridiazole. No data are available on its occurrence in birds dosed with etridiazole. Data on the toxicity of this metabolite to birds, mammals and other aquatic or terrestrial organisms was not submitted. Monochloro-etridiazole may still possess the active moiety.

For this metabolite the same line of reasoning can be followed as for dichloro-etrididazole (large margin of safety calculated for the parent). In addition the estimated logPow of monochloro-etridiazole is 2.5 (Table B.9.1), indicating a low potential for bioaccumulation. The risk is considered acceptable.

3-Hydroxymethyl-etridiazole

3-Hydroxymethyl-etridiazole was a minor metabolite in a water-sediment study (≤3.4% AR in any compartment, see section B.8.4), which was not found in rat (section B.6.1), orally dosed with parent etridiazole. No data are available on its occurrence in birds dosed with etridiazole. Data on the toxicity of this metabolite to birds, mammals and other aquatic or terrestrial organisms was not submitted. This metabolite has lost the last chlorine atom, and is structurally more closely related to etridiazole acid than to parent etridiazole. Its toxicity profile is therefore likely to resemble that of etridiazole acid, rather than that of parent etridiazole.

For this metabolite the same line of reasoning can be followed as for etrididazole-acid (large margin of safety calculated for the parent). In addition the estimated logPow of 3-Hydroxymethyl-etridiazole is 0.78 (Table B.9.1), indicating a low potential for bioaccumulation. The risk is considered acceptable.

B.9.1.6 Risk assessment for mammals

Procedures for risk assessment were identical to those for birds (see B.9.1.5), with the exception of the aspects indicated below.

B.9.1.6.1 Risk of active substance for mammals

Acute risk assessment (standard exposure scenario)

The acute risk assessment of exposure to etridiazole via intake of contaminated surface water is based on a small mammal weighing 10 g, with a daily water intake (DWI) of 1.6 mL/day. The lowest acute LD50 is 945 mg a.s./kg bw for rats (see section B.6). The estimated theoretical exposure (ETE) for the acute time scale is calculated as PECsw*DWI/BW. The PECsw is the initial PECsw value (FOCUS Step 2) taken from Section B.8.6.1. Residue levels in water, and ETE and TERa values are presented in Table B.9.9.

Table B.9.9 Acute Toxicity Exposure Ratios for exposure of mammals to etridiazole due to consumption of contaminated drinking water

appln.	dose (kg as/ha)	LD50 (mg/kg bw)	Route	PEC_{WATER} (mg/L)	ETE (mg/kg bw/d)	TERa
Ornamentals	2 x 7	945	Water	2E-03	4E-04	3E+06
Tomatoes/peppers	2 x 0.56	945	Water	2E-04	3E-05	3E+07
Cucumbers	2 x 0.28	945	Water	9E-05	1E-05	6E+07

TERa values in Table B.9.9 are all far above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to mammals is considered to be acceptable.

Long-term risk assessment (bioaccumulation and food chain behaviour)

Fish

Long-term risk assessment is based on a fish-eating mammal weighing 3000 g with a daily food intake (DFI) of 390 g fish/day. PEC calculations were identical to those for exposure of birds to fish (see B.9.1.5.1).

The NOEC is taken to be 5.3 mg/kg bw/day, originating from the 2-generation study in rat (see section B.6), and is based on decreased pup weight of the first generation offspring. The estimated theoretical exposure (ETE) for the long-term time scale is calculated as PEC*DFI/BW. Residue levels in fish and ETE and TERlt values are presented in Table B.9.10.

Table B.9.10 Long-term Toxicity Exposure Ratios for exposure of mammals to etridiazole due to consumption of contaminated fish

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERlt

Ornamentals	2 x 7	5.3	Fish	0.04	0.01	977
Tomatoes/peppers	2 x 0.56	5.3	Fish	3E-03	4E-04	1.E+04
Cucumbers	2 x 0.28	5.3	Fish	2E-03	2E-04	2.E+04

TERlt values for consumption of contaminated fish in Table B.9.10 are far above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk to mammals due to consumption of contaminated fish is considered to be acceptable.

B.9.1.6.2 Risk of plants metabolites for mammals

B.9.1.6.2.1 Major metabolites

Etridiazole acid

Etridiazole acid was the only major (>10%) metabolite in water (maximum in water 13%, see section B.8.4), which was also found in rat (20-36% AR in 0-24 h urine, section B.6.1), orally dosed with parent etridiazole. Etridiazole acid was also a major metabolite in soil (maximum 31%, section B.8.1). This metabolite is of low acute toxicity to rate (acute oral LD50 >2000 mg/kg bw).

Acute risk

Since the acute risk of etridiazole to mammals was considered acceptable (TERa ≥3E+06), the initial PECsw of etridiazole acid is 6 times lower than that of etridiazole, and the acute toxicity of this metabolite is 2 times lower than that of etridiazole, the acute risk of this metabolite to mammals is considered to be acceptable.

Long-term risk

Since the experimental logPow of etridiazole acid is 0.7 (section B.2.1), and hence <3, the long-term risk to mammals due to consumption of fish contaminated with etridiazole acid is considered to be acceptable.

B.9.1.6.2.2 Minor metabolites

Dichloro-etridiazole

Dichloro-etridiazole was a minor metabolite in a water-sediment study (max. 9.5% AR in water, section B.8.4), which was not found in rat (section B.6.1) orally dosed with etridiazole. Therefore, the toxicity of this metabolite has not been covered by the studies with the parent compound. Data on acute toxicity of dichloro-etridiazole in birds and mammals was not submitted. The acute toxicity of this metabolite to fish and daphnia was a factor of about 3 higher than that of parent etridiazole (LC50 trout 0.77 mg/L *versus* 2.4 mg/L; EC50 daphnia 1.1 mg/L *versus* 3.1 mg/L), whilst its toxicity to algae was a factor of about 2 lower (EbC50 algae 0.62 mg/L *versus* 0.30 mg/L). On the basis of this information dichloro-etridiazole still possesses the active moiety.

Acute risk

Since no toxicity data are available for this metabolite, the LD50 needed to reach TERa<10 is calculated from the initial PECsw (see section B.8.6.1). Residue levels in water and ETE values are presented in Table B.9.11, together with the LD50 that would result in TERa<10.

Table B.9.11 PECwater and ETE values for exposure of mammals to dichloro-etridiazole due to consumption of contaminated drinking water, and LD50 values needed to reach TER<10

appln.	Dose (kg as/ha)	PEC_{WATER} (mg/L)	ETE (mg/kg bw/d)	LD50 (mg/kg bw/d)	Factor more toxic than etridiazole
Ornamentals	2 x 7.0	2E-04	3E-05	3E-04	3E+06
Tomatoes/peppers	2 x 0.56	2E-05	2E-06	2E-05	4E+07
Cucumbers	2 x 0.28	8E-06	1E-06	1E-05	8E+07

Table B.9.11 shows that the TERa of the metabolite would only be below the trigger of 10 if the acute toxicity would exceed that of parent etridiazole by a factor of at least 3E+06. This is considered unlikely, and the risk is considered to be low for these applications.

Long-term risk

Since the estimated logPow of dichloro-etridiazole is 2.7 (Table B.9.1), and hence <3, the long-term risk to mammals due to consumption of fish contaminated with dichloro-etridiazole is considered to be acceptable.

Monochloro-etridiazole

Monochloro-etridiazole was a minor (<10%) metabolite in a water-sediment study (≤4.0% AR in any compartment, see section B.8.4), which was not found in rat (section B.6.1), orally dosed with parent etridiazole. Therefore, the toxicity of this metabolite has not been covered by the studies with the parent compound. Data on the toxicity of this metabolite to birds, mammals and other aquatic or terrestrial organisms was not submitted. Monochloro-etridiazole may still possess the active moiety.

Acute risk

The maximum residue level of this metabolite in water, corrected for the difference in molecular mass with parent etridiazole and for the maximum percentage of formation, will be a factor of 35 lower than for the parent. The TERa of the metabolite would only be below the trigger value of 10 if the acute toxicity would exceed that of parent etridiazole by a factor of at least 9E+06. This is considered unlikely, and the acute risk is considered to be low.

Long-term risk

Since the estimated logPow of monochloro-etridiazole is 2.5 (Table B.9.1), and hence <3, the long-term risk to mammals due to consumption of fish contaminated with monochloro-etridiazole is considered to be acceptable.

3-Hydroxymethyl-etridiazole

3-Hydroxymethyl-etridiazole was a minor metabolite in a water-sediment study (≤3.4% AR in any compartment, see section B.8.4), which was not found in rat (section B.6.1), orally dosed with parent etridiazole. Therefore, the toxicity of this metabolite has not been covered by the studies with the parent compound. Data on the toxicity of this metabolite to birds, mammals and other aquatic or terrestrial organisms was not submitted. This metabolite has lost the last chlorine atom, and is structurally more closely related to etridiazole acid than to parent etridiazole. Its toxicity profile is therefore likely to resemble that of etridiazole acid, rather than that of parent etridiazole.

Acute risk

The maximum residue level of this metabolite in water, corrected for the difference in molecular mass with parent etridiazole and for the maximum percentage of formation, will be a factor of 45 lower than for the parent. The TERa of the metabolite would only be below the trigger value of 10 if the acute toxicity would exceed that of parent etridiazole by a factor of at least 1E+07. This is considered unlikely, and the acute risk is considered to be low.

Long-term risk

Since the estimated logPow of 3-hydroxymethyl-etridiazole is 0.78 (Table B.9.1) and hence <3, the long-term risk to mammals due to consumption of fish contaminated with 3-hydroxymethyl-etridiazole is considered to be acceptable.

B.9.2 Effects on aquatic organisms (IIA 8.2, IIIA 10.2)

B.9.2.1 Acute toxicity to aquatic life (IIA 8.2)

Studies were conducted in compliance with relevant OECD and/or EC guidelines, unless stated.

B.9.2.1.1 Acute toxicity of the active substance

Study 1

Characteristics

reference	:	Dionne E. (2002a)	exposure duration	:	96 hours
type of study	:	Acute toxicity study	nominal concentrations		0, 1.3, 2.2, 3.6, 6.0 and 10 mg/L
year of execution	:	2002			
GLP statement	:	yes	exposure regime	:	Flow-through, stock solution in acetone
guideline	:	OECD 203, OPPTS 850.1075, EEC C1	acceptability	:	Acceptable
test substance	:	Etridiazole, Lot no. 1RC012320	LC50	:	2.4 mg/L
purity	:	97.9%			
species	:	Rainbow trout (*Oncorhynchus mykiss*)			

Methods

A 96-hour acute toxicity test on rainbow trout (*Oncorhynchus mykiss*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with etridiazole at nominal test concentrations of 1.3, 2.2, 3.6, 6.0 and 10 mg/L, with untreated and solvent-control. Based on pretest results, where measured concentrations of etridiazole between sampling intervals were not consistent due to precipitation, samples of test solutions from the main study were centrifuged prior to analysis of etridiazole.

Results

The measured concentrations were 0.42, 1.1, 1.5, 2.9 and 4.6 mg/L at test initiation (representing 32-50% of nominal), and 0.65, 1.4, 2.1, 4.3 and 4.7 mg/L at the end of exposure (representing 47-72% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters were: temperature (12-14°C), dissolved oxygen (≥8.2 mg/L) and pH (6.8-7.3). The results are summarised in Table B.9.12.

Table B.9.12 The acute toxicity of etridiazole to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in mg/L	NOEC mg/L
Oncorhynchus mykiss	Flow-through 96 hours (97.9%)	32-72	24 h: > 4.7(A) 48 h: 4.0(A) 72 h: 3.8(A) 96 h: 2.4(A)	24 & 48 h: 1.8(A) 72 & 96 h: 1.3(A)

(A) Based on mean measured concentrations.

Conclusion

96-hour LC50: 2.4 mg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 2

Characteristics

reference	:	Machado M.W. (1993a)	exposure duration	:	96 hours
type of study	:	Acute toxicity study	nominal concentrations	:	0, 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L
year of execution	:	1993			
GLP statement	:	yes	dosing method	:	Flow-through, stock solution in acetone
guideline	:	EPA 72-3			
test substance	:	Terrazole Technical (etridiazole), Lot # 203S026	acceptability LC50	: :	Acceptable 4.0 mg/L
purity	:	97.9%			
species	:	Sheepshead minnow (*Cyprinodon variegatus*)			

Methods

A 96-hour acute toxicity test on sheepshead minnow (*Cyprinodon variegatus*) (2 replicates of ten fish each per concentration) was conducted under flow-through conditions with Terrazole Technical (etridiazole) at nominal test concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations in the duplicate vessels were 87-100% of nominal at 0 hour and 54-95% at 96 hours (overall means were 80-94% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters were: temperature (21-22°C), dissolved oxygen (≥6.0 mg/L) and pH (7.8-8.0). The results are summarised in Table B.9.13.

Table B.9.13 The acute toxicity of Terrazole Technical (etridiazole) to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in mg/L	NOEC mg/L
Cyprinodon variegatus	Flow-through 96 hours (97.9%)	87-100 (0 h) 54-95% (96 h)	24, 48, 72 h: > 4.0(A) 96 h: 4.0(A)	24, 48, 72 & 96 h: 0.91(A)

(A) Based on mean measured concentrations.

Conclusion

96-hour LC50: 4.0 mg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 3

Characteristics

reference	:	Dionne E. (2002b)	species	:	*Daphnia magna*
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2002	nominal concentrations	:	0, 0.94, 1.9, 3.8, 7.5 and 15 mg/L
GLP statement	:	Yes			
guideline	:	EEC C2, OECD 202, OPPTS 850.1010	dosing method	:	Flow-through, stock solution in acetone
test substance	:	Etridiazole, Lot No. 1RC012320	acceptability	:	Acceptable
purity	:	97.9%	EC50	:	3.1 mg/L

Methods

A 48-hour immobilisation test on *Daphnia magna* (2 replicates of 10 Daphnia each per concentration) was conducted under flow-through conditions with etridiazole at nominal test concentrations of 0.94, 1.9, 3.8, 7.5 and 15 mg/L, with untreated and solvent-control. Based on pretest results, where measured concentrations of etridiazole in centrifuged samples were 18% lower than in uncentrifuged samples, samples of test solutions from the main study were centrifuged prior to analysis of etridiazole.

Results

The measured concentrations were 0.60, 1.6, 1.9, 3.8 and 7.8 mg/L at test initiation (representing 50-84% of nominal), and 0.51, 1.5, 2.1, 4.0 and 9.2 mg/L at the end of exposure (representing 53-79% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters were: temperature (19-21°C), dissolved oxygen (≥7.9 mg/L) and pH (7.2-7.5). The results are summarised in Table B.9.14.

Table B.9.14 The acute toxicity of etridiazole to aquatic invertebrates

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in mg/L (95% CL)	NOEC mg/L
Daphnia magna	Flow-through 48 hours (97.9%)	50-84	24 h: 6.3(A) 48 h: 3.1(A)	24 h: 3.9(A) 48 h: 2.0(A)

(A) Based on mean measured concentrations.

Conclusion

48-hour EC50: 3.1 mg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 4

Characteristics

reference	:	Machado M.W. (1993b)	species	:	*Mysidopsis bahia* (marine shrimp)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	1992	nominal concentrations	:	0, 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L
GLP statement	:	Yes			
guideline	:	EPA 72-3	dosing method	:	Flow-through, stock solution in acetone
test substance	:	Terrazole Technical (etridiazole), Lot # 203S026	acceptability	:	Acceptable
purity	:	97.92%	LC50	:	2.5 mg/L

Methods

A 96-hour toxicity test on the salt water shrimp *Mydisopsis bahia* (2 retention chambers with 5 shrimps per replicate aquarium, 2 aquariums per concentration) was conducted under flow-through conditions with Terrazole Technical (etridiazole) at nominal test concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations (range for both replicate test aquariums) were 0.61-0.64, 0.34-0.97, 1.2-1.3, 2.4-2.8 and 4.5-4.5 mg/L at test initiation (representing 54-96% of nominal), and 0.57-0.61, 0.44-0.74, 1.1-1.4, 2.3-2.4 and 4.1-4.2 mg/L at the end of exposure (representing 61-98% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters were: temperature (24-25°C), dissolved oxygen (≥6.4 mg/L), pH (6.4-7.5) and salinity (31-32‰). The results are summarised in Table B.9.15.

Table B.9.15 The acute toxicity of Terrazole Technical (etridiazole) to invertebrates

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in mg/L	NOEC mg/L
Mysidopsis bahia (marine organism)	Flow-through 96 hours (97.92%)	54-98	24 h: 3.6(A) 48 h: 3.3(A) 72 h: 3.0(A) 96 h: 2.5(A)	24, 48, 72 & 96 h: 0.61(A)

(A) Based on mean measured concentrations.

