

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Etridiazole

EC Number: 219-991-8

CAS Number: 2593-15-9

Index Number: 613-133-00-X

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Etridiazole
EC number:	219-991-8
CAS number:	2593-15-9
Annex VI Index number:	613-133-00-X
Degree of purity:	≥ 970 g/kg
Impurities:	Confidential information

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	Directive 67/548/EEC (Dangerous Substances Directive; DSD)
Current entry in Annex VI, CLP Regulation	Carc. 2: H351 Acute Tox. 3 *: H331 Acute Tox. 4 *: H312 Acute Tox. 4 *: H302 Aquatic Acute 1: H400 Aquatic Chronic 1: H410	Carc. Cat. 3; R40 T; R23 Xn; R21/22 N; R50/53
Current proposal for consideration by RAC	Removal of Acute Tox. 3 *: H331 Removal of Acute Tox. 4 *: H312 Removal of (*) from Acute Tox. 4 *H302 STOT SE 3: H335 Skin Sens 1B: H317	Removal of T; R23 Removal of Xn; R21 Xi; R37 Xi; R43

	Acute M-factor 1 Chronic M-factor 1	
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 2: H351 Acute Tox. 4: H302 STOT SE 3: H335 Skin Sens 1B: H317 Aquatic Acute 1: H400 Aquatic Chronic 1: H410 Acute M-factor 1 Chronic M-factor 1	Carc. Cat. 3; R40 Xn; R22 Xi; R37 Xi; R43 N; R50/53

*Minimum classification

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

A review of the available information for etridiazole has revealed that the classification listed in Annex VI of Regulation EC no.1272/2008 (including the 1st ATP) is not in agreement with the data. This proposal seeks to amend the current classification and labelling of etridiazole.

Pursuant to Commission Regulation (EC) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures, a subcategory for skin sensitization, and both acute and chronic M-factors are derived.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification ¹⁾	Reason for no classification ²⁾
2.1.	Explosives	-	-	-	Data conclusive but not sufficient for classification
2.2.	Flammable gases	-	-	-	Data conclusive but not sufficient for classification
2.3.	Flammable aerosols	-	-	-	Data conclusive but not sufficient for classification
2.4.	Oxidising gases	-	-	-	Data conclusive but not sufficient for classification
2.5.	Gases under pressure	-	-	-	Data conclusive but not sufficient for classification
2.6.	Flammable liquids	-	-	-	Data conclusive but not sufficient for classification
2.7.	Flammable solids	-	-	-	Data conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	-	-	-	Data conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	-	-	-	Data conclusive but not sufficient for classification
2.10.	Pyrophoric solids	-	-	-	Data conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	-	-	-	Data conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Data conclusive but not sufficient for classification
2.13.	Oxidising liquids	-	-	-	Data conclusive but not sufficient for classification
2.14.	Oxidising solids	-	-	-	Data conclusive but not sufficient for classification
2.15.	Organic peroxides	-	-	-	Data conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	-	-	-	Data conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox.4 (H302)	-	Acute Tox. 4* (H302)	
	Acute toxicity - dermal	-	-	Acute Tox. 4* (H312)	Data conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	Acute Tox. 3* (H331)	Data conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	-	-	-	Data conclusive but not sufficient for classification

S-phrases:

S2 Keep out of reach of children

S36/37 Wear suitable protective clothing and suitable gloves

S46 If swallowed, seek medical advice immediately and show this container or label.

S60 This material and its container must be disposed of as hazardous waste

S61 Avoid release to the environment. Refer to special instructions/safety data sheet

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Etridiazole was notified as an existing active substance and assessed in accordance to Directive 91/414/EEC concerning the placing of plant protection products on the market, with a view to the possible inclusion of the substance into Annex I to the directive (Draft Assessment Report, April 2007, additional report, December 2009 and subsequent addenda, May and June 2010, RMS the Netherlands).

No registration dossiers are available for Etridiazole checked via REACH-IT on December 21, 2011.

Etridiazole was added to Annex I of Directive 67/548/EEC in the 25th ATP (Commission Directive 98/98/EC of 15 December 1998) with classification Carc. Cat. 3;R40, T;R23, Xn;R21/22, N;R50/53.. Etridiazole was discussed in the TC-C&L in November 1995, November 1996 and May 1997 (Summary records ECBI/94/95, ECBI/45/96, ECBI/27/97, see Annex). The basis for the classification is unknown.

Etridiazole is currently listed (entry 613-133-00-X) in Annex VI of Regulation EC no. 1272/2008 with the same classification as was listed in the 25th ATP to Directive 67/548/EEC.

Since the basis for this classification is unknown due to a lack of information on the studies used, it is not possible to indicate in this report which studies are new information compared to the studies used for the classification in the 25th ATP to Directive 67/548/EEC.

2.2 Short summary of the scientific justification for the CLH proposal

The available data on etridiazole do not support the current harmonised classification for acute toxicity (R21 and R23). According to data presented in the DAR, the lowest oral LD₅₀ values found were 945 mg/kg bw female rats. Etridiazole is considered not acutely toxic via dermal and inhalation routes. In accordance with the CLP regulation 1272/2008, etridiazole should be classified as Acute Tox 4 (H302). The reference indicating minimum classification (*) and the classification with Acute Tox 3 (H331) and Acute tox 4 (H312) is no longer necessary.