Conclusion

96-hour LC50: 2.5 mg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 5

Characteristics

reference	:	Dionne E. (1993)	species	:	*Crassostrea virginica*
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	1993	nominal concentrations	:	0, 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L
GLP statement	:	Yes			
guideline	:	EPA 72-3	dosing method	:	Flow-through, stock solution in acetone
test substance	:	Terrazole Technical (etridiazole), Lot # 203S026	acceptability	:	Acceptable
purity	:	97.92%	LC50	:	3.0 mg/L

Methods

A 96-hour toxicity test on *Crassostrea virginica* (2 replicates of 20 oysters each per concentration) was conducted under flow-through conditions with Terrazole Technical (etridiazole) at nominal test concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations (range for both replicate test aquariums) were 0.49-0.50, 0.86-0.91, 1.3-1.3, 2.2-2.3 and 3.5-3.6 mg/L at test initiation (representing 70-83% of nominal), and 0.56-0.57, 1.0-1.0, 1.5-1.6, 2.7-2.7 and 3.8-4.4 mg/L at the end of exposure (representing 76-91% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters were: temperature (18-20°C), dissolved oxygen (≥6.5 mg/L), pH (7.6-8.0) and salinity (31‰). The results are summarised in Table B.9.16.

Table B.9.16 The acute toxicity of Terrazole Technical (etridiazole) to invertebrates

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in mg/L	NOEC mg/L
Crassostrea virginica (marine organism)	Flow-through 96 hours (97.92%)	70-91	96 h: 3.0 (2.4-3.7)(A)	96 h: 0.94(A)

(A) Based on mean measured concentrations.

Conclusion

96-hour EC50: 3.0 mg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 6

Characteristics

reference	:	Hoberg J.R. (1993a)	exposure duration	:	120 hours
type of study	:	Algal growth inhibition test	nominal	:	0, 0.0024, 0.0081, 0.027, 0.090,
year of execution	:	1993	concentrations		0.30 and 1.0 mg/L
GLP statement	:	Yes	dosing method	:	Static, stock solution in acetone
guideline	:	EPA 122-2, 123-3	acceptability	:	Acceptable
test substance	:	Terrazole Technical (etridiazole), Lot	72h-E_bC_{50}	:	0.30 mg/L
		# 203S026	72h-E_rC_{50}	:	>1.0 mg/L
purity	:	97.92%	72h-NOE_bC	:	0.027 mg/L
species	:	*Selenastrum capricornutum*	72h-NOE_rC	:	0.027 mg/L

Methods

A 120-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per concentration, each containing 0.3 x 10^4 cells/mL at the start) was conducted with Terrazole Technical (etridiazole) at nominal test concentrations of 0.0024, 0.0081, 0.027, 0.090, 0.30 and 1.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations were 0.0020, 0.0094, 0.035, 0.092, 0.29 and 0.95 mg/L at test initiation (representing 83-128% of nominal), which declined to 0.0020, 0.0066, 0.020, 0.066, 0.21 and 0.73 mg/L at the end of exposure (representing 69-82% of nominal). Water quality parameters were: temperature (24-25°C), pH (7.5-9.5), conductivity (80-90 µmhos/cm).

Reported EC50 values (0.39, 0.31 and 0.072 mg/L after 72, 96 and 120 hours respectively) and the 120-hour NOEC value (0.0020 mg/L) were based on absolute values for cell density instead of the area under the growth curve and the specific growth rates as detailed by OECD 202. The 72-hour EC50 and NOEC values for growth inhibition and specific growth rate reduction were calculated by the RMS following the methods in OECD 202, based on the reported raw data and using Toxstat Release 3.5, 1996. Since the initial concentrations were >80% of the nominal concentrations, endpoints were based on nominal concentrations, which is in agreement with the recommendations in the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev. 4 (final) of 17 October 2002). The results are summarised in Table B.9.17.

Table B.9.17 The acute toxicity of Terrazole Technical (etridiazole) to algae

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC50 in mg a.s./L	NOEC mg a.s./L
Selenastrum capricornutum (green alga)	Static 120 hours (97.92%)	69-128	72h-E_bC_{50} 0.30[(A)] 72h-E_rC_{50} >1.0 [(A)]	72h-NOE_bC & 72h-NOE_rC: 0.027[(A)]

(A) Based on nominal concentrations. See also comment below.

Conclusion

72-hour E_bC_{50} and E_rC_{50}: 0.30 and >1.0 mg/L, respectively; 72-hour NOE_bC and NOE_rC: 0.027 mg/L, all based on nominal concentrations.

Guidelines and limitations

The test is acceptable.

Study 7

Characteristics

reference	:	Hoberg J.R. (1993b)	exposure duration	:	120 hours
type of study	:	Algal growth inhibition test	nominal	:	0, 0.063, 0.13, 0.25, 0.50 and
year of execution	:	1993	concentrations		1.0 mg/L
GLP statement	:	Yes	dosing method	:	Static, stock solution in acetone
guideline	:	EPA 122-2, 123-3	acceptability	:	Acceptable
test substance	:	Terrazole Technical (etridiazole), Lot	120h-EbC50	:	0.42 mg/L
		# 203S026	120h-ErC50	:	>1.0 mg/L
purity	:	97.92%	120h-NOEbC	:	0.063 mg/L
species	:	*Anabaena flos-aquae*	120h-NOErC	:	0.063 mg/L

Methods

A 120-hour toxicity test on blue-green algae (*Anabaena flos-aquae*) (3 replicates per concentration, each containing 1.0 x 10^4 cells/mL at the start) was conducted with Terrazole Technical (etridiazole) at nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations were 0.062, 0.13, 0.26, 0.46 and 0.92 mg/L at test initiation (representing 92-100% of nominal), which declined to 0.051, 0.11, 0.19, 0.35 and 0.74 mg/L at the end of exposure (representing 69-88% of nominal). Reported endpoints were based on mean measured concentrations, which is however not in agreement with the recommendations in the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev. 4 (final) of 17 October 2002).

Cell growth in the control was somewhat irregular during the test, with 24-hour intervals of rapid growth alternating with 24-hour intervals of slower growth. The most sensitive endpoints were obtained by evaluation of the 120-hour test results. The 120-hour EC50 and NOEC values for growth inhibition and specific growth rate reduction were calculated by the RMS following the methods in OECD 202, based on the reported raw data and using Toxstat Release 3.5, 1996. Since the initial concentrations were >80% of the nominal concentrations, endpoints were based on nominal concentrations. Water quality parameters were: temperature (24-25°C), pH (7.3-9.0). The results are summarised in Table B.9.18.

Table B.9.18 The acute toxicity of Terrazole Technical (etridiazole) to algae

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC50 in mg a.s./L	NOEC mg a.s./L
Anabaena flos-aquae (blue-green alga)	Static 120 hours (97.92%)	69 - 100	120h-E_bC_{50}: 0.42(A) 120h-E_rC_{50}: >1.0(A)	120h-NOE_bC & 120h-NOE_rC: 0.063(A)

(A) Based on nominal concentrations.

Conclusion

120-hour EbC50 and ErC50: 0.42 and >1.0 mg/L, respectively; 120-hour NOEbC and NOErC: 0.063 mg/L; all based on nominal concentrations.

Guidelines and limitations

The test is acceptable.

Study 8

Characteristics

reference	:	Hoberg J.R. (1993c)	nominal concentrations	:	0, 1.6, 3.1, 6.3, 13, 25 and 50 mg/L
type of study	:	Duckweed growth inhibition test			
year of execution	:	1993	dosing method	:	Static, stock solution in acetone/test medium
GLP statement	:	Yes			
guideline	:	EPA 122-2, 123-2	acceptability	:	Acceptable
test substance	:	Terrazole Technical (etridiazole), Lot # 203S026	14-day E_bC_{50}	:	7.3 mg/L
			14-day E_rC_{50}	:	14 mg/L
purity	:	97.92%	14-day NOE_bC	:	5.7 mg/L
species	:	*Lemna gibba*	14-day NOE_rC	:	5.7 mg/L
exposure duration	:	14 days			

Methods

A 14-day toxicity test on the growth of duckweed (*Lemna gibba*) (3 replicates per concentration, each containing five plants with three fronds each) was conducted with Terrazole Technical (etridiazole) at nominal test concentrations of 1.6, 3.1, 6.3, 13, 25 and 50 mg/L, with untreated control.

Results

The measured concentrations were 1.4, 2.9, 5.7, 12, 23 and 49 mg/L at test initiation (representing 88-98% of nominal), which declined to <0.11, 0.22, 0.39, 1.1, 2.7 and <3.5 mg/L at the end of exposure (representing <7-11% of nominal). Water quality parameters were: temperature (24-25°C), pH (5.0-6.2).

Endpoints were based on initial measured concentrations, which is acceptable. The reported 14-day EC- and NOEC-values for biomass were based on frond dry weight at the end of the test, which is acceptable. In addition, the report contained 14-day EC- and NOEC-values that were calculated from the percentage inhibition of absolute values for frond density at study end. This is not in agreement with the recommendations in the OECD 221 draft Guideline (July 2002) for testing on *Lemna gibba*. According to this guideline, frond density should be used to calculate either the area under the curve or the average growth rate. RMS calculated these parameters according to the methods outlined in the OECD 221 draft Guideline (July 2002), based on the reported raw data for frond number on all samplings. Since the area under the curve resulted in a higher EbC50 value (20 mg/L) than the EbC50 based on final biomass (7.3 mg/L), the latter will be used for risk assesment. Further, the ErC50 was 14 mg/L. The 14-day NOErC was calculated using Toxstat Release 3.5, 1996 using the Bonferroni t-test. The results are summarised in Table B.9.19.

Table B.9.19 The toxicity of Terrazole Technical (etridiazole) to aquatic plants

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in mg/L	NOEC mg/L
Lemna gibba (duckweed)	Static 14 days (97.92%)	88 – 98 (start) <7 – 11 (end)	14d-E_bC_{50}: 7.3(A) 14d-E_rC_{50}: 14(A)	14d-NOE_bC: 2.9(A) 14d-NOE_rC: 5.7(A)

(A) Based on initial measured concentrations.

Conclusion

The 14-day EbC50 and ErC50 are 7.3 and 14 mg/L, respectively; 14-day NOEbC and NOErC: 2.9 and 5.7 mg/L, respectively; all based on initial measured concentrations.

Guidelines and limitations

The test is acceptable.

B.9.2.1.2 Acute toxicity of metabolites

Study 1

Characteristics

reference	:	Sousa J.V. (1998)	species	:	Rainbow trout (*Oncorhynchus mykiss*)
type of study	:	Acute toxicity study			
year of execution	:	1998	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concentrations	:	0, 0.26, 0.43, 0.72, 1.2 and 2.0 mg/L
guideline	:	OECD 203, EEC C1			
test substance	:	5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03), Lot No. 2333-92-BTF	dosing method	:	Flow-through, stock solution in DMF
			acceptability	:	Acceptable
purity	:	99.75%	LC50	:	0.77 mg/L

Methods

A 96-hour acute toxicity test on rainbow trout (*Oncorhynchus mykiss*) (1 replicate with ten fish per concentration) was conducted under flow-through conditions with 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03, metabolite of etridiazole also referred to as dichloro-etridiazole) at nominal test concentrations of 0.26, 0.43, 0.72, 1.2 and 2.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations were 0.29, 0.50, 0.77, 1.3 and 2.1 mg/L at test initiation (representing 105-116% of nominal), and 0.19, 0.32, 0.55, 0.90 and 1.7 mg/L at the end of exposure (representing 73-85% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters were: temperature (11-12°C), dissolved oxygen (≥9.2 mg/L) and pH (6.9-7.4). The results are summarised in Table B.9.20.

Table B.9.20 The acute toxicity of 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in mg/L	NOEC mg/L
Oncorhynchus mykiss	Flow-through 96 hours (99.75%)	73-116	24 h: >1.9(A) 48 h: 1.1(A) 72&96 h: 0.77(A)	24 h: 0.66(A) 48&72 h: 0.41(A) 96 h: 0.24(A)

(A) Based on mean measured concentrations.

Conclusion

96-hour LC50: 0.77 mg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 2

Characteristics

reference	:	Cafarella M.A. (2000a)	species	:	Rainbow trout (*Oncorhynchus mykiss*)
type of study	:	Acute toxicity study			
year of execution	:	2000	exposure duration	:	96 hours
GLP statement	:	Yes	nominal concentrations	:	0, 6.3, 13, 25, 50 and 100 mg/L
guideline		OECD 203			
test substance	:	5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02), Lot No. AGD-1785-057	dosing method	:	Static, stock solution in dilution water
			acceptability	:	Acceptable
purity	:	99.8%	LC50	:	>100 mg/L

Methods

A 96-hour acute toxicity test on rainbow trout (*Oncorhynchus mykiss*) (1 replicate with ten fish per concentration) was conducted under static conditions with 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02, metabolite of etridiazole also referred to as etridiazole acid) at nominal test concentrations of 6.3, 13, 25, 50 and 100 mg/L, with untreated control.

Results

The measured concentrations were 6.8, 13, 23, 48 and 98 mg/L at test initiation (representing 92-108% of nominal), and 6.5, 13, 23, 48 and 95 mg/L at the end of exposure (representing 92-103% of nominal). Endpoints were based on analytically confirmed nominal concentrations, which is acceptable. Water quality parameters were: temperature (13-14°C), dissolved oxygen (≥7.6 mg/L) and pH (5.3-7.2). The results are summarised in Table B.9.21.

Table B.9.21 The acute toxicity of 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in mg/L	NOEC mg/L
Oncorhynchus mykiss	Static 96 hours (99.8%)	92-108	24, 48, 72 & 96 h: >100[A)]	24, 48, 72 & 96 h: 100[(A)]

(A) Based on analytically confirmed nominal concentrations.

Conclusion

96-hour LC50: > 100 mg/L, based on analytically confirmed nominal concentrations.

Guidelines and limitations

The test is acceptable.

Study 3

Characteristics

reference	:	Putt A.E. (2001)	species	:	*Daphnia magna*
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2000	nominal concentrations	:	0, 0.26, 0.43, 0.72, 1.2 and 2.0 mg/L
GLP statement	:	Yes			
guideline	:	EEC C2, OECD 202	dosing method	:	Flow-through, stock solution in DMF
test substance	:	5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03), Lot No. 2333-92-BTF	acceptability	:	Acceptable
			EC50	:	1.1 mg/L
purity	:	99.75%			

Methods

A 48-hour immobilisation test on *Daphnia magna* (2 replicates of 10 Daphnia each per concentration) was conducted under flow-through conditions with 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03, metabolite of etridiazole, also referred to as dichloro-etridiazole) at nominal test concentrations of 0.26, 0.43, 0.72, 1.2 and 2.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations in centrifuged samples collected from the midpoint of each vessel were 0.37, 0.56, 0.62, 0.97 and 1.6 mg/L at test initiation (representing 80-142% of nominal), and 0.41, 0.65, 0.69, 1.0 and 1.7 mg/L at the end of exposure (representing 83-158% of nominal). At 2.0 mg/L nominal, the concentrations in uncentrifuged samples from 0 and 48 hours (1.5 and 1.8 mg/L respectively) were comparable to those in centrifuged samples (1.6 and 1.7 mg/L respectively). The overall mean measured concentrations were 0.39, 0.61, 0.65, 0.99 and 1.6 mg/L, with immobility rates after 48 hours of 5%, 10%, 10%, 25% and 90%, respectively. Immobility in the control was 0%. Endpoints were based on mean measured concentrations, which is acceptable.

Water quality parameters were: temperature (20°C), dissolved oxygen (≥5.5 mg/L) and pH (6.9-7.3). The results are summarised in Table B.9.22.

Table B.9.22 The acute toxicity of 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) to invertebrates

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in mg/L	NOEC mg/L
Daphnia magna	Flow-through 48 hours (99.75%)	80-158	24 h: > 1.6(A) 48 h: 1.1(A)	24 h: 0.39(A) 48 h: < 0.39(A)

(A) Based on mean measured concentrations.

Conclusion

48-hour EC50: 1.1 mg/L, based on mean measured concentrations.

Guidelines and limitations

The water solubility of the test substance was not reported, but is presumable comparable to or lower than that of etridiazole (about 9 mg/L in buffer of pH 7 and 10 at 25°C). It was reported that throughout the exposure period no visible undissolved substance was observed in the diluter system and the test solutions. Undissolved substance however may have been present, based on the presence of daphnids on the surface of all test solutions, but not in the control. The report stated that several of the mobile daphnids were trapped on the surface of the test solutions after 24 and 48 hours at all concentrations. If this was due to a film of undissolved substance on the surface, exposure of trapped daphnids to the test substance may have been higher than that of daphnids inside the solution. In case of a significant contribution of exposure of trapped daphnids to undissolved test substance, immobility percentages are expected to be somewhat erratic, and there may be large differences between replicates. There was however a well-defined dose-response relationship, and differences between replicates were considered to be not unusual. The EC50 is therefore considered to be acceptable.

Study 4

Characteristics

reference	:	Cafarella M.A. (2000b)	species	:	*Daphnia magna*
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2000	nominal concentrations	:	0, 63, 130, 250, 500 and 1000 mg/L
GLP statement	:	Yes			
guideline	:	OECD 202	dosing method	:	Static, stock solution in dilution water
test substance	:	5-ethoxy-1,2,4-thiadazole-3-carboxylic acid (T-02), Lot No. RJS 1732-104	acceptability	:	Acceptable
			EC50	:	350 mg/L
purity	:	100%			

Methods

A 48-hour immobilisation test on *Daphnia magna* (4 replicates of 5 Daphnia each per concentration) was conducted under static conditions with 5-ethoxy-1,2,4-thiadazole-3-carboxylic acid (T-02, metabolite of etridiazole, also referred to as etridiazole acid) at nominal test concentrations of 63, 130, 250, 500 and 1000 mg/L, with untreated control.

Results

The measured concentrations were 62, 130, 260, 510 and 1000 mg/L at test initiation (representing 98-104% of nominal), and 65, 130, 260, 520 and 1100 mg/L at the end of exposure (representing 100-110% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Immobility was 0%, 0%, 0%, 5%, 100% and 100% at nominal concentrations of 0, 63, 130, 250, 500 and 1000 mg/L, respectively. The test substance is an acid, and at the start of the test the pH was 8.1, 7.4, 7.0, 6.4, 3.4 and 2.8 at nominal concentrations of 0, 63, 130, 250, 500 and 1000 mg/L, respectively. The effects observed at the two highest exposure levels may have been caused by the low pH of these solutions. At the end of the test, the pH was 7.5-7.9 in the control and the three lowest test concentrations. Other water quality parameters were: temperature (20-21°C), dissolved oxygen (≥8.6 mg/L). The results are summarised in Table B.9.23.

Table B.9.23 The acute toxicity of 5-ethoxy-1,2,4-thiadazole-3-carboxylic acid (T-02) to aquatic invertebrates

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in mg/L	NOEC mg/L
Daphnia magna	Static 48 hours (100%)	98-110	24 h: 360(A) 48 h: 350(A)	24 h: 260(A) 48 h: 130(A)

(A) Based on mean measured concentrations.

Conclusion

48-hour EC50: 350 mg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 5

Characteristics

reference	:	Hoberg J.R. (1998)	exposure duration	:	72 hours
type of study	:	Algal growth inhibition test	nominal	:	0, 0.010, 0.026, 0.064, 0.16, 0.40
year of execution	:	1998	concentrations		and 1.0 mg/L
GLP statement	:	Yes	dosing method	:	Static, stock solution in DMF
guideline	:	OECD 201, EEC C3	acceptability	:	Acceptable
test substance	:	5-ethoxy-3-dichloromethyl-1,2,4-	72h-E_bC_{50}	:	0.62 mg/L
		thiadiazole (T-03, 3-dichloromethyl	72h-E_rC_{50}	:	>0.98 mg/L
		terrazole), Lot No. CP-TER-1-3	72h-NOE_bC	:	0.069 mg/L
purity	:	92.6%	72h-NOE_rC	:	0.15 mg/L
species	:	*Selenastrum capricornutum*			

Methods

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per concentration, each containing 1.0 x 10^4 cells/mL at the start) was conducted with 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03, metabolite of etridiazole, also referred to as dichloro-etridiazole) at nominal test concentrations of 0.010, 0.026, 0.064, 0.16, 0.40 and 1.0 mg/L, with untreated and solvent-control.

Results

The measured concentrations at test initiation and at the end of exposure were the same: 0.011, 0.028, 0.069, 0.15, 0.38 and 0.98 mg/L (representing 94-110% of nominal). Water quality parameters were: temperature (24-25°C) and pH (7.6-9.9). Endpoints were based on mean measured concentrations (which were identical to initial measured concentrations), which is acceptable. The results are summarised in Table B.9.24.

Table B.9.24 The acute toxicity of 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) to algae

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC50 in mg a.s./L	NOEC mg a.s./L
Selenastrum capricornutum (green alga)	Static 72 hours (92.6%)	94 - 110	72h-E_bC_{50} 0.62[(A)] 72h-E_rC_{50} > 0.98[(A)]	72h-NOE_bC: 0.069[(A)] 72h-NOE_rC: 0.15[(A)]

(A) Based on mean measured concentrations (which were identical to initial measured concentrations).

Conclusion

72-hour E_bC_{50} and E_rC_{50}: 0.62 and >0.98 mg/L, respectively; 72-hour NOE_bC and NOE_rC: 0.069 and 0.15 mg/L, respectively; all based on mean measured concentrations (which were identical to initial measured concentrations).

Guidelines and limitations

The test is acceptable.

Study 6

Characteristics

reference	:	Hoberg J.R. (2000)	exposure duration	:	72 hours
type of study	:	Algal growth inhibition test	nominal	:	0, 6.4, 13, 25, 50 and 100 mg/L
year of execution	:	1999	concentrations		
GLP statement	:	Yes	dosing method	:	Static, stock solution in test
guideline	:	OECD 201			medium
test substance	:	5-ethoxy-1,2,4-thiadiazole-3-	acceptability	:	Acceptable
		carboxylic acid (T-02) , Lot No. RJS	72h-EbC50	:	27 mg/L
		1732-100	72h-ErC50	:	29 mg/L
purity	:	100%	72h-NOEbC	:	12 mg/L
species	:	*Selenastrum capricornutum*	72h-NOErC	:	12 mg/L

Methods

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per concentration, each containing 1.0 x 10^4 cells/mL at the start) was conducted with 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02, metabolite of etridiazole, also referred to as etridiazole acid) at nominal test concentrations of 6.4, 13, 25, 50 and 100 mg/L, with untreated control.

Results

The measured concentrations were 5.6, 12, 24, 48 and 100 mg/L at test initiation (representing 88-100% of nominal), and 6.2, 12, 24, 48 and 99 mg/L at the end of exposure (representing 92-99% of nominal). The test substance is an acid, and at the start of the test the pH was 6.5, 6.3, 6.3, 4.4 and 3.7 for the mean measured concentrations of 5.9, 12, 24, 48 and 100 mg/L, respectively. The absence of algal growth in the two highest exposure concentrations may have been caused by the low pH of these solutions. Other water quality parameters were: temperature (24°C), conductivity (80-170 µmhos/cm).

Endpoints were based on mean measured concentrations, which is acceptable. The inhibition of biomass relative to the control was 2%, 5%, 21%, 100% and 100% at the mean measured concentrations of 5.9, 12, 24, 48 and 100 mg/L, respectively. The reported EbC50 value (22 mg/L) should be incorrect as only 21% inhibition was observed at 24 mg/L (and 100% at the next tested concentration of 48 mg/L). The EbC50 value in the table below (27 mg/L) was calculated by the RMS following the methods in OECD 202, based on the reported raw data and using Toxstat Release 3.5, 1996, and was higher than the reported value. The results are summarised in Table B.9.25.

Table B.9.25 The acute toxicity of 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) to algae

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC/EC50 in mg a.s./L	NOEC mg a.s./L
Selenastrum capricornutum (green alga)	Static 72 hours (100%)	92 - 100	72h-E_bC_{50} 27(A) 72h-E_rC_{50}: 29(A)	72h-NOE_bC & 72h-NOE_rC: 12(A)

(A) Based on mean measured concentrations.

Conclusion

72-hour EbC50 and ErC50: 27 and 29 mg/L, respectively; 72-hour NOEbC and NOErC: 12 mg/L; all based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

B.9.2.1.3 Acute toxicity of the plant protection product (IIIA 10.2.1)

No data were submitted.

B.9.2.2 Chronic toxicity

B.9.2.2.1 Chronic toxicity of the active substance

B.9.2.2.1.1 Fish (IIA 8.2.2)

Characteristics

reference	:	Machado M.W. (1993c)	exposure duration	:	90 days
type of study	:	Chronic toxicity study (ELS)	nominal concentrations	:	0, 0.031, 0.063, 0.13, 0.25 and 0.50 mg/L
year of execution	:	1993			
GLP statement	:	Yes	dosing method	:	Flow-through, stock solution in acetone
guideline	:	EPA 72-4			
test substance	:	Terrazole Technical (etridiazole), Lot # 203S026	acceptability	:	Acceptable
			NOEC	:	0.12 mg/L
purity	:	97.92%			
species	:	Rainbow trout (*Oncorhynchus mykiss*)			

Methods

A 90-day fish early life stage flow-through study was undertaken with rainbow trout (*Oncorhynchus mykiss*). Newly fertilised eggs (3.75 hours post-fertilisation, two replicates/concentration, 100 eggs/replicate) were exposed to Terrazole Technical (etridiazole, purity 97.92%) at nominal concentrations of 0.031, 0.063, 0.13, 0.25 and 0.50 mg/L plus control and solvent control (acetone). Mean measured concentrations were 0.032, 0.060, 0.12, 0.24 and 0.42 mg/L, representing 84 to 103% of nominal. Water quality parameters were: temperature (10-14°C), dissolved oxygen (7.7-12.9 mg/L), pH (6.7 to 7.3). The total duration of the exposure period was 90 days. Survival and fish behaviour was monitored daily and fish length and weight at 60 days post-hatch.

Results

Egg hatchability, time to hatch and time to initiation of swim-up was not affected at any test concentration when compared to the pooled control group. Survival at hatch was slightly reduced compared to the pooled control at the two highest test concentrations (not statistically significant). No test substance related abnormalities were noted. Fish body length was reduced at 0.42 mg/L, while fish body weight was reduced at 0.24 and 0.42 mg/L.

Conclusion

Based on reduced body weight, the NOEC was 0.12 mg/L and the LOEC was 0.24 mg/L.

Guidelines and limitations

The study was conducted in accordance with GLP and EPA 72-4 and is acceptable.

B.9.2.2.1.2 Invertebrates (IIA 8.2.5)

Characteristics

reference	:	Putt A.E. (1993)	species	:	*Daphnia magna*
type of study	:	Chronic toxicity study	exposure duration	:	21 days
year of execution	:	1992-1993	nominal concns	:	0, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L
GLP statement	:	Yes	dosing method	:	Flow-through, stock solution in acetone
guideline	:	EPA 72-4			
test substance	:	Terrazole Technical (etridiazole), Lot # 203S026	acceptability	:	Acceptable
			NOEC	:	0.37 mg/L
purity	:	97.92%			

Methods

The chronic toxicity of Terrazole Technical (etridiazole, purity 97.92%) to *Daphnia magna* was assessed in a 21-day flow-through study. First instar daphnids (≤24 hours old, 40 per treatment, 10 daphnia per replicate vessel) were used to initiate the study. The nominal concentrations were 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L plus a blank- and solvent-control (acetone). Mean measured concentrations of Terrazole Technical (etridiazole) were 0.37, 0.54, 0.97, 2.0 and 3.6 mg/L, representing 72 to 119% of nominal. Water quality parameters were: temperature (18-21°C), dissolved oxygen (7.4-8.6 mg/L), pH (8.1-8.2), alkalinity (110-118 mg/L) and hardness (168-180 mg/L).

Results

Survival of adult daphnia was statistically significantly reduced by 7% and 35% at 2.0 and 3.6 mg/L. The reduction of 7% at 2.0 mg/L is considered to be without biological relevance as the OECD guidelines for a Daphnia reproduction test (202(II) and 211) accept 20% mortality in the control. A delay in time to first brood was not observed at any test concentration. Reproductive success, as measured by the mean number of live young per adult, was significantly reduced at and above 0.97 mg/L when compared to the pooled control group. Mean total body length after 21 days was significantly reduced at and above 2.0 mg/L, while mean dry body weight was reduced at and above 0.54 mg/L.

Conclusion

The NOEC for parental survival was identified as 2.0 mg/L. The NOEC for reproduction was 0.54 mg/L. The NOEC for effects on growth was 0.37 mg/L based on mean dry body weight. Based on dry body weight, the LOEC was 0.54 mg/L.

Guidelines and limitations

The test is acceptable.

B.9.2.2.1.3 Effects on sediment dwelling organisms (IIA 8.2.7)

No data were required, since the maximum level of etridiazole in sediment was 0.5% AR.

B.9.2.2.2 Chronic toxicity of metabolites

No data were submitted.

B.9.2.3 Bioaccumulation (IIA 8.2.3)

Characteristics

reference	:	Schocken M.J. (1994)	species	:	Bluegill sunfish (*Lepomis macrochirus*)
type of study	:	Bioconcentration and metabolism in fish	exposure duration	:	42 days
year of execution	:	1993-1994	nominal concentrations	:	0 and 0.050 mg a.s./L
GLP statement	:	Yes			
guideline	:	EPA 165-4	dosing method	:	Flow-through, stock solution in acetone
test substance	:	Unlabeled etridiazole (Terrazole®), Lot No. AC-1322-17; [14C]-etridiazole, Lot No. CSL-92-359-77-25, sp. act. 24.35 mCi/mmol	acceptability	:	Acceptable
			BCF		Etridiazole: 165 L/kg (whole fish)
purity	:	Unlabeled etridiazole: 98.7%; [^{14}C]-etridiazole: radiochemical purity ≥98%			

Methods

Two groups of 205 Bluegill sunfish (*Lepomis macrochirus*) were exposed to [^{14}C]-etridiazole (radiochemical purity ≥98%) for 42 days in a flow-through system, followed by 14 days of depuration. One group was used for determination of the bioconcentration factor and the other group was used for metabolite identification. The nominal concentration was 0.050 mg/L for both replicates, plus solvent control (4% hexane in acetone, 0.05 mL/L). The mean measured radioactivity concentrations in the water during exposure were 0.051 and 0.050 mg eq./L (standard deviation 0.004 and 0.010 mg eq./L). Taking into consideration the mean percentage of parent compound determined in water samples on day 21 during the uptake phase (90.4% and 97.7%), the mean measured concentrations of etridiazole in the water during exposure were 0.046 and 0.049 mg a.s./L. Water quality parameters were: temperature (17-20°C), dissolved oxygen (≥60% of saturation) and pH (6.4-7.7). Water was sampled on days 0, 1, 3, 7, 10, 14, 21, 28, 33, 36, 37, 40 and 42 of uptake and 1, 3, 7, 10 and 14 of depuration. Five fish were sampled on days 1, 3, 7, 10, 14, 21, 28, 33, 36, 40 and 42 of uptake and 1, 3, 7, 10 and 14 of depuration. Control water and fish were sampled on days 0 and 42 of exposure and day 14 of depuration. Total radioactive residues in water (by LSC), and edible and non-edible tissue from five fish (by combustion/LSC), were determined at all sampling times. Lipid content of fish was not determined.

Extraction and metabolite identification was performed on samples of water from day 21 of uptake and on edible and non-edible tissues of 301 fish sampled on day 42 of uptake. The edible and non-edible tissue samples were extracted with methanol, leaving 19% (edible tissue) or 20-22% (viscera) of the initial Total Radioactivity Residues (TRR) in post-extraction solids (PES). Methanol/water extraction of PES from edible and non-edible tissue, respectively, released only 2% and 3-4% of the ^{14}C, but

subsequent protease treatment solubilised 7-8% and 6-7%. Methanol extracts of viscera were analysed by direct reversed-phase HPLC. Methanol extracts of edible tissues were subjected to two different work-up methods prior to HPLC: C18 SPE and rotary evaporation. Conjugated metabolites in extracts were identified by enzyme hydrolysis (β-glucuronidase and protease) followed by reversed-phase, ion-exclusion, anion-exchange and cation exchange HPLC. Confirmation of metabolite identity was performed by LC-MS and GC-MS. All water samples were analysed by LSC, and on day 21 of exposure by HPLC-UV and HPLC-RAM. Compound identity was based on chromatography of reference standards.

Results

No abnormal behaviour was observed for control and test fish throughout the study. Two exposed fish died on day 28, and two control fish were removed since they were lethargic. Six fish died during maintenance handling.

Concentrations of radioactivity in whole fish were 2.1, 2.0, 2.5, 4.2, 4.4, 6.3, 7.4, 9.3, 9.0, 8.9 and 9.3 mg eq.kg on day 1, 3, 7, 10, 14, 21, 28, 33, 36, 40 and 42 of uptake, respectively. The author of the report stated that a steady state situation was reached by day 28, based on the absence of a statistically significant difference between the values for three consecutive samplings (ANOVA, p = 0.05, and Tukey's test). Details of the actual statistical analysis of the residue values in fish were however not reported. Visual inspection of the residue concentrations in fish strongly suggest that a steady state was reached on day 33. The fact that statistical analysis concluded a steady state situation by day 28, is largely due to the fact that the variation between residues in fish on day 28 was much higher than on adjacent sampling times (for day 14-42 of uptake: SD on day 28 2.4 mg eq./kg versus 0.8-1.5 mg eq./kg for all other days). It is concluded therefore that the steady state was reached by day 33.