According to the data presented in the DAR, etridiazole causes irritation of the respiratory tract and is positive in a Maximisation Test for skin sensitisation. Therefore, etridiazole should be classified with STOT SE 3 (H335) and Skin Sens 1B (H317).

The available data in the DAR support the current classification with Carc 2 (H351), Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410).

Information on reproductive toxicity is also presented in the CLH dossier since the issue of possible classification based on fetal malformations should be flagged to RAC according to the discussion in the Pesticide Risk Assessment Peer Review Unit (PRAPeR) meeting.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Table 5 Current Annex VI table 3.1 classification and labelling

Classification	Labelling
----------------	-----------

Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)
Carc.2	H351	GHS06	H351	
Acute Tox 3*	H331	GHS08	H331	
Acute Tox 4*	H312	GHS09	H312	
Acute Tox 4*	H302	Dgr	H302	
Aquatic Acute 1	H400		H410	
Aquatic Chronic 1	H410			

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Table 6 Current Annex VI table 3.2 classification and labelling

Classification	Labelling
Carc. Cat.3; R40 T; R23 Xn; R21/22 N; R50/53	T; N R: 21/22-23-40-50/53 S: 1/2-36/37-38-45-60-61

2.4 Current self-classification and labelling

Not applicable.

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Not applicable.

2.4.2 Current self-classification and labelling based on DSD criteria

Not applicable.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Etridiazole is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (CLP, article 36.2).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

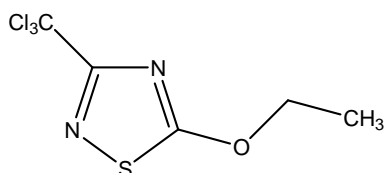
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	219-991-8
EC name:	5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole
Iso name:	etridiazole
CAS number (EC inventory):	2593-15-9
CAS number:	2593-15-9
CAS name:	1,2,4-Thiadiazole, 5 ethoxy-3-(trichloromethyl)-
IUPAC name:	5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole
CLP Annex VI Index number:	613-133-00-X
Molecular formula:	C ₅ H ₅ Cl ₃ N ₂ OS
Molecular weight range:	247.5

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Etridiazole	Minimum 970 g/kg	-	-

Current Annex VI entry:

Table 3.1: Carc. 2 (H351), Acute Tox 3* (H331), Acute Tox 4* (H312), Acute Tox 4* (H302), Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410)

Table 3.2: Carc.Cat.3;R40, T;R23, Xn;R21/22, N;R50/53

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
			All impurities have been claimed confidential. However, based on the DAR there are no (eco)toxicological relevant impurities present.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
				Not applicable

1.3 Physico-chemical properties

Table 9 Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Pure: semisolid (mixture of a clear, colourless solid and a clear colourless liquid). Colourless crystalline powder with no characteristic odour Technical: semisolid (mixture of a clear, colourless solid and a yellow liquid).	DAR	
Melting/freezing point	22.0°C	DAR	measured
Boiling point	113°C at reduced pressure (0.53 kPa)	DAR	measured
Relative density	1.497 at 25°C.	DAR	measured
Vapour pressure	0.01073 mm Hg at 25°C (equivalent to 1.43 Pa, calculated by RMS)	DAR	measured
Surface tension	64.26 mM/m at 20°C (saturated aq. soln.)	DAR	measured
Water solubility	117.1 mg/L in water at 25°C; 85.8 mg/L in buffer pH 4 at 25°C; 88.9 mg/L in buffer pH 7 at 25°C; 89.7 mg/L in buffer pH 10 at 25°C.	DAR	measured
Partition coefficient n-octanol/water	Log Pow (aqueous pH7 buffer/n-octanol) at 26°C to 27.5°C: 3.37.	DAR	measured
Flash point	111°C		measured
Flammability	not applicable for liquids.	DAR	measured
Explosive properties	No explosive properties	DAR	measured
Self-ignition temperature	342°C	DAR	measured
Oxidising properties	No oxidising properties	DAR	From structural reasons the test substance has no oxidising properties
Granulometry	not applicable for liquids		
Stability in organic solvents and identity of relevant degradation products	At 20°C Etridiazole is miscible in all proportions with n-Heptane, toluene, xylenes, dichloromethane, 1,2-dichloroethane, methanol, acetone, ethyl acetate and n-octanol	DAR	measured The active substance as manufactured didn't include any organic solvent
Dissociation constant	Pka = 2.77 at 25°C	DAR	measured
Viscosity	Pure material at 25°C: liquid with low viscosity.	DAR	measured

DAR = Draft Assessment Report

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Etridiazole is used as a fungicide (to control soil and root fungi) in the treatment of substrate grown tomato, cucumber, pepper, and ornamentals (non-soil bound), and is applied in glasshouses by drip-irrigation.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of etridiazole were assessed in the Draft Assessment Report and the Addendum to the Draft Assessment Report prepared in the context of the possible inclusion of etridiazole in Annex I of Council Directive 91/414/EEC (DAR April 2007, additional report December 2009 and the addendum May 2010 RMS The Netherlands). Since the basis for the current classification is unknown due to a lack of information on the studies used, it is not possible to indicate in this CLH report which studies are new information compared to the studies used for the classification in the 25th ATP to Directive 67/548/EEC.

No changes in the classification for the physico-chemical endpoints are proposed in this dossier. For this reason, it is considered not warranted to present the data relating on physical hazards in this dossier and no consideration by RAC on the physico-chemical properties is required.