Reported steady-state bioconcentration factors (BCFs) were calculated for the day 28-42 interval (85, 280 and 174 L/kg wet weight for edible tissue, non-edible tissue, and whole fish, respectively). The RMS recalculated the BCFs for radioactivity by dividing the mean radioactivity concentration during steady state (day 33-42) in edible tissue, non-edible tissue and whole fish (4.4, 14.9 and 9.1 mg eq./kg, respectively) by the overall mean radioactivity concentration in water during day 33-42 (0.052 mg eq./L), respectively. The BCFs for radioactivity calculated this way were 85, 288 and 176 L/kg for edible tissue, non-edible tissue and whole fish, respectively. After 14 days in uncontaminated water, 83, 87 and 86% of the radioactivity present at the end of the uptake phase was cleared from edible tissue, non-edible tissue and whole fish, respectively. The CT50 (edible tissue, non-edible tissue and whole fish) was <1 day. CT90 values were >14 days.

The radioactivity from the treated day 21 water samples consisted mainly of etridiazole (90.4% and 97.7% in the tank for bioconcentration and metabolite identification, respectively).

Direct HPLC analysis of methanol extracts of fish samples showed that etridiazole was the main component (79-82% TRR) in non-edible portions of fish, whilst the metabolite 3-dichloromethyl etridiazole represented 18-21% TRR.

Due to volatilisation of radioactivity during rotary evaporation of methanol extracts of edible tissue, extensive losses occurred, resulting in low recovery (29%) for this purification step. In the concentrated extracts, the main component was etridiazole (90% of recovered RA), whilst low levels (11% of recovered RA) of the metabolite 5-hydroxy etridiazole were identified. Due to the low recovery of this step, the only firm conclusion from this work-up method is that extracts of edible tissues contain the metabolite 5-hydroxy etridiazole at a level of at least 3% TRR.

The recovery of SPE purification of the methanol extracts of edible tissue was 86% (14% of the radioactivity was unretained by the column and not further identified). HPLC of the retained activity following elution from the column showed that only parent etridiazole was present (hence representing at least 86% TRR).

In protease hydrolysates of edible and non-edible tissue, respectively, the metabolite 3-carboxylic acid was detected at levels of 0.6 and 1.8% TRR, and an unknown polar metabolite at levels of 5.6 and 1.6% TRR.

The BCFs for parent etridiazole are derived by correction the BCFs for radioactivity for the percentage of parent compound in water during uptake (90.4% in the replicate for determination of the BCF) and in fish. Parent compound represented on average 81% TRR for non-edible tissue. In edible tissue, parent compound was at least 86% TRR, presumably more (see above). Based on the level of the only identified metabolite in edible tissue (5-hydroxy etridiazole, at least 3% TRR), the level of parent compound in edible tissue is set to be 97% TRR, which gives the highest (i.e. worst case) value for the BCF of etridiazole. Based on these corrections, the BCFs for radioactivity in edible tissue, non-edible tissue and whole fish (85, 288 and 176 L/kg wet weight, respectively) correspond with values of 92, 256 and 165 L/kg wet weight, respectively, for the active substance.

Conclusion

The BCFs for etridiazole in edible tissue, non-edible tissue and whole fish are 92, 256 and 165 L/kg wet weight, respectively. The depuration CT50 for radioactivity in edible tissue, non-edible tissue and whole fish is <1 day, CT90 values are >14 days.

Guidelines and limitations

The study was in accordance with GLP and EPA 165-4, and is acceptable.

B.9.2.4 Risk assessment

B.9.2.4.1 Risk assessment of the active substance

B.9.2.4.1.1 Acute risk

Measurement of treatment concentrations during the course of the flow-through tests with technical etridiazole in fish (2 species), *Daphnia magna*, *Mysidopsis bahia* and *Crassostrea virginica* showed that concentrations of etridiazole were maintained within acceptable limits, and results were based on mean measured concentrations. In two static tests in green and blue-green algae (*Selenastrum capricornutum* and *Anabaena flos-aquae*), etridiazole was not stable, and results were based on analytically confirmed nominal (initial) concentrations. In the static test with etridiazole in *Lemna gibba*, concentrations were not stable, and endpoints based on initial measured concentrations were calculated.

Tests with the proposed Aaterra formulation were not submitted. The formulation contains emulsifying agents that could increase the toxicity of the active substance. However, the safety margins calculated for the active substance were high (see table below). In addition, no established EU scenario for emission from greenhouses is available. This issue is therefore referred to member state level.

For the initial risk assessment the following endpoints will be used:
96 hr LC50 for rainbow trout (*Oncorhynchus mykiss*) of 2.4 mg a.s./L;
48 hr EC50 for *Daphnia magna* of 3.1 mg a.s./L;
96 hr LC50 for shrimp (*Mysidopsis bahia*) of 2.5 mg a.s./L
72 hr EC50 for *Selenastrum capricornutum* of 0.30 mg a.s./L;
14 day EC50 for *Lemna gibba* of 7.3 mg a.s./L.

The acute TERs for fish, *Daphnia magna*, shrimp, algae and *Lemna* are shown in Table B.9.26. The PECsw value was taken from section B.8.6.1. These values were calculated for two applications at 7, 0.56 and 0.28 kg a.s./ha in ornamentals, tomatoes/peppers and cucumbers, respectively. No drift percentages are available at EU level for the greenhouse scenario. In the Netherlands an emission rate to surface water of 0.1% is used (emission via condensation water and evaporation/volatilisation). PECsw values for the greenhouse applications were calculated for this emission rate of 0.1% using FOCUS STEP 2 (as opposed to FOCUS STEP 1 no run off/drainage, which is considered appropriate for greenhouse treatments).

Table B.9.26 Acute TERs for etridiazole for fish, *Daphnia*, shrimp, algae and *Lemna*

Crop	dose (kg a.s./ha)	LC/EC50 (µg as/L)					PECsw (µg a.s./L)	TER				
		Fish	*Daphnia*	Shrimp	Algae	*Lemna*		Fish	*Daphnia*	Shrimp	Algae	*Lemna*
O	2 x 7	2400	3100	2500	300	7300	2.343	1024	1323	1067	128	3116
T	2 x 0.56	2400	3100	2500	300	7300	0.187	1E+04	2E+04	1E+04	1604	4E+04
C	2 x 0.28	2400	3100	2500	300	7300	0.094	1E+04	3E+04	3E+04	3191	8E+04

O: ornamentals; T: tomatoes/peppers; C: cucumbers

The acute TERs for fish, *Daphnia magna* and shrimp are all far above the relevant Annex VI triggers of 100, and the TERs for algae and *Lemna* are far above the relevant Annex VI triggers of 10. Hence the acute risk from the proposed uses should be low for fish, *Daphnia*, shrimp, algae and aquatic plants.

B.9.2.4.1.2 Long-term risk

The proposed use in ornamentals, tomatoes/peppers and cucumbers involves two applications at 7, 0.56 and 0.28 kg a.s./ha, respectively. In a water-sediment study, etridiazole was not detected in sediment at levels above 0.2% AR, hence <10% AR (see section B.8.4.3.2). Therefore, testing with sediment-dwelling organisms is not required. In the same water-sediment study, etridiazole dissipated from the water column with a DT50 of 1.3days, hence <2 days. According to the Guidance Document on Aquatic Ecotoxicology (Working Document, Sanco/3268/2001), chronic exposure to etridiazole is therefore not likely to occur. Chronic toxicity data for the technical substance were however submitted and are summarised in Table B.9.27.

Table B.9.27 The chronic toxicity of etridiazole to aquatic life

Species	Type of test	NOEC (µg a.s./L)
Oncorhynchus mykiss	90-day fish early life stage test	120
Daphnia magna	21-day study	370

The long-term TERs based on the initial PECsw (covering the worst-case situation that chronic effects are caused by the peak concentration directly after application, and taken from section B.8.6.1), and based on the NOEC values of the active substance, are shown in Table B.9.28.

Table B.9.28 Long-term TERs for etridiazole assuming constant exposure to the initial PECs

Crop	Dose (kg a.s./ha)	NOEC (µg as/L)		PECsw (µg a.s./L)	TER	
		fish	*Daphnia*		Fish	*Daphnia*
Ornamentals	2 x 7	120	370	2.343	51	158
Tomatoes/peppers	2 x 0.56	120	370	0.187	642	1979
Cucumber	2 x 0.28	120	370	0.094	1277	3936

The long-term TER for fish and *Daphnia* are all above the Annex VI trigger of 10. Therefore, the long-term risk is considered to be acceptable.

B.9.2.4.1.3 Bioaccumulation

In a bio-concentration study with 14C-etridiazole under flow-through conditions, the BCFs for radioactivity were 85, 288 and 176 L/kg wet weight for edible tissue, non-edible tissue and whole fish, respectively, and the corresponding BCFs for etridiazole were 92, 256 and 165 L/kg wet weight. The Annex VI trigger factor is 100 L/kg for non-readily biodegradable substances. Etridiazole was not readily biodegradable in a closed bottle test. However, etridiazole does not exceed the triggers for a fish ELS or FLC test (EC50 >0.1 mg/L), nor that for evaluation of biomagnification in aquatic food chains (DT90 in water/sediment system 5.9-6.4 days, hence <10 days). In addition, the risk of poisoning of birds and mammals due to intake of contaminated fish was evaluated and found to be low (see section B.9.1.5 and B.9.1.6). The risk for bioaccumulation is therefore considered to be of no concern.

B.9.2.4.2 Risk assessment of metabolites

Etridiazole acid was shown to be the only major (>10%) metabolite in the water phase of a water/sediment system (Section B.8.4.3.2), with a maximum level in water of 13% (and of 8.3% in sediment). Dichloro-etridiazole was a minor (<10%) metabolite in water (maximum 9.5% and 1.4% AR in water and sediment, respectively; section B.8.4.3.2). In addition, monochloro-etridiazole and 3-hydroxymethyl etridiazole were found at ≤4.0% in any compartment. The risk of these metabolites must be considered.

B.9.2.4.2.1 Acute risk

Etridiazole acid

The LC/EC50s for etridiazole acid are summarised in Table B.9.29.

Table B.9.29 The acute toxicity of etridiazole acid to aquatic life

Species	Test duration	LC/EC50 (mg/L)
Oncorhynchus mykiss	96 hours	>100
Daphnia magna	48 hours	350
Selenastrum capricornutum	72 hours	27

Acute risk assessment will be based on these LC/EC50 values. The worst case TERs for this metabolite are summarised in Table B.9.30. The PECsw presented in the table are taken from section B.8.6.1.

Table B.9.30 Acute TERs for etridiazole acid

Crop	LC/EC50 (µg/L)			PECsw	TER		
	fish	*Daphnia*	Algae	(µg/L)	fish	*Daphnia*	Algae
Ornamentals	>100000	350000	27000	0.419	>2E+05	8E+05	6E+04
Tomatoes/peppers	>100000	350000	27000	0.034	>3E+06	1E+07	8E+05
Cucumber	>100000	350000	27000	0.017	>6E+06	2E+07	2E+06

The acute TERs of etridiazole for fish, *Daphnia* and algae are all far above the relevant Annex VI triggers (100, 100 and 10, respectively). Hence the acute risk from the proposed uses should be low.

Dichloro-etridiazole

The LC/EC50s for dichloro-etridiazole are summarised in Table B.9.31.

Table B.9.31 The acute toxicity of dichloro-etridiazole to aquatic life

Species	Test duration	LC/EC50 (mg/L)
Oncorhynchus mykiss	96 hours	0.77
Daphnia magna	48 hours	1.1
Selenastrum capricornutum	72 hours	0.62

Acute risk assessment will be based on these LC/EC50 values. The worst case TERs for this metabolite are summarised in Table B.9.32. The PECsw presented in the table are taken from section B.8.6.1.

Table B.9.32 Acute TERs for dichloro-etridiazole

Crop	LC/EC50 (µg/L)			PECsw	TER		
	fish	*Daphnia*	Algae	(µg/L)	fish	*Daphnia*	Algae
Ornamentals	770	1100	620	0.193	3990	5699	3212
Tomatoes/peppers	770	1100	620	0.015	5E+04	7E+04	4E+04
Cucumber	770	1100	620	0.008	1E+05	1E+05	8E+04

The acute TERs of etridiazole for fish, *Daphnia* and algae are all far above the relevant Annex VI triggers (100, 100 and 10, respectively). Hence the acute risk from the proposed uses should be low.

Monochloro-etridiazole and 3-hydroxymethyl etridiazole

No studies on the aquatic toxicity of monochloro-etridiazole or 3-hydroxymethyl etridiazole are available. The maximum level of any single metabolite in water and sediment was 4.0% AR. High safety margins were calculated for the acute risk for parent etridiazole (TERa ≥1024 for fish, daphnia and shrimp and ≥128 for algae and aquatic plants) and for the metabolites etridiazole acid and dichloro-etridiazole (TERa ≥3990 for fish and daphnia and ≥3212 for algae and aquatic plants). Given this fact, together with the low levels of the metabolites monochloro-etridiazole and 3-hydroxymethyl etridiazole, and the structural similarity between these two metabolites and those for which a very low risk was calculated, the acute risk of these minor metabolites is considered to be low.

B.9.2.4.2.2 Long-term risk

According to the Guidance Document on Aquatic Ecotoxicology (Working Document, Sanco/3268/2001), chronic toxicity testing is required when the persistence trigger (DT50 <2 days) is surpassed for the metabolite, but only where the metabolite is acutely more toxic than the parent compound. For metabolites in sediment, it is stated that testing is required where a metabolite is present in the sediment at a level of more than 10% of the parent applied radioactivity at day 14 or later.

Etridiazole acid

Etridiazole acid is acutely not more toxic than parent etridiazole. Therefore, chronic testing is not required for this metabolite. Etridiazole acid was a minor metabolite in sediment (max. 8.3% AR, see section B.8.4.3.2). Therefore, testing with sediment-dwelling organisms is not required.

Dichloro-etridiazole

The acute toxicity of dichloride-etridiazole to fish and *Daphnia* is slightly higher (about a factor of 3) than that of parent etridiazole. The DT50 for dissipation from the water column of a water-sediment study is 1.4-2.9 days, hence slightly higher than 2 days, and chronic exposure should be considered. The safety margins for chronic exposure to parent etridiazole were large, (TERlt ≥51) and the initial concentrations of dichloro-etridiazole are much lower than those of the parent etridiazole (0.193, 0.015 and 0.008 µg/L as opposed to 2.343, 0.187 and 0.094 µg/L). Taking this into consideration, as well as the fact that the DT50 for dissipation from the water column is only slightly higher than 2 days, it is not expected that exposure to dichloro-etridiazole will lead to an unacceptable long-term risk. Therefore the chronic risk of this metabolite should be low. Dichloro-etridiazole was a minor metabolite in sediment (max. 1.4% AR, section B.8.4.3.2). Therefore, testing with sediment-dwelling organisms is not required.

Monochloro-etridiazole

Monochloro-etridiazole was a minor metabolite in water and sediment (max. 4% AR). Therefore, testing with sediment-dwelling organisms is not required. No studies on the acute toxicity of monochloro-etridiazole are available. Considering the close similarity between the molecular structures of monochloro-etridiazole, etridiazole and dichloro-etridiazole, the low chronic risk expected for etridiazole and dichloro-etridiazole, and the lower initial concentrations of monochloro-etridiazole compared to those of etridiazole and dichloro-etridiazole, it is not considered likely that the long-term risk due to exposure to monochloro-etridiazole will be higher than that of etridiazole or dichloro-etridiazole. The chronic risk of this metabolite to aquatic organisms should therefore be low.

3-Hydroxymethyl etridiazole

3-Hydroxymethyl etridiazole was a minor metabolite in water and sediment (max. 3.4% AR). Therefore, testing with sediment-dwelling organisms is not required. No studies on the acute toxicity of 3-hydroxymethyl etridiazole are available. Considering the close similarity between the molecular structures of 3-hydroxymethyl etridiazole and etridiazole acid, the large safety margin for the chronic risk of etridiazole acid and the lower initial concentrations of 3-hydroxymethyl etridiazole compared to those of etridiazole acid, it is not considered likely that the long-term risk due to exposure to 3-hydroxymethyl etridiazole will be higher than that of etridiazole acid. The chronic risk of this metabolite to aquatic organisms should therefore be low.

Conclusion long-term risk assessment of metabolites

Chronic exposure to metabolites is considered to be of no concern.

B.9.2.4.2.3 Bioaccumulation

No experimentally determined BCF values in fish are available for etridiazole acid, dichloro-etridiazole, monochloro-etridiazole and 3-hydroxymethyl etridiazole. An experimentally determined logPow value is available for etridiazole acid. LogPow values for dichloro-etridiazole, monochloro-etridiazole and 3-hydroxymethyl etridiazole were estimated by the RMS using EPA EPI Suite software (see Table B.9.1). The BCF for fish can be estimated according to the formula logBCF=0.85*logPow-0.7 (USES 2.0). The logPow values and estimated BCF values are presented in Table B.9.33.

Table B.9.33 LogPow and BCF-values for metabolites of etridiazole

Metabolite	logPow	BCF (L/kg)
Etridiazole acid	0.7	0.79
Dichloro-etridiazole	2.7 (A)	39
Monochloro-etridiazole	2.5 (A)	27
3-Hydroxymethyl etridiazole	0.78 (A)	0.92

(A) Estimated by RMS using EPA EPI Suite software

The estimated BCF values are all below the Annex VI trigger of 100 and the risk of the above metabolites for bioaccumulation should be low.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3)

No data were submitted.

B.9.4 Effects on bees (Annex IIA 8.3.1, IIIA 10.4)

B.9.4.1 Toxicity

No data were submitted.

B.9.4.2 Risk assessment

The notifier provided a statement for the risk assessment for bees and bumblebees. It was stated that: *honeybees are normally never kept in glasshouses as pollinators, but only outdoors and that implies that they never will be at risk since there is no exposure. Further, etridiazole is applied directly to the root zone of the plants and consequently there will be no spray residues on plants that could lead to any direct exposure. From the root zone, the active substance can be taken up and transported in the plant, but etridiazole is*

-not a truly systemic product that will be evenly distributed through the plant

-rapidly metabolised if taken up in plants.

For bumblebees, which are sometimes used in glasshouses as pollinators, it was stated that it will be evident that there will be no exposure as a result of direct plant treatment. In view of the rapid plant metabolism and the method of application (on substrate only), it is also unlikely that pollinating bumblebees will come into contact with appreciable concentrations of the test substance through contact with soil, pollen or nectar. Also the summary of all data on effects on other non-target species (Annex IIA, point 8.6) and the results of the studies conducted on other non-target arthropods did not give any indication of etridiazole having insecticidal properties that would lead to concerns on safety to bumblebees.

In addition to their first statement, notifier submitted a short review of their plant metabolism studies and a publication of the University of California on pesticide hazards to honey bees. According to notifier, etridiazole should be considerd as a 'group II – pesticide', a group indicated in the publication of the University of California, consisting of pesticides essentially non-toxic to bees at dosages above 11 µg/bee (based on field research with over 600 pesticides). According to notifier, fungicides in general can be considered to be essentially non-toxic to bees, showing no insecticidal properties.

Reaction RMS: RMS can agree with notifier that honey bees are normally not used as pollinators in the proposed glasshouse uses in cucumber, peppers and tomatoes. In cucumber, no pollination is used since this will result in large seeds in the cucumbers. In peppers honey bees are sometimes used to gather pollen in order to decrease the exposure of workers, because of possible pollen allergy. This practice differs within and between member states and RMS therefore considers this an issue to be addressed at member state level. Therefore it can be concluded that the risk for honey

bees in the proposed uses in cucumber and peppers is acceptable. In tomatoes however, pollination always takes place by the use of bumblebees. RMS considers this as general practice in all member states. Therefore, the risk to bumblebees for the use in tomatoes should be addressed.

If etridiazole can be considered as non-systemic, exposure of bumblebees is considered not to take place. But etridiazole was found in tomatoes in a supervised residue trial (see section B.7.6.2). And etridiazole was also found in cucumbers after treatment with etridiazole in a hydroponic growth system (see section B.7.1.1, study 2). Although amounts found were low (max 23% TRR / 0.1 mg/kg and 0.034 – 0.023 mg/kg in cucumber and tomatoe respectively), and the amounts in flowers/pollen/nectar are unknown, this shows that etridiazole is absorbed by the roots of the plant and translocates to the fruits. Thus, etridiazole seems to have some systemic activity. Therefore it cannot be excluded that bumblebees used as pollinators in glasshouses will be exposed to etridiazole. Also, it cannot be excluded that bumblebees enter glasshouses, unless measures are taken to avoid entry of pollinators into glasshouses (e.g. by placing bee mesh in front of the openings). For the proposed use in glasshouse in tomatoes, bumblebees may therefore be exposed to etridiazole or its metabolites. No toxicity studies with honey bees or bumblebees were submitted, therefore the risk cannot be evaluated. RMS is of the opinion that a general statement on all fungicides being non-toxic to bees is not acceptable for etridiazole (moreover, etridiazole was not included in the referred to research of the University of California). Also it is unclear if this would be applicable to bumblebees. Therefore, the notifier is requested to provide an acute oral toxicity study with bumblebees with the a.s. etridiazole, in order to enable a (first tier) risk assessment for the use in tomatoes.

(Reference for a bumblebee test could be for example: Steen JJM, Gretenkord C & Schaefer H (1996): Method to determine the acute oral LD50 and acute contact LD50 of pesticides for bumble bees (*Bombus terrestris* L.). Proc. 6th Int. Symp. on the hazard of pesticides for bumble bees (ICPBR), September 17-19, Braunschweig, Germany, appendix 28.)

B.9.5 Effects on other arthropod species (IIA 8.3.2, IIIA 10.5)

B.9.5.1 Laboratory toxicity studies

Study 1

Characteristics

reference	:	Vinall S. (2002a)	exposure duration	:	48 hours
type of study	:	Laboratory toxicity study	nominal	:	0, 720, 1980, 3240, 4500 and 5760
year of execution	:	2001	concentrations		g a.s./ha
GLP statement	:	Yes	exposure method	:	Dry residues on glass plates
guideline	:	Mead-Briggs *et al.* (2000)	acceptability	:	Acceptable
test substance	:	AATERRA ME, Lot No. AM1B06F001, Ref. SI 7821	LR50	:	1494 g a.s./ha
			ER50	:	>720 g a.s./ha
a.s. content	:	720 g etridiazole/L			
species	:	*Aphidius rhopalosiphi*			

Methods

The effect of fresh residues of AATERRA ME (720 g etridiazole/L) on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi*, confined under laboratory conditions over laboratory-treated glass plates, was determined.

Treatments with AATERRA ME at 720, 1980, 3240, 4500 and 5760 g a.s./ha and a positive control (dimethoate, 0.1 g a.s./ha) were tested in 200 L spray solution/ha against water-treated controls. Laboratory treated glass plates were allowed to dry and incorporated into test units (3/treatment), forming floor and ceiling respectively. Ten adult wasps were added to each test unit. The test units were kept in a controlled environment room until study end. Mortality was assessed during 48 hours. Surviving females of the control and the 720 g a.s./ha treatment were removed and individually confined over untreated aphid-infested barley plants for 24 hours. The wasps were then removed and the plants were left for a further 10 days before the number of aphid mummies was recorded.

Results

Mortality and reproduction data are summarised in table B.9.34. Reproduction success was not significantly reduced at 720 g a.s./ha. The reference substance gave the expected response.

Table B.9.34 Effects of AATERRA ME on *Aphidius rhopalosiphi* under laboratory conditions

treatment	Dose (g a.s./ha)	Mortality (%)	Corrected mortality (%)[(A)]	Mean number of mummies per female	Reduction of reproduction (%)[(B)]
Water control	-	0	-	43.9	-
AATERRA ME	720	10	10	33.5	24
AATERRA ME	1980	59	59	Not tested	-
AATERRA ME	3240	100	100	Not tested	-
AATERRA ME	4500	100	100	Not tested	-
AATERRA ME	5760	100	100	Not tested	-
Dimethoate	0.1	100	100	Not tested	-

(A) According to Abott formula
(B) Reduction = 100%*(1-R), where R=Rt/Rc, Rt and Rc being the average number of mummies per female.

Conclusion

LR50 1494 g a.s./ha, ER50 >720 g a.s./ha.

Guidelines & Limitations

The study was conducted without major deviations from the guideline by Mead-Briggs *et al.* (2000) and is acceptable. Reported dose levels (700, 1925, 3150, 4375 and 5600 g a.s./ha) and LR50 (1452 g a.s./ha) were based on the nominal a.s. content of the test product, which was 700 g a.s./L. The dose levels and LR50 and ER50 values in the above summary were based on the reported measured a.s. content of the test product, which was 720 g a.s./L.

Study 2

Characteristics

reference	:	Taruza S. (2002a)	exposure duration	:	14 days
type of study	:	Laboratory toxicity study	nominal	:	0, 72, 360, 720, 1440 and 2880 g
year of execution	:	2002	concentrations		a.s./ha
GLP statement	:	Yes	exposure method	:	Dry residues on glass plates
guideline	:	Blümel *et al*. (2000)	acceptability	:	Acceptable
test substance	:	AATERRA ME, Lot No.	LR50	:	5003 g a.s./ha
		AM1B06F001, Ref. SI 7821	ER50	:	4200 g a.s./ha
a.s. content	:	720 g etridiazole/L			
species	:	*Typhlodromus pyri*			

Methods

The effect of fresh residues of AATERRA ME (720 g etridiazole/L) on survival and reproduction of the predatory mite *Typhlodromus pyri*, confined under laboratory conditions over laboratory-treated glass plates, was determined.

Two studies were performed. Treatments with AATERRA ME at 72, 360, 720, 1440 and 2880 g a.s./ha and a positive control (dimethoate, 6 g a.s./ha) were tested in 200 L spray solution/ha against water-treated controls in the first study. A second study followed with AATERRA ME treatments at 1440, 2880, 4320, 5760 and 7200 g a.s./ha and a positive control (dimethoate, 6 g a.s./ha). Laboratory treated glass plates were allowed to dry and incorporated into test units (3/treatment), forming floor and shelter respectively. Twenty protonymphs were added to each test unit. The test units were kept in a controlled environment room until study end. Survival was assessed over a 7-day period. Fecundity assessments were carried out in the control and all AATERRA ME treatments during a second week in the same treated test units.

Results

Reported dose levels and LR50 were based on the nominal a.s. content of the test product, which was 700 g a.s./L. The dose levels and LR50 and ER50 values in the present summary were based on the reported measured a.s. content of the test product, which was 720 g a.s./L.

Test results are shown in the table below. The reference substance gave the expected response. Mortality rates were significantly increased (at 1% level) without a rate-response relation at 4320 and 7200 g a.s./ha. The LR50 was based on the results of the second test and is 5003 g a.s./ha. No statistically significant effects on mite fecundity were observed up to treatment rates of 2880 g a.s./ha. The ER50 was not reported. The RMS calculated the ER50 using the reported percentages reduction of reproduction of both tests (data from 1440 g a.s./ha upwards) and linear regression using log-transformed rate-values.

Table B.9.35 Effects of AATERRA ME on *Typhlodromus pyri* under laboratory conditions

treatment	Dose (g a.s./ha)	Mortality (%)	Corrected mortality (%)[(A)]	Mean number of eggs per female	Reduction of reproduction (%)[(B)]

treatment	Dose (g a.s./ha)	Mortality (%)	Corrected mortality (%)[A]	Mean number of eggs per female	Reduction of reproduction (%)[B]
First test:					
Water control	-	15	-	6.7	-
AATERRA ME	72	15	0	6.6	1
AATERRA ME	360	27	14	6.2	7
AATERRA ME	720	32	20	6.9	-3[C]
AATERRA ME	1440	18	4	5.6	16
AATERRA ME	2880	35	24	3.7	45
Dimethoate	6	97*	96	Not tested	-
Second test:					
Water control	-	18	-	7.2	-
AATERRA ME	1440	28	12	5.6	22
AATERRA ME	2880	33	18	5.9	18
AATERRA ME	4320	67*	60	Not tested	-
AATERRA ME	5760	37	23	2.5*	65
AATERRA ME	7200	60*	51	Not tested	-
Dimethoate	6	90*	88	Not tested	-

* Significantly different from the control at 5% level.
(A) According to the Abott formula.
(B) Reduction = 100%*(1-R), where R = Rt/Rc, Rt and Rc = average number of eggs per female in treated (t) and control (c).
(C) Note: negative %, hence no adverse effect.

Conclusion

LR50 5003 g a.s./ha, ER50 4200 g a.s./ha.

Guidelines and limitations

The study was conducted without major deviations from the guideline by Blümel *et al.* (2000) and is acceptable.

Study 3

Characteristics

reference	:	Vinall S. (2002b)	exposure duration	:	48 hours
type of study	:	Extended laboratory toxicity study	nominal concentrations	:	0, 360, 2520, 5040, 7200 and 14400 g a.s./ha
year of execution	:	2002			
GLP statement	:	Yes	exposure method	:	Dry residues on barley seedlings
guideline	:	Barrett *et al*. (1994)	acceptability	:	Acceptable
test substance	:	AATERRA ME, Lot No. AM1B06F001, Ref. SI 7821	LR50	:	8280 g a.s./ha
			ER50	:	>7200 g a.s./ha
a.s. content	:	720 g etridiazole/L			
species	:	*Aphidius rhopalosiphi*			

Methods

The effect of fresh residues of AATERRA ME (720 g etridiazole/L) on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi*, confined under laboratory conditions over laboratory-sprayed barley seedlings, was determined.

Treatments with AATERRA ME at 360, 2520, 5040, 7200 and 14400 g a.s./ha and a positive control (dimethoate, 6 g a.s./ha) were tested in 400 L spray solution/ha against water-treated controls. Laboratory-sprayed barley seedlings were allowed to dry and enclosed within cylindrical, ventilated collars (5 replicates/treatment). Five female <48 h old wasps were added to each plant. The test units were kept in a controlled environment room until study end. Mortality was assessed during 48 hours. Fifteen healthy females of the control and the 2520, 5040 and 7200 g a.s./ha treatment were removed and individually confined over untreated aphid-infested barley plants for 21 hours. The wasps were then removed and the plants were left for a further 10 days before the number of aphid mummies was recorded.

Results

Observations during the first 2½ hours of exposure showed significant reductions (at 5% level) in settling rates at and above 3600 g a.s./ha as compared to the control, hence residues of AATERRA ME were repellent at these dose rates. Other results are shown in the table below. Reproduction success was statistically significantly reduced at 5040 and 7200 g a.s./ha at the 5% level. The reference substance gave the expected response.

Table B.9.36 Effects of AATERRA ME on *Aphidius rhopalosiphi* under extended laboratory conditions

treatment	Dose (g a.s./ha)	Mortality (%)	Corrected mortality (%)[(A)]	Mean number of mummies per female	Reduction of reproduction (%)[(B)]
Water control	-	0	-	11.6	-
AATERRA ME	360	0	0	Not tested	-
AATERRA ME	2520	16	16	8.2	29
AATERRA ME	5040	12	12	8.1*	30
AATERRA ME	7200	20	20	6.9*	41
AATERRA ME	14400	96	96	Not tested	-
Dimethoate	6	100	100	Not tested	-

* Significantly different from the control at 5% level.
(A) According to Abott formula
(B) Reduction = 100%*(1-R), where R=Rt/Rc, Rt and Rc being the average number of mummies per female.

Conclusion

LR50 8280 g a.s./ha, ER50 >7200 g a.s./ha.

Guidelines and limitations

The study was conducted without major deviations from the guideline by Barrett *et al.* (1994) and is acceptable. Reported dose levels and LR50 were based on the nominal a.s. content of the test product, which was 700 g a.s./L. The dose levels and LR50 and ER50 values in the above summary were based on the reported measured a.s. content of the test product, which was 720 g a.s./L.

Study 4

Characteristics

reference	:	Taruza S. (2002b)	exposure duration	:	14 days

type of study	:	Extended laboratory toxicity study	nominal concentrations	:	0, 72, 360, 720, 3600, 7200 and 14400 g a.s./ha
year of execution	:	2002			
GLP statement	:	Yes	exposure method	:	Dry residues on isolated French bean leaves
guideline	:	Blümel *et al*. (2000)			
test substance	:	AATERRA ME, Lot No. AM1B06F001, Ref. SI 7821	acceptability	:	Acceptable
			LR50	:	6292 g a.s./ha
a.s. content	:	720 g etridiazole/L	ER50	:	>3600 g a.s./ha
species	:	*Typhlodromus pyri*			

Methods

The effect of fresh residues of AATERRA ME (720 g etridiazole/L) on survival and reproduction of the predatory mite *Typhlodromus pyri*, exposed under laboratory conditions on laboratory-treated leaf discs excised from French bean plants, was determined.