4 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazards of etridiazole were assessed in the Draft Assessment Report and additional report of the Netherlands prepared in the context of the possible inclusion of the active substance etridiazole in Annex I of Council Directive 91/414/EEC (DAR April 2007 and additional report December 2009, RMS The Netherlands).

Based on a review of the available data on acute and repeated toxicity, an update in the classification is needed. The summaries included in this proposal are copied from the additional report, which includes all the text from the DAR. Details of some of the summaries were not included when not considered important for a decision on the classification and labelling of this substance. References to individual studies are not included. For more details the reader is referred to the DAR, additional report and its addenda. These are available at: <http://www.efsa.europa.eu/en/efsajournal/pub/1823.htm>

In this proposal we include information related to the following hazard class, acute toxicity, irritation and skin sensitization. Information on reproductive toxicity is also presented since the issue of possible classification based on fetal malformations should be flagged to ECHA according to the discussion in the Pesticide Risk Assessment Peer Review Unit (PRAPeR) meeting. In addition, to provide an overview of the substance information related to the toxicokinetics of etridiazole is included.

Etridiazole was also discussed in the TC-C&L in November 1995, November 1996 and May 1997 (Summary records ECBI/94/95, ECBI/45/96, ECBI/27/97, see Annex). Since the basis for the current classification is unknown due to a lack of information on the studies used, it is not possible to indicate in this CLH report which studies are new information compared to the studies used for the classification in the 25th ATP to Directive 67/548/EEC. Based on the differences in results in e.g. the acute toxicity studies in the DAR compared to the current classification it is concluded that the studies in the DAR were not available to the TC-C&L in 1995-1997, despite that these studies were conducted in 1994.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The summary from the additional report (Volume 3, B.6.1.4) is copied below.

Absorption

In one study in rats, at 168 hours after a single oral dose of 5 and 150 mg/kg bw, or repeated oral dosing at 5 mg/kg bw, 58-73% Administered radioactivity (AR), 14-16% AR and 4.2-7.4% AR, respectively, was excreted with urine, faeces and expired air, whilst 2.4-3.9% AR was retained in tissues. Excretion and retention after single intravenous dose administration was comparable to that for oral dosing. Radioactivity excreted with faeces following oral dosing may therefore be assumed to represent absorbed radioactivity, excreted via the biliary pathway. From the results of this study, oral absorption is estimated to be 100%.

In another study in rats, peak ¹⁴C-concentrations in blood were observed after 4 hours in both sexes after a single oral dose of 5 mg/kg bw, but only after 8 and 21 hours in males and females, respectively, that received a single oral dose of 150 mg/kg bw.

Elimination

In one study in rats, at 168 hours after a single oral dose of 5 and 150 mg/kg bw, or repeated oral dosing at 5 mg/kg bw, the majority of administered radioactivity was excreted in urine (58-73% AR), whilst radioactivity in faeces accounted for 14-16% AR and in expired air for 4.2-7.4% AR. Pre-treatment for 14 days did not affect the pattern of excretion and retention.

In another study in rats, the elimination half-life in blood after a single oral dose of 5 mg/kg bw (14 hours for both sexes) was much shorter than after a single oral dose of 150 mg/kg bw (36 and 60 hours in males and females, respectively). At 168 hours after a single oral dose of 5 and 150 mg/kg bw, the majority of administered radioactivity was excreted in urine (62-78% AR), whilst radioactivity in faeces accounted for 14-21% AR. At the high dose, the rate of excretion in urine was slower in females than in males (33% and 50% AR after 24 hours, respectively).

Distribution

In one study in rats, at 168 hours after a single oral dose of 5 mg/kg bw, the highest radioactivity concentrations were found in liver (0.83-0.97 mg eq./kg), kidney (0.77-0.86 mg eq./kg) and lung (0.45-0.47 mg eq./kg), and the lowest in brain (0.08-0.09 mg eq./kg) and fat (0.05-0.06 mg eq./kg). Comparable concentrations were found in tissues and organs of rats after multiple oral dosing with 5 mg/kg bw. After a single oral dose of 150 mg/kg bw, the pattern of distribution was comparable to that after a single oral dose of 5 mg/kg bw, but the concentrations in high dose male and female rats were on average a factor of 39 and 32, respectively, higher than in low dose rats (hence roughly proportional to the dose).

In another study in rats, radioactivity in tissues (including the residual carcass) at 168 hours post a single oral dose of 5 or 150 mg/kg bw represented 3.2-4.7% AR. At the low dose, at $\frac{1}{2} t_{max}$ and 168 hours post-dose, respectively, radioactivity concentrations in tissues with quantifiable residues were on average 41-54% and 8-9%, and at the high dose 34-43% and 12-15%, of those at t_{max} . At t_{max} , $\frac{1}{2} t_{max}$ and 168 hours post-dose, respectively, the concentrations in tissues with quantifiable residues in rats receiving the high dose were on average a factor of 28-29, 14-18 and 30-34, respectively, higher than in low dose rats (hence roughly proportional to the dose). There were no remarkable differences between tissue levels and depletion rates of male and female rats.