Treatments with AATERRA ME at 72, 360, 720, 3600, 7200 and 14400 g a.s./ha and a positive control (dimethoate, 12 g a.s./ha) were tested in 200 L water/ha against water-treated controls. Treatments were applied to leaf discs excised from French bean plants (BBCH stage 12-13, 3 replicate discs per treatment) under laboratory conditions (measured spray deposit within 10% from target). The residue was allowed to dry, the discs were laid on water-saturated cotton wool in Petri dishes, and 20 <24 h old *T. pyri* protonymphs were added to each test arena. The test units were kept in a controlled environment room until study end. Survival was assessed over a 7-day period, and fecundity assessments were carried out in the control and at 72, 360, 720 and 3600 g a.s./ha during a second week in the same treated test arenas.

Results

The results are summarised in the table below. There was a statistically significant increase in mortality at 7200 and 14400 g a.s./ha, but reproduction success was not statistically significantly affected at dose rates up to and including 3600 g a.s./ha. The reference substance gave the expected response.

Table B.9.37 Effects of AATERRA ME on *Typhlodromus pyri* under extended laboratory conditions

treatment	Dose (g a.s./ha)	mortality (%)	Corrected mortality (%)[A]	Mean number of eggs per female	Reduction of reproduction[B]
Water control	-	15	-	7.6	-
AATERRA ME	72	18	4	7.3	4
AATERRA ME	360	12	0	9.5	-25[C]
AATERRA ME	720	12	0	8.8	-16[C]
AATERRA ME	3600	15	0	6.7	12
AATERRA ME	7200	65*	59	Not tested	-
AATERRA ME	14400	97*	96	Not tested	-
Dimethoate	12	93*	92	Not tested	-

(A) According to Abott formula.
(B) Reduction = 100%*(1-R), where R=Rt/Rc, Rt and Rc being the average number of eggs per female.
(C) Note: negative %, hence no adverse effect.

Conclusion

LR50 6292 g a.s./ha; ER50 >3600 g a.s./ha.

Guidelines & Limitations

The study was conducted without major deviations from the guideline by Blümel *et al.* (2000), and is acceptable. Reported dose levels and LR50 were based on the nominal a.s. content of the test product, which was 700 g a.s./L. The dose levels and LR50 and ER50 values in the above summary were based on the reported measured a.s. content of the test product, which was 720 g a.s./L.

Study 5

Characteristics

reference	:	Manley B. (2002)	exposure duration	:	30 days
type of study	:	Extended laboratory toxicity study	nominal	:	0, 5112, 8208, 11304 and 14400 g
year of execution	:	2002	concentrations		a.s./ha
GLP statement	:	Yes	exposure method	:	Dry residues on isolated French
guideline	:	Vogt *et al*. (2000)			bean leaves
test substance	:	AATERRA ME, Lot No.	acceptability	:	Acceptable
		AM1B06F001, Ref. SI 7821	LR50	:	>14400 g a.s./ha
a.s. content	:	720 g etridiazole/L	ER50	:	>14400 g a.s./ha
species	:	*Chrysoperla carnea* Steph.			

Methods

The effect of fresh residues of AATERRA ME (720 g etridiazole/L) on survival and reproduction of the green lacewing *Chrysoperla carnea*, confined under laboratory conditions on laboratory-treated leaves excised from French bean seedlings, was determined.

Treatments with AATERRA ME at 5112, 8208, 11304 and 14400 g a.s./ha and a positive control (dimethoate, 83.5 g a.s./ha) were tested in 200 L water/ha against water-treated controls. Treatments were applied to the upper side of leaves excised from French bean plants (BBCH stage 12, 40 replicate discs per treatment) under laboratory conditions (measured spray deposit within 10% from target). The residue was allowed to dry, the leaves (treated side facing upwards) were used to line the floor of test arenas (40 per treatment), and one *C. carnea* larva (2-3 days old) was added to each test arena. The test was performed in a controlled environment room. Pre-imaginal mortality was assessed. Developing pupae from each treatment were transferred to large plastic storage boxes, and emerging adults were transferred to a polystyrene box for assessment of egg laying (two 24-h periods within one week) and egg viability.

Results

The results are summarised in the table below. None of the differences in mortality between the AATERRA ME treatments and the water control were statistically significant at the 5% level. No significant effect of AATERRA ME on reproduction was observed up to the highest tested rate of 14400 g a.s./ha.

Table B.9.38 Effects of AATERRA ME on *Chrysoperla carnea* under extended laboratory conditions

Treatment	Dose (g a.s./ha)	pre-imaginal mortality (%)	Corrected mortality (%)(A)	Mean number of eggs/ female/day	Egg viability (%)	Reduction of reproduction (%)(B)
Water control	-	13	-	34.6	95	-
AATERRA ME	5112	10	0	Not tested	-	-
AATERRA ME	8208	8	0	Not tested	-	
AATERRA ME	11304	15	3	42.5	92	-19(C)
AATERRA ME	14400	13	0	30.3	92	16
Dimethoate	83.5	90	89	Not tested	-	-

(A) According to Abbott formula
(B) Reduction = 100%*(1-R), where R = Rt/Rc, Rt and Rc = average number of viable eggs per treated or control female.
(C) Note: negative effect %, hence no adverse effect.

Conclusion

LR50 and ER50: >14400 g a.s./ha.

Guidelines and limitations

The study was conducted without major deviations from the guideline by Vogt *et al.* (2000) and is acceptable. Reported dose levels were based on the nominal a.s. content of the test product, which was 700 g a.s./L. The dose levels in the above summary were based on the reported measured a.s. content of the test product, which was 720 g a.s./L.

B.9.5.2 Semi-field and field studies

No study was submitted.

B.9.5.3 Risk assessment

B.9.5.3.1 Risk of the active substance

Etridiazole was systemic in cucumber after treatment of the hydroponic culture (see section B.7.1.1). For the proposed uses in glasshouses (application through (drip-) irrigation system to non-soil bound ornamentals and substrate grown peppers, tomatoes and cucumbers), non-target arthropods may therefore be exposed to etridiazole or its metabolites. Exposure of soil is not relevant for the intended non-soil bound uses. A risk assessment for the active substance etridiazole is provided below.

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002) and ESCORT 2.

As the proposed use involves two treatments with a 14-day interval, the maximum recommended dose for glass substrate during dose-response laboratory tests for parasitiods and predatory mites will be 11900 g a.s./ha (applying a MAF of 1.7). Table B.9.39 below summarises the results of laboratory testing with non-target arthropod species.

Table B.9.39 Summary of effects of Aaterra ME on non-target arthropod species during laboratory testing

Species	Test type, Substrate	Max. recommended test dose (g a.s./ha)	Actual dose tested (g a.s./ha)	Effect (%) at respective dose in test and LR50 and ER50 (reproduction) values
Aphidius rhopalosiphi	Laboratory, glass	11900	720; 1980; 3240; 4500; 5760	10; 59; 100; 100; 100 (% mortality) 24; n.a. (A); n.a. (A); n.a. (A); n.a. (A) (% reduction of reproduction) LR50 (g a.s./ha): 1494 ER50 (g a.s./ha): >720
Typhlodromus pyri	Laboratory, glass	11900	72; 360; 720; 1440; 1440 (D); 2880 (D); 2880; 4320; 5760; 7200	0; 14; 20; 4; 12; 24; 18; 60; 23; 51(% mortality) 1; 7; -3 (B); 16; 22; 45; 18; n.a. (A); 65; n.a. (A) (% reduction of reproduction) LR50 (g a.s./ha): 5003 ER50 (g a.s./ha): 4200
Aphidius rhopalosiphi	Extended laboratory, barley seedlings	11900	360; 2520; 5040; 7200; 14400	0; 16; 12; 20; 96 (% mortality) n.a. (C); 29; 30; 41; n.a. (A) (% reduction of reproduction) LR50 (g a.s./ha): 8280 ER50 (g a.s./ha): >7200
Typhlodromus pyri	Extended laboratory, plant leafs	11900	72; 360; 720; 3600; 7200; 14400	4; 0; 0; 0; 59; 96 (% mortality) 4; -25 (B); -16 (B); 12; n.a. (A); n.a. (A) (% reduction of reproduction) LR50 (g a.s./ha): 6292 ER50 (g a.s./ha): >3600
Chrysoperla carnea	Extended laboratory, plant leaves	11900	5112; 8208; 11304; 14400	0; 0; 3; 0 (% mortality) n.a. (C); n.a. (C); -19 (B); 16 (% reduction of reproduction) LR50 & ER50 (g a.s./ha): >14400

(A) n.a. = not applicable (insufficient survivors from initial phase to assess reproduction).
(B) Note: negative effect %, hence no adverse effect.
(C) n.a. = not applicable (not tested)

(D) This dose was tested twice.

According to Annex II of 91/414, data must be submitted on two sensitive species and two species that are relevant to the intended use. Annex II refers to the SETAC Guidance Document on Regulatory Testing Procedures for Pesticides with Non-target Arthropods (Barrett *et al*., 1994) as a source of guidance for testing. Since several limitations have been identified in this Guidance document it is proposed that the risk to non-target arthropods should be adequately addressed according to the recommendations of ESCORT 2. Data have been submitted on *Typhlodromus pyri* and *Aphidius rhopalosiphi* and one additional species: *Chrysoperla carnea*.

Off-field exposure is considered to be irrelevant for glasshouse applications. A Tier I assessment is performed using the data from the laboratory studies with *T. pyri* and *A. rhopalosiphi*. For this standard risk assessment, the key input is the nominal field application rate supplemented by the Multiple Application Factor (MAF):
in-field exposure= Application rate * MAF

As the proposed worst-case use of the Aaterra ME formulation involves 2 applications with a 14-day interval at the highest dose (ornamentals, 7000 g a.s./ha), the multiple application factor (MAF) for the maximum dose is 1.7.
The LR50 for *Typhlodromus pyri* and *Aphidius rhopalosiphi* on fresh dried residue on glass was determined as 5003 and 1494 g a.s./ha, respectively. These values were used for hazard quotient (HQ) calculation for in-field exposure: in-field HQ= in-field exposure/LR50. ESCORT 2 states that the risk is considered acceptable if HQ <2. Table B.9.40 below summarises the results.

Table B.9.40 Risk to *Typhlodromus pyri* and *Aphidius rhopalosiphi* as a result of in-field (0 m) exposure of the proposed Aaterra ME formulation in ornamentals, tomatoes/peppers and cucumbers (Hazard Quotient Approach), **based on LR50 values from inert substrate**

Crop / species	dose g as/ha	distance (m)	% drift	Exposure (g a.s./ha)	LR50 (g a.s./ha)	HQ	trigger value
Typhlodromus pyri							
Ornamentals	2 x 7000	0	-	11900	5003	**2.4**	2
Tomatoes/ peppers	2 x 560	0	-	952	5003	0.2	2
Cucumbers	2 x 280	0	-	476	5003	0.1	2
Aphidius rhopalosiphi							
Ornamentals	2 x 7000	0	-	11900	1494	**8.0**	2
Tomatoes/ peppers	2 x 560	0	-	952	1494	0.6	2
Cucumbers	2 x 280	0	-	476	1494	0.3	2

The HQs for application in tomatoes/peppers and cucumbers were all below 2, and an in-field risk is not present for these applications. Since the HQs for in-field exposure for *T. pyri* and *A. rhopalosiphi* for application in ornamentals are above 2, an in-field risk is assumed to be present.

The guidance document (Sanco/10329/2002 rev 2 final, referring to ESCORT 2) states that when an in-field risk is present for both indicator species, both species plus one additional species need to be tested in higher tier tests. In these tests, the guidance document states that both lethal and sublethal effects of <50% are considered acceptable (i.e. HQ<1). To address the concern for in-field exposure, arisen from the Tier I assessment, data from the extended laboratory studies with *T. pyri*, *A. rhopalosiphi* and *C. Carnea* are used for a higher tier risk assessment.
For this higher tier risk assessment, scenarios are similar to those used in the Tier I assessment.

In the extended laboratory study with *C. carnea* exposed to laboratory-sprayed leaves, no effects on survival and reproduction were noted at the highest test dose of 14400 g a.s./ha, hence effects on this species should be negligible. In the extended laboratory study with *A. rhopalosiphi* exposed to laboratory-sprayed plants, the ER50 and LR50 values for fresh residues were >7200 and 8280 g a.s./ha, respectively. In the extended laboratory study with *T. pyri* exposed to laboratory-treated leaf discs, the ER50 and LR50 values for fresh residues were >3600 and 6292 g a.s./ha, respectively. These values were used for hazard quotient calculation for in-field exposure as described above. The risk is considered acceptable if HQ <1. Table B.9.41 below summarises the results.

Table B.9.41 Risk to *Typhlodromus pyri* and *Aphidius rhopalosiphi* as a result of in-field (0 m) exposure of the proposed Aaterra ME formulation in ornamentals (Hazard Quotient Approach), **based on LR50 and ER50 values on plant leaves**

Crop / species	dose g as/ha	distance (m)	% drift	Exposure (g a.s./ha)	LR50 (g a.s./ha)	ER50 (g a.s./ha)	HQ lethal	HQ sublethal	trigger value
Typhlodromus pyri									
Ornamentals	2 x 7000	0	-	11900	6292	>3600	**1.9**	**3.3**	1
Aphidius rhopalosiphi									
Ornamentals	2 x 7000	0	-	11900	8280	>7200	**1.4**	**1.7**	1

The HQs for in-field exposure are above the trigger of 1 for *A. rhopalosiphi* and *T. pyri.* On the basis of this assessment there is still a risk.

However, all of the above calculations assume that the arthropods are exposed to the entire dose as if the product was sprayed on the leaves. This is not the case, since the product is applied by dripping onto the substrate. The relevant exposure route for non-target arthropods will therefore be, due to the systemic behaviour of etridiazole, exposure of insects parasitizing or predating on 'sap sucking' insects that are contaminated with etridiazole.
The worst case application rate is 7 kg a.s./ha for ornamentals. For the risk assessment it is assumed that the concentration of etridiazole in the sap sucking insects is equal to that in the plants. However, assuming that the total dose added to the water is absorbed by the plants is an unrealistic worst case assumption. Data for refinement can be found in study 2 of section B.7.1, which describes the fate of etridiazole in cucumbers after treatment of the water in a hydroponic growth system. The lowest test dose of 2x21.3 mg per plant was in agreement with that of the proposed GAP. The maximum TRR in

harvested cucumber was 0.911 mg eq./kg at 21 days after treatment. Data on plant and cucumber weight were not provided in the study report, but assuming that (1) each plant contains in total 8 kg of cucumbers (likely to be an unrealistic worst-case), (2) the remaining weight of the cucumber plant is 2 kg, (3) the residues are evenly distributed over the whole plant; the total residue in the entire plant with fruits is 10 X 0.911 = 9.11 mg eq.. This would suggest that the absorbed dose represents about (9.11/(2x21.3)=) 20% of the amount dosed into the water. Further, parent etridiazole represented at the most 23% of the TRR, 3 days after the first treatment, when the TRR (0.442 mg eq./kg) was much lower than the maximum TRR.

After correction of the exposure by the absorption rate of 20%, all HQ values would be below the trigger of 1 (lowered by factor 5), and they would be lower when degradation is taken into consideration. Although there are uncertainties associated with the above refinement (e.g. extrapolation from cucumber to ornamentals, even distribution of residues in whole plant), it should also be taken into account that both ER50 values were lower limit values, where no effect on reproduction was noted at the stated highest tested dose. Higher doses were not tested for effects on reproduction because of the high mortality. This suggests that the LR50 provides a good estimate to test effects on reproduction, and the trigger value of 1 was only slightly exceeded for evaluation using lethal effects.

It is reasonable to assume therefore, that the risk to non-target arthropods from the proposed applications will be acceptable.

B.9.5.3.2 Risk of metabolites

Major metabolites in cucumbers treated with etridiazole were 5-hydroxyethoxy etridiazole acid (max. 33% TRR), 3-hydroxymethyl etridiazole (max. 12% TRR), etridiazole acid (max. 18% TRR) and the glucose conjugate of 3-hydroxymethyl etridiazole (max. 17% TRR), while dichloro-etridiazole was a minor metabolite (max. 3.9% TRR). These major metabolites were present at 2.8-18%% TRR 3 days after application, and all non-conjugated metabolites had reached levels >10% TRR within 11 days, implicating that these metabolites were covered in the extended studies. Given the low risk for parent etridiazole, the risk of metabolites is considered low for the application through (drip) irrigation in the glasshouses.

B.9.6 Effects on earthworms (IIA 8.4, IIIA 10.6.1)

B.9.6.1 Toxicity

B.9.6.1.1 Toxicity of the active substance

No data were submitted.

B.9.6.1.2 Toxicity of metabolites (IIA 8.4)

Characteristics

reference	:	Teixeira D. (2000)	species	:	*Eisenia fetida*
type of study	:	Acute toxicity to earthworms	exposure duration	:	14 days
year of execution	:	1999-2000	nominal concentrations	:	0, 63, 130, 250, 500 and 1000 mg a.s./kg dry soil
GLP statement	:	Yes			
guideline	:	OECD 207	dosing method	:	mixed with silica sand
test substance	:	5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02), Lot No. AGD1785-057	acceptability	:	Acceptable
			LC50	:	>1000 mg a.s./kg dry soil
purity	:	99.8%			

Methods

In an acute toxicity study, earthworms (*Eisenia fetida*) were exposed to 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02; metabolite of etridiazole, also referred to as etridiazole acid) for 14 days in artificial soil. The test was conducted in 1 L glass jars covered with perforated plastic wrap containing 750 g of moist soil. The test substance was added to the soil as a mixture with silica sand. Nominal soil concentrations of 63, 130, 250, 500 and 1000 mg a.s./kg dry soil and a blank and solvent control were tested in 4 replicates of 10 worms each. Jars were maintained at 19-20°C under continuous light. Test conditions were: pH (6.2-6.8) and moisture content (22-25%).

Results

Two worms were lethargic at 500 mg a.s./kg dry soil. Other results are summarised in Table B.9.44. The reference product (2-chloroacetamide), tested in a separate test, gave an adequate response (LC50 25 mg a.s./kg dry soil).

Table B.9.44 Acute toxicity of 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) to *Eisenia fetida*

Nominal concentration (mg a.s./kg soil d.w.)	Mortality at 14 days (%)	Mean weight change survivors over 14 days (%)
Blank control	0	+2.7
Solvent control	0	+4.5
63	0	+3.5
130	0	+3.7
250	0	+2.6
500	0	+3.3
1000	0	+3.0

Conclusion

The NOEC is ≥1000 mg a.s./kg dry soil. The LC50 is >1000 mg a.s./kg dry soil.

Guidelines and limitations

The study was conducted according to OECD 207 and GLP and is acceptable.

B.9.6.1.3 Toxicity of the plant protection product (IIIA 10.6.1)

Characteristics

reference	:	Engelhard E.K. (1998)	species	:	*Eisenia fetida*
type of study	:	Acute toxicity to earthworms	exposure duration	:	14 days
year of execution	:	1998	nominal	:	0, 62.5, 125, 250, 500 and 1000
GLP statement	:	Yes	concentrations		mg/kg dry soil
guideline	:	OECD 207	dosing method	:	stock solution in water
test substance	:	Aaterra ME, Lot No. AE F017408 00	acceptability	:	Acceptable
		1H55 A101	LC50	:	353 mg/kg dry soil ⇔
a.s. content	:	710 g etridiazole/L			198 mg a.s./kg dry soil

Methods

In an acute toxicity study, earthworms (*Eisenia fetida*) were exposed to Aaterra ME (containing 710 g etridiazole/L) for 14 days in artificial soil. The test was conducted in 1.5 L glass beakers covered with loose-fitting glass lids containing 750 g of moist soil. The test substance was added to the soil as a solution in deionised water. Nominal soil concentrations of 62.5, 125, 250, 500 and 1000 mg/kg dry soil and a control were tested in 4 replicates of 10 worms each. Jars were maintained at 19-21°C under continuous light.

Results

Burrowing times were significantly increased by 14, 298, 195 and 502% at 125, 250, 500 and 1000 mg/kg dry soil on day 7, and by 733% at 500 mg/kg dry soil on day 14. Other results are summarised in Table B.9.45. The reference product (2-chloroacetamide), tested in a separate test, gave an adequate response (LC50 21.6 mg a.s./kg dry soil).

Table B.9.45 Acute toxicity of Aaterra ME to *Eisenia fetida*

Nominal concentration (mg/kg soil d.w.)	Mortality at 14 days (%)	Mean weight change survivors over 14 days (%)
control	0	+5.9
62.5	0	+7.1
125	0	+4.9
250	0	-9.9*
500	100*	
1000	100*	

* Significantly different from control at 5% level.

Conclusion

Based on significant effects on mean weight change over 14 days, the NOEC is 125 mg/kg dry soil. The LC50 is 353 (250-500) mg/kg dry soil. The corresponding concentrations corrected for etridiazole content are 70 and 198 (140-280) mg a.s./kg dry soil, respectively.

Guidelines and limitations

The study was conducted according to OECD 207 and GLP and is acceptable.

B.9.6.2 Risk assessment

Acute toxicity studies with Aaterra ME and etridiazole acid were submitted. However, since the proposed uses are non-soil bound and in glasshouses, it is unlikely that earthworms will be exposed to etridiazole or its metabolites. Therefore the risk to earthworms is acceptable for these uses.

B.9.7 Effects on soil non-target macro-organisms (IIIA 10.6.2)

Since the proposed uses are non-soil bound and in glasshouses, it is unlikely that soil non-target macro-organisms will be exposed to etridiazole or its metabolites. Therefore the risk to soil non-target macro-organisms is acceptable for these uses.

B.9.8 Effects on soil non-target micro-organisms (IIA 8.5, IIIA 10.7)

B.9.8.1 Toxicity

B.9.8.1.1 Toxicity of the active substance (IIA 8.5)

No data were submitted.

B.9.8.1.2 Toxicity of metabolites (IIA 8.5)

No data were submitted.

B.9.8.1.3 Toxicity of the plant protection product (IIA 8.5, IIIA 10.7)

Characteristics

reference	:	Van der Kolk J. (1998)	exposure duration	:	98-100 days
type of study	:	Soil microflora test (respiration and nitrification)	nominal concentrations	:	3.36 and 33.6 mg a.s./kg dry soil (loamy sand)
year of execution	:	1998			3.49 and 34.9 mg a.s./kg dry soil (sandy loam)
GLP statement	:	Yes			
guideline	:	BBA VI, 1-1 (1990)	dosing method	:	mixed with water
test substance	:	Aaterra ME, Lot no. AE F017408 00 1H55 A101	acceptability	:	Acceptable
			respiration	:	NOEC ≥33.6 & ≥34.9 mg a.s./kg

a.s. content	:	710 g etridiazole/L			(loamy sand & sandy loam)
test organism	:	Soil microflora	nitrate formation	:	NOEC 3.36 & 3.49 mg a.s./kg (loamy sand & sandy loam); persistent inhibitory effects at ≥33.6 & ≥34.9 mg a.s./kg

Methods

Data were submitted from a laboratory study on the effect of Aaterra ME (710 g etridiazole/L) on microbial respiration and nitrification in a loamy sand and a sandy loam soil.

Batches of a loamy sand soil (12.0, 38.0 and 50.0% particles in the <2, 2-63 and >63 µm size class, pH (KCl) 7.0, 0.9% organic carbon, microbial biomass 271 mg C/kg, 2 mm sieved) and of a sandy loam soil (24.8, 30.1 and 45.1% particles in the <2, 2-63 and >63 µm size class, pH (KCl) 5.9, 1.96% organic carbon, microbial biomass 820 mg C/kg, 2 mm sieved) were treated with the test substance mixed with water at 3.36 and 33.6 mg a.s./kg d.w. (loamy sand) or 3.49 and 34.9 mg a.s./kg d.w. (sandy loam). Control soil was left untreated. For nitrification, the soil was amended with lucerne meal (0.5%). Aliquots (99.0 and 95.5 g d.w. for the loamy sand and the sandy loam soil, respectively) were dispensed into 200 ml glass erlenmeyer flaks closed with a ground-glass stopper (24 replicates per treatment), which were incubated at 18.0-21.5°C. After two days, the glass-ground stoppers were replaced by porous cellulose paper plugs to allow air exchange. Soil moisture was maintained at 55% and 51% MWHC for the loamy sand and the sandy loam soil, respectively. On day 0, and after 14, 28, 56-57, 83 and 100 days (loamy sand) and after 14 and 28 days (sandy loam), an aliquot of 49.1-49.5 g d.w. (loamy sand) and 47.3-47.7 g d.w. (sandy loam) was removed from each bottle, amended with glucose (2020-2037 and 2516-2537 mg/kg soil for loamy sand and sandy loam, respectively), and O_2 consumption was measured for 20 hours.

On day 0, and after 14, 28, 56, 83 and 100 days (loamy sand) and after 14, 28, 56, 81 and 98 days (sandy loam), an aliquot of 19.8 g d.w. (loamy sand) and 18.9-19.1 (sandy loam) was removed from each bottle and extracted with potassium chloride. Nitrate, nitrite and ammonium were analysed by means of ion chromatogram (limits of detection 2.6, 1.2 and 0.4 mg/kg d.w. for nitrate, nitrite and ammonium respectively).

Results

Respiration

The respiration rate in the control loamy sand soil was reported as 19.1 mg O_2/3-6 hour/kg soil d.w. on day 0. This rate increased to 42.3 mg O_2/2-9 hour/kg soil d.w. on day 28 and then decreased to 23.5 mg O_2/2-9 hour/kg soil d.w. on day 100. The respiration rate in the control sandy loam soil was reported as 195.8 mg O_2/3-11 hour/kg soil d.w. on day 0. This rate decreased to 139.1 mg O_2/2-8 hour/kg soil d.w. on day 28. Effect percentages for Aaterra ME are summarised in Table B.9.46.

Table B.9.46 Effect of Aaterra ME on the respiration rate in a loamy sand soil and a sandy loam soil

Treatment rate (mg a.s./kg soil d.w.)	% deviation from control on day:					
	0	14	28	57	83	100
Loamy sand soil						
3.36	+2	+28*	+42*	+15	+14	+35*
33.6	-5	+20*	+27*	+6(A)	-12	-1
Sandy loam soil						
3.49	0	+2	0			
34.9	+1	+13*	+12*			

* significantly different from control at 5% level

(A) Measured after 56 days

Short-term respiration of treated loamy sand soil differed from untreated soil by 42 and 27% after 28 days, and by 35 and 1% after 100 days. Since the deviation from the control at 3.36 mg a.s./kg d.w. after 100 days was higher than after 57 and 83 days (no time-related trend), and since the response was also not dose related, the result after 100 days was considered not treatment related.
Short-term respiration of treated sandy loam soil differed from untreated soil by ≤13% at all sampling points.

Nitrification

Nitrite
In untreated and treated loamy sand soil, nitrite levels were 0.9-1.4 mg/kg on day 0 and decreased to levels below the limit of detection within 14 days. Nitrite levels in untreated and treated sandy loam soil were 2.0-2.4 mg/kg at day 0, and also decreased to levels below the limit of detection within 14 days.

Nitrate
Nitrate levels in untreated loamy sand and sandy loam soil were respectively 19.5 and 19.7 mg/kg at day 0, and increased to 51.1 and 63.3 mg/kg after 28 days and to 81.5 and 101.6 mg/kg after 98-100 days. The OECD 216 guideline for the nitrification test states that evaluation should be based on nitrate formation rates. In the report however, evaluation was based on absolute nitrate values. Nitrate formation rates were calculated by the reviewer from the reported absolute nitrate levels. Results for Aaterra ME based on nitrate levels and nitrate formation rates are summarised in Tables B.9.47 and B.9.48. The variation (CV) between nitrate levels of control replicates was ≤12% at any sampling and for both soils.

The following evaluation of the test results was made by the RMS. The final conclusion reached by the RMS agreed with that by the author of the report.

Nitrification, loamy sand soil
After 28 days, nitrate concentrations and nitrate formation rates in treated soils were much lower (73-99%) than in untreated soil. A strong inhibition of nitrate concentrations and nitrate formation rates

(99-128%) was still apparent at the last sampling (day 100) at the high dose. At the low dose, however, nitrate formation rates were much higher than in untreated soil during the day 28-56 and day 56-83 intervals, and this resulted in comparable nitrate concentrations (13% difference) in treated and untreated soil on the last study day for this soil (day 83). The typical inhibitory effects of the test material were therefore considered to be reversible at the low test dose.

Nitrification, sandy loam soil

After 14 days, nitrate concentrations and nitrate formation rates in treated soils were much lower (61-133%) than in untreated soil. A strong inhibition of nitrate concentrations and nitrate formation rates (65-88%) was still apparent at the last sampling (day 98) at the high dose. At the low dose, however, nitrate formation rates were much higher than in the untreated soil during all subsequent intervals, and this resulted in comparable nitrate concentrations (6% difference) in treated and untreated soil on the last study day for this soil (day 81). Also in this soil, the typical inhibitory effects of the test material were therefore considered to be reversible at the low test dose.

Ammonium

Ammonium levels in untreated loamy sand and sandy loam soil, respectively, were 3.3 and 1.1 mg/kg at day 0, and decreased to levels below the limit of detection within 28 and 14 days, respectively.

In the loamy sand soil treated at the low dose, ammonium levels increased from 2.8 mg/kg at day 0 to 23 mg/kg after 28 days, and then declined to 19 mg/kg on day 56 and <LOD at study end (day 83). In the loamy sand soil treated at the high dose, however, ammonium levels increased from 2.9 mg/kg at day 0 to a maximum of 57 mg/kg on day 83 (54 mg/kg at study end, day 100).

In the sandy loam soil treated at the low dose, ammonium levels increased from 1.2 mg/kg on day 0 to a maximum of 5.1 mg/kg on day 14, but they were below the limit of detection for the remainder of the study. At the high dose, however, ammonium levels steadily increased throughout the study from 1.4 mg/kg on day 0 to 44 mg/kg after 100 days.

Hence in both soils, the inhibition of nitrate formation was accompanied by accumulation of ammonium.

Table B.9.47 Effect of Aaterra ME on nitrification in a loamy sand soil

Treatment rate	nitrate levels (mg/kg) on day:					
(mg a.s./kg soil d.w.)	0	14	28	56	83	100
0 (control)	19.5	29.0	51.1	79.1	78.6	81.5
3.36	19.6	1.1	7.1	52.5	89.1	
33.6	19.8	0.2	0.5	9.9	1.5	0.7
Treatment rate	% deviation (nitrate levels) from control on day :					
(mg a.s./kg soil d.w.)	0	14	28	56	83	100
3.36	0	-96*	-86*	-34*	+13	
33.6	+1	-99*	-99*	-87*	-98*	-99*
Treatment rate	% deviation (nitrate formation rate) from control on day:					
(mg a.s./kg soil d.w.)	0	14	28	56	83	100
3.36	n.a.	-295	-73	62	-7420[A]	
33.6	n.a.	-306	-99	-66	1580	-128

* significantly different from control at 5% level

(A) This apparent negative effect was caused by a small decrease in the untreated soil compared to a relatively high increase in the treated soil.

Table B.9.48 Effect of Aaterra ME on nitrification in a sandy loam soil

Treatment rate	nitrate levels (mg/kg) on day:					
(mg a.s./kg soil d.w.)	0	14	28	56	81	98
0 (control)	19.7	47.2	63.3	77.4	87.3	101.6
3.49	19.0	18.6	47.5	64.8	82.3	
34.9	18.7	9.5	10.9	13.3	6.9	11.9
Treatment rate	% deviation (nitrate levels) from control on day :					
(mg a.s./kg soil d.w.)	0	14	28	56	81	98
3.49	-4	-61*	-25*	-16*	-6	
34.9	-5	-80*	-83*	-83*	-92*	-88*
Treatment rate	% deviation (nitrate formation rate) from control on day:					
(mg a.s./kg soil d.w.)	0	14	28	56	81	98
3.49	n.a.	-101	80	23	77	
34.9	n.a.	-133	-91	-83	-165	-65

* significantly different from control at 5% level

Conclusion

Loamy sand and sandy loam soil, respectively:

NOEC for effects on short-term respiration ≥33.6 and ≥34.9 mg a.s./kg soil.

NOEC for effects on nitrate formation 3.36 and 3.49 mg a.s./kg soil (persistent inhibitory effects at 33.6 and 34.9 mg a.s./kg).

Guidelines and limitations

The study was conducted according to BBA VI, 1-1 (1990) and in accordance with GLP, and is acceptable.

B.9.8.2 Risk assessment

A study on the respiration and nitrification processes in soil treated with Aaterra ME was submitted. However, since the proposed uses are non-soil bound and in glasshouses, it is unlikely that soil non-target micro-organisms will be exposed to etridiazole or its metabolites. Therefore the risk to soil non-target micro-organisms is acceptable for these uses.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6, IIIA 10.8)

B.9.9.1 Review of screening tests

Characteristics

reference	:	Lengen M. (2004)	exposure duration	:	Various
type of study	:	Literature data	nominal	:	Various
year of execution	:	Not reported	concentrations		

GLP statement	:	No	dosing method	:	Various
guideline	:	Not reported	acceptability	:	Acceptable
test substance	:	Etridiazole			
purity	:	Not reported			
species	:	Various			

The submitted paper presented available data to assess biological activity of etridiazole, which originated from bioscreen assays and a comprehensive search of published literature.