Metabolism

In 0-24 hour urine of rats treated with ¹⁴C-etridiazole (single oral dose of 5 and 150 mg/kg bw, or repeated oral dosing at 5 mg/kg bw), which contained 28-60% AR, the metabolite pattern was essentially the same for sexes and dosing regimes. Parent compound was not identified in urine. The main metabolite in urine was etridiazole carboxylic acid (20-36% AR, 53-71% TRR), which was also the main (and only identified) component in faeces. Other metabolites identified in urine were N-carbethoxy oxamic acid (4.1-12% AR, 14-20% TRR), ethyl (aminocarbamyl) carbamate (0.5-4.3% AR, 1.0-7.2% TRR), N-acetyl cysteinyl conjugate of etridiazole (0.3-2.0% AR, 0.9-3.6% TRR) and, tentatively, oxalic acid, at low levels (presumably <1% AR, <2% TRR). A multitude of unidentified polar components, each present at low levels, eluted close to natural urinary compounds such as uric acid, urea, hippuric acid etc. Metabolite identification in urine is acceptable for Annex I inclusion in 91/414/EEC for the major fractions only (metabolite 1, multi-component mixture of polar fractions; etridiazole carboxylic acid (20-36% AR, 53-71% TRR), and N-carbethoxy oxamic acid (4.1-12% AR, 14-20% TRR). It is uncertain whether the minor fractions (<7% AR) in urine are the result of metabolism, or that they were already present in the test material used for treating the rats, which was of low radiochemical purity (93-97%).

4.1.2 Human information

Not available

4.1.3 Summary and discussion on toxicokinetics

For more details the reader is referred to the additional report December 2009, Volume 3, B.6.1.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD 401 Acute oral toxicity	LD ₅₀ male: 1141 mg/kg bw LD ₅₀ female: 945 mg/kg bw	Rat: Sprague-Dawley CrI:CD BR VAF/Plus	DAR, Annex IIA, 5.2.1/01, 1994
OECD 402 Acute dermal toxicity	LD ₅₀ > 5000 mg/kg bw (male and female)	Rabbit, New Zealand White	DAR, Annex IIA, 5.2.2/01, 1994
OECD 403 Acute inhalation toxicity	LC ₅₀ > 5.7 mg/L	Rat: Sprague-Dawley CrI:CD BR VAF/Plus	DAR, Annex IIA, 5.2.3/01, 1994

For the current classification, etridiazole was discussed in the TC-C&L in November 1995, November 1996 and May 1997 (Summary records ECBI/94/95, ECBI/45/96, ECBI/27/97: see Annex). Since the basis for the current classification is unknown due to a lack of information on the studies used, it is not possible to indicate in this CLH report which studies are new information compared to the studies used for the classification in the 25th ATP to Directive 67/548/EEC. Based on the differences in results in e.g. the acute toxicity studies in the DAR/additional report compared to the current classification it is concluded that the studies in the DAR/additional report were not available to the TC-C&L in 1995-1997, despite that these studies were conducted in 1994.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In this study in rats, performed according to OECD 401, doses of 700, 850, 1000 and 1400 mg/kg bw were used. Mortality occurred in 2/5 males and 3/5 females given 1000 mg/kg bw within 6 days after treatment, and another 2 females were found dead on day 7 after treatment. 4/5 males and 5/5 females given 1400 mg/kg bw were found dead within 6 days after treatment. No further mortality occurred. Clinical signs were observed in nearly all animals at all dose levels (decreased activity and decreased defecation), which was normal again within 10 days after treatment in surviving animals. Other symptoms at 850 mg/kg and above were hunched posture, ataxia, dry red material around the eyes, mouth or nose, anogenital staining, or no stool. These findings generally disappeared before the end of the experiment. Body weight loss or decreased body weight gain was observed at 850 mg/kg bw and above. No significant pathology observation were seen at necropsy in the surviving animals. Some animals that had died during the study revealed foci of the glandular and/or nonglandular stomach mucosa, red or yellow foci of the liver, tan discoloration of the liver, and red discoloured lung. The acute oral LD₅₀ of etridiazole was found to be 1141 mg/kg bw in male rats and 945 mg/kg bw in female rats.

4.2.1.2 Acute toxicity: inhalation

In the additional report (Volume 3, B.6.2.1 and B.6.3.3) one acute and one subacute repeated dose inhalation toxicity study were available. From these studies it appeared that etridiazole is irritating to the respiratory tract (see 4.4.3). Moreover, the current classification for toxic by inhalation is no longer necessary, based on the new acute inhalation study. Therefore the study evaluation is copied from the additional report:

STUDY

Characteristics

reference	: DAR, Annex II, 5.2.3/01	exposure	: 4 hours; nose only
type of study	: Acute inhalation toxicity study	doses	: 1.2 and 5.7 mg/l (actual concentrations); MMAD 1.9-3.8 µm ; GSD 1.60-1.81 µm
year of execution	: 1993	vehicle	: None
test substance	: Terrazole Technical (Etridiazole), Lot no. 208S052, purity 99.4%	GLP statement	: Yes
route	: Inhalation	guideline	: In accordance with OECD 403 (1981)
species	: Rat, Sprague-Dawley derived CrI:CD BR VAF/Plus	acceptability	: Acceptable
group size	: 5/sex/dose	LC ₅₀	: > 5.7 mg/l

Study design

The study was performed in accordance with OECD 403 (1981).

Results

Mortality: No mortality was observed.

Symptoms of toxicity: Immediately after exposure (within 1 day) to 5.7 mg/l, decreased activity (M: 4/5; F:1/5), abnormal gait (M:1/5; F:4/5), laboured breathing (M:1/5; F:5/5) and increased salivation (M:1/5; F:2/5) was observed. After day 1, rapid respiration (M:3/5; F:5/5), hair loss (M:1/5; F:2/5), laboured breathing (F:2/5) and abnormal gait (F:1/5) was observed, and these effects disappeared before the termination of the experiment. Immediately after exposure (within 1 day) to 1.2 mg/l, decreased activity (M:5/5; F:3/5), rapid respiration (M:5/5; F:5/5) and abnormal gait (F:1/5) was observed. After day 1, one male showed decreased activity, one female showed rapid respiration and another female showed hair loss. These effects were reversible.

Body weight: No effects on body weight were observed in the 1.2 mg/l group; body weight gain was normal. In the 5.7 mg/l group, body weight gain was decreased after 8 days, but was normal again after 15 days.

Pathology: No treatment-related macroscopic observations were seen.

Conclusions

The acute inhalation LC₅₀ of etridiazole in rats was found to be greater than 5.7 mg/l for both males and females.

4.2.1.3 Acute toxicity: dermal

In this study, performed according to OECD 402, a limit dose of 5000 mg/kg bw was used. No mortality occurred. Clinical signs observed consisted of inappetance and decreased defecation and/or no stool. These effects disappeared within a week after treatment. Body weight loss was observed during the first 8 days after treatment in 4/5 males and 4/5 females. No significant pathology observation were seen at necropsy. The acute dermal LD₅₀ of etridiazole was found to be >5000 mg/kg bw in male and female rabbits.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

In an acute oral toxicity in rats, the LD₅₀ for etridiazole was 1141 mg/kg bw/d in male rats and 945 mg/kg bw in female rats). Etridiazole did not produce mortality in dermal and inhalation toxicity studies in rats (LD₅₀ >5000 mg/kg bw and LC₅₀ >5.7 mg/L respectively).

4.2.4 Comparison with criteria

Etridiazole has an oral LD₅₀ in female rats of 945 mg/kg bw. In 67/548/EEC this is considered harmful (200 < LD₅₀ ≤ 2 000 mg/kg bw). According to EC 1272/2008 etridiazole falls within category 4 (300 < Category 4 ≤ 2 000 mg/kg bw). The minimum classification Acute Tox Cat 4*, is considered no longer necessary.

No classification is necessary for acute dermal toxicity, since the LD₅₀ (>5000 mg/kg bw) is above the limits in 67/548/EEC (400 < LD₅₀ ≤ 2 000 mg/kg bw) and EC 1272/2008 (1000 < Category 4 ≤ 2 000 mg/kg bw).

No classification is necessary for acute inhalation toxicity, since the LC₅₀ (>5.7 mg/L) is above the limits in 67/548/EEC for aerosols and particulates (1 < LC₅₀ ≤ 5 mg/litre/4h) and EC 1272/2008 category 4 for dust/mist (1.0 < Category 4 ≤ 5.0 mg/L).

4.2.5 Conclusions on classification and labelling

According to 67/548/EEC, etridiazole should be classified as Xn; R22. According to EC 1272/2008 etridiazole should be classified as Acute Tox. 4: H302.

4.3 Specific target organ toxicity – single exposure (STOT SE)

The available data indicate that etridiazole does not need to be classified for specific target organ toxicity, with the exemption of respiratory tract irritation (see 4.4.3).

4.4 Irritation

4.4.1 Skin irritation

No skin irritation was observed in the skin irritation study in rabbits, performed according to OECD 404. Etridiazole does not need to be classified on the basis of its skin irritation. This is in accordance with the current classification. No consideration of RAC on the skin irritating properties is required.

4.4.2 Eye irritation

Only slight eye irritation was observed in the eye irritation study in rabbits, performed according to OECD 405. Etridiazole does not need classification for eye irritation. This is in accordance with the current classification. No consideration of RAC on the eye irritating properties is required.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In the additional report (Volume 3, B.6.2.1 and B.6.3.3) one acute and one subacute repeated dose inhalation toxicity study were available. From these studies it appeared that etridiazole is irritating to the respiratory tract. Therefore the evaluation of the acute toxicity inhalation study is copied under 4.2.1.2 and the evaluation of the repeated dose toxicity study is copied under 4.7.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

In the acute inhalation study (see 4.2.1.2) laboured breathing, rapid respiration were observed immediately after exposure (within 1 day) at 1.2 mg/L and at 5.7 mg/L. After day 1 only 1 female showed rapid respiration. No treatment-related macroscopic observations were seen.

In the repeated inhalation toxicity study (see 4.7) no NOAEL could be derived for local effects, because squamous metaplasia of the larynx mucosa was observed at all dose levels (LOAEL 15 mg/m³ = 0.015 mg/L).

4.4.3.4 Comparison with criteria

In 67/548/EEC substances could be classified as irritating to the respiratory tract (R37) based on positive results from appropriate animal tests. Positive results from appropriate animal tests may include data obtained in a general toxicity test, including histopathological data from the respiratory system.

On the other hand, substances should be classified with R48 Danger of serious damage to health by prolonged exposure when serious damage (clear functional disturbance or morphological change which has toxicological significance) is likely to be caused by repeated or prolonged exposure. Substances and preparations are classified at least as harmful when these effects are observed at levels of the order of ca < 0.75 mg/l, 6h/day (subacute inhalation, rat).