SCREENING TESTS

Spring wheat seeds treated with etridiazole (dose not reported) did not result in phytotoxicity, and there was good germination (no further information). Larvae of Southern armyworm and Colorado beetle became moribund after direct application of etridiazole (dose not stated), and unhatchability of cotton leafworm eggs treated with a 50% EC formulation (containing 45% xylene) at 10-5000 ppm ranged from 20-93% (etridiazole dose not stated).

PUBLIC LITERATURE

Mammals

Literature data indicate that the acute oral LD50 of etridiazole technical to rat is 1028 mg/kg bw, and the acute dermal toxicity to rabbit is >5000 mg/kg bw.

Earthworms

Following immersion in a fungicide solution containing 0.35% etridiazole, no survival was observed in earthworms (*Eisenia foetida*) after 7 days. Survival of earthworms fed grass clippings treated with 0.035% etridiazole was decreased by 76% after 101 days. Survival of worms exposed to treated soil was decreased by 48 and 44% after 10 and 84 days at 120 mg a.s./kg. The 14-day LC50 of Aaterra ME for earthworm was 353 mg/kg d.w.. Survival of another earthworm species, *Amynthas rodericensis*, was decreased by 25-54% after 28 days of exposure to plants treated with 35 kg a.s./ha.

Other non-target terrestrial species

The median tolerance limit of etridiazole technical to tadpole was 12 mg/L.

Insects

Etridiazole was not harmful to 4 species of parasitic wasps, 3 species of predatory mites and 5 species of predatory insects used as biological control agents (dose not reported).

Plants

Phytotoxicity was not observed in herbaceous and woody ornamental plants following treatment of the growth medium with Banrot 8G, a formulation containing 30 g etridiazole/kg and 50 g thiophanate-methyl/kg, at a dose of 0.73 g/m^2. After repeated (probably 3-4X) application of Truban (a formulation

containing 30% etridiazole) with 8 week intervals at a dose of 0.78 mL etridiazole (volume of treated surface not specified), stem calliper, top weight and root weight of fir seedlings were not affected, while a reduction in the number of stem cankers as compared to the control was observed. In field trials, establisment of soybean plants treated as seeds at doses of 1000 and 3000 mg a.s./kg seed was not affected, while a slight reduction of nodulation was observed at both doses. In glasshouse trials, nodulation of soy beans was not affected at a dose of 2000 mg a.s./kg seed. In a test with tomatoes, a very slight and a slight reduction in yield were observed at doses of 30 and 60 mg Aaterra/kg, respectively.

Microorganisms

Etridiazole inhibits nitrification by preventing the conversion of ammonium into nitrite by *Nitrosomonas* spp. Etridiazole was toxic to *Nitrosominas* at a concentration of 0.5 mg a.s./L, but not to *Nitrobacter* at a concentration of 25 mg a.s./L. EC50 values for beneficial soil fungi were above 100 mg a.s./L except for *Chaetonium globosum* and *Trichoderma viride* (EC50 of 28 and 51 mg a.s./l, respectively). EC50 values for actinomycetes were 10 (*Actinomyces sterptomycini*) or >120 mg a.s./L (*Norcardia corrallina*). Etridiazole was toxic to bacteria, with EC50 values ranging from 3 (*B. subtilis*) to 141 mg a.s./L (*B. fluorescens*). EC50 values for yeast ranged from 10 (*Hansenula anomala*) to >60 mg a.s./L (*Rhodotorula* spp.). The toxicity to microbial species was claimed to be due to a bacteriostatic effect rather than to a bacteriocidal effect.

In four soils, urea hydrolysis was not affected after treatment of the soil with Terrazole (95 g/L) at doses of 1 and 50 mg/kg soil, but nitrification was significantly inhibited at both doses.
In laboratory studies with liquid medium inoculated with soil filtrate, Terrazole significantly inhibited denitrification at doses of 10, 50 and 100 mg a.s./L. In greenhouse studies with soil and corn, Terrazole did not affect corn growth at a dose of 2.5 mg a.s./kg soil, but nitrate levels in soil were significantly higher compared to the control. In 2-year field studies, corn yield was increased by 78% and 25% in the first and second year, respectively, at a dose of 0.6 kg Terrazole/ha.

Guidelines and limitations

The paper presented the available data on biological activity of etridiazole, which is acceptable. The information submitted however does not cover the data requirement for effects on non-target plants, outlined in point 7.1 of the Guidance Document on Terrestrial Ecotoxicology, SANCO/10329/2002 rev 2 final of 17 October 2002. According to this guidance document, data should be provided for at least 6 plant species of different taxa, exposed to the highest application rate. The data for Banrot 8G do not comply with this requirement since the tested dose was lower than the highest application rate. The data for Truban cannot be used since the tested dose is unclear (volume of treated surface not specified). Lastly, in the studies with soybean and tomato (seed treatment), effects were found at the lowest test dose.

B.9.9.2 Risk assessment

Since the proposed uses are non-soil bound and in glasshouses, it is unlikely that terrestrial non-target organisms will be exposed to etridiazole or its metabolites. Therefore the risk to terrestrial non-target organisms is acceptable for these uses.

For the tadpole, a non-target aquatic species, the exposure is negligible compared to the effect concentration (highest initial PECsw 2.343 µg/L as opposed to a median tolerance limit of 12 mg/L). Therefore the risk to this non-target organism is acceptable.

B.9.10 Effects on biological methods for sewage treatment (IIA 8.7)

B.9.10.1 Effects of the active substance

Characteristics

reference	:	Mead C. (2002)	exposure duration	:	30 minutes and 3 hours
type of study	:	Activated sludge, respiration inhibition test	nominal concentrations	:	0, 1.0, 3.2, 10, 32 and 100 mg/L
year of execution	:	2002	dosing method	:	Etridiazole was added to the test vessels as a solution in water (1.0-32 mg/L) or as a suspension in water (100 mg/L)
GLP statement	:	Yes			
guideline	:	OECD 209			
test substance	:	Etridiazole Technical, batch ORC159316, SI 7843	acceptability	:	Acceptable
purity	:	99.8%	EC50	:	30-min: 105 mg/L
species	:	Activated sludge			3-hour: 32 mg/L

Methods

The effect of etridiazole (99.8% purity) on the rate of respiration of bacterial populations from activated sludge was determined after contact times of 30 minutes and 3 hours. Etridiazole was added to the test vessels as a solution in water (1.0, 3.2, 10 and 32 mg/L) or as a suspension in water (100 mg/L) and toxicity was tested at 21°C in one flask/concentration, with untreated controls in duplicate flasks. The sludge was obtained from a water treatment works containing effluent from predominantly domestic origin (final concentration in test 1.64 g solids/L). At the end of the contact time, oxygen consumption was measured for 10 minutes.

Results

After 30 minutes, the respiratory rate in the duplicate control flasks was 40.2 and 44.4 mg O_2/L/hour. At 100 mg/l, the inhibition percentage of the respiratory rate was 48%. The EC50 was estimated to be 105 mg/L.

After 3 hours, the respiratory rate in the duplicate control flasks was 37.2 and 40.2 mg O_2/L/hour. At 1.0, 3.2, 10, 32 and 100 mg/L, the inhibition percentages of the respiratory rate were 1, 7, 30, 50 and 67%, respectively. The EC50 is 32 mg/L.

The concurrently determined EC50 of the reference product 3,5-dichlorophenol was in the acceptable range of 5 to 30 mg/L.

Conclusion

The 30-minute EC50 is 105 mg/L. The 3-hour EC50 is 32 mg/L.

Guidelines and limitations

The study was conducted according to OECD 209 and in compliance with GLP.

B.9.10.2 Risk assessment

Given the nature of the proposed uses, it is unlikely that etridiazole will reach sewage treatment works in significant levels to have an impact on water treatment procedures (taking into consideration 30 min and 3 hour EC50 values for bacterial respiration of 105 and 32 mg a.s./L). Discharge of contaminated water from greenhouses may vary within the EU. This issue should be further addressed at MS level.

B.9.11 References relied on

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
8.1.1/01	Fletcher, D.	1972a	Acute oral toxicity study with Olin Terrazole in Mallard ducks Report [redacted] No.IBT J891 TER – 138 Non-GLP, Unpublished	Y	CHEM
8.1.1/02	Fletcher, D.	1972b	Acute oral toxicity study with Olin Terrazole, D8364 fog in Bobwhite quail Report [redacted] No.IBT J890 TER - 139 Non-GLP, Unpublished	Y	CHEM
8.1.2/01	Fletcher, D.W.	1981a	8-day dietary LC50 study with Terrazole technical in Bobwhite quail Report [redacted], [redacted] No.BLAL 80 QC 4 TER - 140 Non-GLP, Unpublished	Y	CHEM
8.1.2/02	Fletcher, D.W.	1981b	8-day dietary LC50 study with Terrazole technical in Mallard ducklings Report [redacted], [redacted] No.BLAL 80 DG 4 TER - 141 Non-GLP, Unpublished	Y	CHEM
8.1.3/01	Pedersen, C.A., Solatycki, A.M.	1995	Toxicity and reproduction study with Terrazole technical in Mallard ducks Report [redacted], [redacted] No.109-022-08 TER - 144 GLP, Unpublished	Y	CHEM
8.1.3/02	Pedersen, C.A., Solatycki, A.M.	1995	Toxicity and reproduction study with Terrazole technical in Bobwhite quail Report [redacted] No.109-021-07 TER - 145 GLP, Unpublished	Y	CHEM
8.2.1/01	Dionne, M.E.	2002	Etridiazole - acute toxicity to rainbow trout (Oncorhynchus mykiss) under flow-through conditions. Report [redacted] No.41.6177 TER - 67 GLP, Unpublished	Y	CHEM
8.2.1/02	Machado, M.W.	1993	Terrazole technical acute toxicity to sheepshead minnow (Cyprinodon variegatus) under flow-through conditions Report [redacted], [redacted] No.93-3-4675 TER - 176 GLP, Unpublished	Y	CHEM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
8.2.1/03	Sousa, J.V.	1998	5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) - acute toxicity to rainbow trout (Oncorhynchus mykiss) under flow-through conditions Report [redacted] No.98-3-7284 TER - 172 GLP, Unpublished	Y	CHEM
8.2.1/04	Cafarella, M.A.	2000	T-02 - acute toxicity to rainbow trout (Oncorhynchus mykiss) under static conditions Report [redacted], [redacted] No.41.6166 TER – 170 GLP, Unpublished	Y	CHEM
8.2.2.2/01	Machado, M.W.	1993	Terrazole technical - the toxicity to rainbow trout (Oncorhynchus mykiss) during an early life-stage exposure Report [redacted], [redacted] No.93-5-4767 TER – 173 GLP, Unpublished	Y	CHEM
8.2.3/01	Schocken, M.J.	1994	Etridiazole (Terrazole) - bioconcentration exposure with bluegill sunfish (Lepomis macrochirus) and identification of resulting metabolites Report [redacted], [redacted] No.9356 TER - 178 GLP, Unpublished	Y	CHEM
8.2.4/01	Dionne, M.E.	2002	Etridiazole - acute toxicity to daphnids (Daphnia magna) under flow-through conditions. Report Springborn Smithers Laboratories, U.S.A. No.41.6176 TER - 66 GLP, Unpublished	Y	CHEM
8.2.4/02	Putt, A.E.	2001	5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) - acute toxicity to daphnids (Daphnia magna) under flow-through conditions Report Springborn Laboratories Inc., U.S.A. No.41.6167 TER - 183 GLP, Unpublished	Y	CHEM
8.2.4/03	Cafarella, M.A.	2000	T-02 - acute toxicity to daphnids (Daphnia magna) under static conditions Report Springborn Laboratories Inc., U.S.A. No.41.6165 TER - 185 GLP, Unpublished	Y	CHEM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
8.2.4/04	Machado, M.W.	1993	Terrazole technical - acute toxicity to the mysid shrimp (Mysidopsis bahia) under flow-through conditions Report Springborn Laboratories Inc., U.S.A. No.93-2-4622 TER - 179 GLP, Unpublished	Y	CHEM
8.2.4/05	Dionne, M.E.	1993	Terrazole technical acute toxicity to eastern oyster (Crassostrea virginica) under flow-through conditions Report Springborn Laboratories Inc., U.S.A. No.93-2-4650 TER – 180 GLP, Unpublished	Y	CHEM
8.2.5/01	Putt, A.E.	1993	Terrazole technical - the chronic toxicity to daphnia magna under flow-through conditions Report Springborn Laboratories Inc. U.S.A., No.93-3-4662 TER – 187 GLP, Unpublished	Y	CHEM
8.2.6/01	Hoberg, J.R.	1993	Terrazole technical - toxicity to the freshwater green algae Selenastrum capricornutum Report Springborn Laboratories Inc. U.S.A., No.93-5-4757 TER - 188 GLP, Unpublished	Y	CHEM
8.2.6/02	Hoberg, J.R.	1998	5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) - toxicity to the freshwater green alga, Pseudokirchneriella subcapitata (Selenastrum capricornutum) Report Springborn Laboratories Inc., U.S.A. No.98-3-7287 TER - 184 GLP, Unpublished	Y	CHEM
8.2.6/03	Hoberg, J.R.	2000	T-02 - toxicity to the freshwater green alga, Pseudokirchneriella subcapitata Report Springborn Laboratories Inc., U.S.A. No.41.6162 TER – 186 GLP, Unpublished	Y	CHEM
8.2.6/04	Hoberg, J.R.	1993	Terrazole technical toxicity to the freshwater blue-green alga Anabaena flos-aquae Report Springborn Laboratories Inc. U.S.A., No.93-3-4710 TER – 190 GLP, Unpublished	Y	CHEM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
8.2.8/01	Hoberg, J.R.	1993	Terrazole technical - toxicity to duckweed, Lemna gibba Report Springborn Laboratories Inc. U.S.A., No.93-4-47-14 TER – 191 GLP, Unpublished	Y	CHEM
8.3.2/01	Vinall, S.	2002	A rate-response laboratory test to determine the effects of Aaterra ME (a 700 g/L formulation of etridiazole) on the parasitic wasp, Aphidius rhopalosiphi Report Mambo-Tox Ltd., United Kingdom No.UNI-01-3 TER - 20 GLP, Unpublished	Y	CHEM
8.3.2/02	Taruza, S.	2002	A rate-response laboratory test to determine the effects of Aaterra ME (a 700 g/L formulation of etridiazole) on the predatory mite, Typhlodromus pyri Report Mambo-Tox Ltd., United Kingdom No.UNI-01-4 TER – 12 GLP, Unpublished	Y	CHEM
8.3.2/03	Vinall, S.	2002	A rate-response extended laboratory test to determine the effects of Aaterra ME (a 700 g/L formulation of etridiazole) on the parasitic wasp, Aphidius rhopalosiphi Report Mambo-Tox Ltd., United Kingdom UNI-02-5 TER – 21 GLP, Unpublished	Y	CHEM
8.3.2/04	Taruza, S.	2002	A rate-response extended laboratory test to determine the effects of Aaterra ME (a 700 g/L formulation of etridiazole) on the predatory mite, Typhlodromus pyri Report Mambo-Tox Ltd., United Kingdom No.Uni-02-6 TER - 30 GLP, Unpublished	Y	CHEM
8.3.2/05	Manley, B.	2002	A rate-response extended laboratory test to determine the effects of Aaterra ME (a 700g/L formulation of etridiazole) on the green lacewing, Chrysoperla carnea Report Mambo-Tox Ltd., United Kingdom TER - 125 GLP, Unpublished	Y	CHEM

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
8.4.1/01	Engelhard, E.K.	1998	Aaterra ME: 14-day acute toxicity test with the earthworm (Eisenia foetida) Report Springborn Laboratories (Europe) A.G., Switzerland No.98-001-1055 TER - 171 GLP, Unpublished	Y	CHEM
8.4.1/02	Teixeira, D.	2000	T-02 acute toxicity to earthworms (Eisenia foetida) Report Springborn Laboratories Inc., U.S.A. No.41.6163 TER - 169 GLP, Unpublished	Y	CHEM
8.5/01	Kolk, J. Van Der	1998	Aaterra ME: the effects of the respiration and nitrification of soil microflora Report Springborn Laboratories (Europe) A.G., Switzerland No.97065 TER - 177 GLP, Unpublished	Y	CHEM
8.6/01	Lengen, M.	2004	Review of the effects of etridiazole on non-target fauna and flora Crompton Co, Bethany, Ct, U.S.A. No. Terrazole 127 TER - 223 Non-GLP, Unpublished	Y	CHEM
8.7/01	Mead, C.	2002	Etridiazole: assessment of the inhibitory effect on the respiration of activated sewage sludge Report Safepharm Laboratories Ltd., United Kingdom No.1133/013 TER - 23 GLP, Unpublished	Y	CHEM