R48 is more appropriate for systemic effects and therefore not appropriate for the local effects observed in the larynx mucosa. According to the criteria for R37, conditions normally leading to classification with R37 are reversible and usually limited to the upper airways. The local effects found in the study (minimal metaplasia in the ventral seromucinous glands of the larynx) was considered an adaptive and non-adverse direct local effect of the test substance. The epithelium on the ventral floor of the larynx is especially sensitive to inhaled materials. Therefore, this is considered to be a target site for histopathological evaluations following inhalation exposure to particulates, vapours and aerosols. Literature data indicate that the chemicals, such as etridiazole, that induce α_{2u} -globulin accumulation (CIGA) do not appear to react with DNA and are generally negative in short term tests for genotoxicity¹. CIGA binding to α_{2u} -globulin is reversible and not covalent in nature. Hence the metaplasia is not induced by genotoxicity, but is a local effect. Based on this information classification for etridiazole with R37 is considered more appropriate.

Similarly, according to EC 1272/2008 a substance can be classified for specific target organ toxicity after a single dose or after repeated doses. The observed findings (breathing difficulties, squamous metaplasia) are considered signs of reversible respiratory tract irritation. Hence classification with specific target organ toxicity after a single dose (STOT SE Cat.3, H335) is more appropriate.

4.4.3.5 Conclusions on classification and labelling

According to 67/548/EEC, etridiazole should be classified as Xi; R37. According to EC 1272/2008 etridiazole should be classified as STOT SE Cat.3, H335.

4.5 Corrosivity

The results of the available acute dermal toxicity study and the skin irritation study gave no indication for corrosivity of the test substance. Etridiazole does not need classification for corrosivity. This is in accordance with the current classification.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 11: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406 Skin sensitisation, Magnusson & Kligman	Skin sensitising (100% of animals)	Guinea-pig, Dunkin/Hartley	DAR, Annex IIA, 5.3.2/01, 1993

4.6.1.1 Non-human information

The study was performed in accordance with OECD 406 and conducted according to Magnusson and Kligman. Only female Dunkin/Hartley Guinea pigs were used (20 control and 20 test animals). Formalin was used as the positive control, Alembical D as the vehicle. Dose levels were based on the results of a range-finding study: intradermal induction 20%, topical induction 100%, and challenge 100 and 50% (first challenge) and 20, 10, and 5% (second challenge). Sodium lauryl sulphate was used 1 day before topical induction. Skin reactions were observed after the induction

¹ Gordon C. Hard, Imogene Sevin Rodgers, Karl P. Baetcke, William L. Richards, Robert E. McGaughy, and Lawrence R. Valcovic; Hazard Evaluation of Chemicals that cause Accumulation of α_{2u} -globulin, Hyaline Droplet Neuropathy, and Tubule Neoplasia in the Kidneys of Male Rats; Environmental Health Perspectives, Vol. 99, pp. 313-349, 1993.

phase. After topical challenge, no to moderate erythema and no to well-defined oedema was observed in the treated animals. Also thickening, dryness and sloughing of the epidermis was noted. The effects were more marked after challenge with undiluted etridiazole, compared to 50% concentration. All treated animals were sensitised. After topical rechallenge with 20, 10, 5, and 1% etridiazole, the number of sensitised animals was 20, 16, 8, and 1, respectively. Topical challenge and rechallenge in control animals did not induce any dermal reaction. Results of the positive control were not reported.

4.6.1.2 Human information

From medical surveillance data of the manufacturing plant, it is evident that allergic skin reactions may be possible if inappropriate personal protection measures are taken. All workers in an Etridiazole plant were subjected to medical examination. Five workers showed long lasting eczemas (weeks to months), clinically due to a type IV sensitization (DAR Annex IIA 5.9, 2004). In another medical survey in 2002, 42% of the respondents indicated the experience of a dry skin or eczema, while the reference group had a score of 17%. A repeated of the survey in 2005 in the same plant did not result in respondents indicating the experience of dry skin or eczema (DAR Annex IIA 5.9, 2009).

4.6.1.3 Summary and discussion of skin sensitisation

Etridiazole is considered sensitising to the skin, as all 20 animals treated with etridiazole in a Magnusson & Kligman test showed skin sensitisation after challenge, compared to no skin reaction in the control animals.

4.6.1.4 Comparison with criteria

Etridiazole showed skin reactions in 100% of the animals, in an adjuvant type guinea pig test method for skin sensitisation. A response of at least 30% of the animals is considered positive according to 67/548/EEC and EC 1272/2008. The induction concentrations were >1%, which lead to Skin.sens. Cat 1B according to EC 1272/2008.

4.6.1.5 Conclusions on classification and labelling

According to 67/548/EEC, etridiazole should be classified as Xi; R43. According to EC 1272/2008 etridiazole should be classified as Skin Sens. Cat. 1B. H317.

4.6.2 Respiratory sensitisation

No studies were submitted. Etridiazole does not need classification for respiratory sensitisation, since no relevant information was available. This is in accordance with the current classification.

4.7 Repeated dose toxicity

Etridiazole should be classified for respiratory irritation based on the findings in the acute and repeated dose inhalation studies (see 4.4.3 and 4.7); the repeated dose inhalation study evaluation is copied from the additional report and included in 4.7. In the repeated dose toxicity studies via the oral and dermal route there were no indication for classification. Etridiazole does not need classification for repeated dose toxicity. This is in accordance with the current classification.

Table 12: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
OECD 410: 28-d dermal toxicity	NOAEL _{local} : 1000 mg/kg bw/d NOAEL _{sys} : 20 mg/kg bw/d	Rats, Sprague Dawley CrI:CD(SD)IGS BR	DAR, Annex IIA, 2002
OECD 412: 28-d inhalation toxicity	NOAEC _{local} : <15 mg/m ³ NOAEC _{sys} : 15 mg/m ³	Rats, Sprague Dawley CrI:CD(SD)IGS BR	DAR, Annex IIA, 2002
OECD 408: 90-d oral toxicity	NOAEL male: 2.7 mg/kg bw/d NOAEL female: 3.3 mg/kg bw/d	Rats, Sprague Dawley CrI:CD(SD)IGS BR	DAR, Annex IIA, 5.3.2/01 1994
OECD 452: 1-y oral toxicity	NOAEL male: 3.1 mg/kg bw/d NOAEL female: 4.3 mg/kg bw/d	Dog, Beagle	DAR, Annex IIA, 5.3.2/03, 2002

With regard to effects on the respiratory tract, in the additional report (Volume 3, B.6.2.1 and B.6.3.3) one acute and one subacute repeated dose inhalation toxicity study were available. From these studies it appeared that etridiazole is irritating to the respiratory tract (see 4.4.3). Therefore the acute study evaluation from the additional report is copied under 4.2.1.2 and the repeated study evaluation is copied below:

STUDY

Characteristics

reference	: DAR, Annex IIA, 5.3.3/01	exposure	: 6 hours/day, 5 days/week, 4 weeks; nose only
type of study	: 28-d inhalation toxicity	doses	: 0, 15, 75 and 200 mg/m ³ (nominal) ¹ , MMAD 2.92 µm, GSD 2.0 µm
year of execution	: 2002	vehicle	: None
test substance	: Etridiazole techn., lot no 1RC012320, purity 97.9%	GLP statement	: Yes
route	: Inhalation	guideline	: In accordance with OECD 412
species	: Rat, Sprague-Dawley: (CD) CrI: CD (SD)IGS BR	acceptability	: Acceptable
group size	: 10/sex/dose	NOAEL local	: <15 mg/m ³
		NOAEL systemic	: 15 mg/m ³

¹ equivalent to 0, 4.0, 20.2 and 54.0 mg/kg bw/day for males and females (using 45 L/kg bw/h (rat respiration rate), see the Guidance Document AOEL)

Study design

The study was performed in accordance with OECD 412 (1981). Larynx, nasopharyngeal tissue, trachea, lungs, liver and gross lesions and masses were investigated for all 4 groups, other tissues were investigated in control group and at 200 mg/m³. Doses were based on a 1-week range-finding study (01-6136; not available), in which effects on body weights, food consumption, clinical signs and organ weights at 1000 mg/m³ and minimal effects at 100 mg/m³ were observed.

Results

The results are summarised in table 6.3.3.1.

Table 6.3.3.1

Dose (mg/m ³)	0		15		75		200		dr
	m	f	m	f	m	f	m	f	
Mortality	none								
Clinical signs - nasal discharge	1/10	1/10	4/10	2/10	4/10	4/10	6/10	6/10	m/f
Body weight (gain)						dc		dc	
Food consumption	No treatment-related findings								
Ophthalmoscopy	No treatment-related findings								
Functional observations - forelimb grip strength							dc		
Motor activity	No treatment-related findings								
Haematology	No treatment-related findings								
Clinical chemistry - sodium - potassium - calcium						ic	ic ic ic		
Organ weights - liver			ic ^{a,r}	i ^a ,ic ^r	i ^a ,ic ^r	i ^a ,ic ^r	i ^r	i ^r	
Pathology									
<u>Macroscopy</u>	No treatment-related findings								
<u>Microscopy</u> - liver: centrilobular hypertrophy	0/10	0/10	0/10	0/10	0/10	0/10	10/10	7/10	
- larynx mucosa: epithelium-squamous/squamoid metaplasia	0/10	0/10	6/10	8/10	8/10	5/10	8/10	7/10	

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

Conclusions

Rats inhaled 0, 15, 75 or 200 mg/m³ etridiazole 6 hours/day for 5 days/week for 4 weeks. No mortality occurred. Nasal discharge (red) was the only treatment-related clinical observation. Body weight gain was significantly decreased in females at 75 and 200 mg/m³ (75-78% of control) at the end of the exposure period. No effect on food consumption was observed.

No treatment-related effects on motor activity were observed. Functional observation of forelimb grip strength showed a significant decrease in males at 200 mg/m³ (83% of control). Since no effect was observed on hindlimb grip strength in males and no effect on grip strength in females, this effect is not considered to be toxicologically relevant.

No treatment-related effects were observed on haematological parameters. Sodium and calcium were slightly, but significantly increased at 200 mg/m³ in males (102 and 104% of control, resp.). Potassium was significantly increased at 75 and 200 mg/m³ in males (110 and 109% of control), but

no renal histopathological changes were noted. In the absence of correlates, the toxicological significance of the changes in clinical parameters remains unclear.

Absolute liver weight was slightly, but significantly increased at 15 mg/m³ in males (113% of control) and increased at 75 mg/m³ in males (107% of control) and at 15 and 75 mg/m³ in females (106-107% of control). Relative liver weight was significantly increased at 15 and 75 mg/m³ in both sexes (109-111% of control) and increased at 200 mg/m³ in both sexes (106% of control). No dose-relationship was observed for increased absolute or relative liver weight. Macroscopically no treatment-related effects were observed. Histopathological examination revealed centrilobular hypertrophy of hepatocytes in all males and most females of both sexes at 200 mg/m³ only.

In the ventral seromucinous glands of the larynx minimal squamous/squamoid metaplasia of epithelium was observed in all test substance exposed groups and not in the control group. In the study report, this minimal metaplasia was considered an adaptive and non-adverse direct local effect of the test substance. The epithelium on the ventral floor of the larynx is especially sensitive to inhaled materials. Therefore, this is considered to be a target site for histopathological evaluations following inhalation exposure to particulates, vapours and aerosols. Based on all genotoxicity studies it is concluded that etridiazole is not genotoxic *in vivo* (see B.6.4.3), and therefore the squamous/squamoid metaplasia in the larynx mucosa is considered not to be induced by genotoxicity. Furthermore, literature data indicate that the chemicals that induce α_{2u} -globulin accumulation (CIGA) do not appear to react with DNA and are generally negative in short term tests for genotoxicity². CIGA binding to α_{2u} -globulin is reversible and not covalent in nature. Hence the metaplasia is not induced by genotoxicity, but is a local effect.

Based on the increased incidence in squamous/squamoid metaplasia in the larynx mucosa of all dosed animals a NOAEL for local effects could not be established.

Based on decreased body weight gain and changes in clinical biochemistry the NOAEL for systemic effects is set at 15 mg/m³ (equivalent to 4.0 mg/kg bw/d).

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

The available data indicate that etridiazole does not need to be classified for specific target organ toxicity, with the exemption of respiratory tract irritation (see 4.4.3) .

4.9 Germ cell mutagenicity (Mutagenicity)

Etridiazole does not need to be classified for mutagenicity. In the genotoxicity studies there were no indications for classification. This is in accordance with the current classification.

4.10 Carcinogenicity

In de additional report (Volume 3, B.6.5) carcinogenicity studies in rats and mice were presented. These studies indicated that etridiazole needs to be classified on the basis of its carcinogenicity. This is in accordance with the current classification: Carc. Cat. 3 Xn; R40 according to 67/548/EEC, and to EC 1272/2008 etridiazole is classified as Carc. Cat. 2: H351.

² Gordon C. Hard, Imogene Sevin Rodgers, Karl P. Baetcke, William L. Richards, Robert E. McGaughy, and Lawrence R. Valcovic; Hazard Evaluation of Chemicals that cause Accumulation of α_{2u} -globulin, Hyaline Droplet Neuropathy, and Tubule Neoplasia in the Kidneys of Male Rats; Environmental Health Perspectives, Vol. 99, pp. 313-349, 1993.

As additional information in light of the discussions on carcinogenicity in the TC-C&L in November 1995, November 1996 and May 1997 (Summary records ECBI/94/95, ECBI/45/96, ECBI/27/97, see Annex) the rat and mouse chronic/carcinogenicity studies and the mechanistic studies are copied below from the additional report (Volume 3, B.6.5 and B.6.8 respectively).

STUDY 1

Characteristics

reference	: DAR, Annex IIA, 5.5/03 (1988)	exposure	: 104 weeks, diet
type of study	: 104-week carcinogenicity study	doses	: 0, 100, 640, 1280 mg/kg food ¹
year of execution	: 1985-1987	Vehicle	: Not applicable
test substance	: Terrazole [®] Technical (etridiazole), batch no. S035012, purity 98.8%	GLP statement	: Yes
route	: oral	Guideline	: Predominantly in accordance with OECD 453
species	: rat, CrI: CD [®] BR	Acceptability	: Acceptable
group size	: 50/sex/dose	NOAEL	: < 5 mg/kg bw/day, oncogenic for rat liver, thyroid, kidney and testis

¹ equal to 0, 5, 30 and 63 mg/kg bw/day in males and 0, 6, 38, 84 mg/kg bw/day in females.

Study design

The study was predominantly performed in compliance with OECD guideline 453. No clinical biochemistry and urinalysis were performed. The notifier also submitted historical control data (Freeman, E., 2006a).

Results

The results are summarised in table 6.5.1.1.

Table 6.5.1.1

Dose (mg/kg food)	0		100		640		1280		dr
	m	f	m	f	m	f	m	f	
Mortality (n=50)	20	26	28	30	25	26	31	25	
Clinical signs	No treatment related findings								
Body weight (gain)						dc	d	dc	f
Food consumption						dc		dc	f
Haematology									
Week 103:									
Segmented neutrophils (absolute and/or relative)						ic		ic	f
Lymphocytes% WBC (absolute and corrected)						dc ic		dc ic	f
RBC and HCT						dc		dc	
HGB								dc	
Organ weights									
Terminal body weight						dc		dc	f
Liver					ic ^{ar}	ic ^r	ic ^{ar}	ic ^{ar}	m,f
Pathology									
<u>macroscopy</u> (n=50)									
Liver:									
-enlarged	3	1	5	1	11	2	9	3	
-raised area	1	2	5	1	1	1	2	6	

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Dose (mg/kg food)	0		100		640		1280		dr
	m	f	m	f	m	f	m	f	
-thickened lobe	1	2	5	3	4	4	6	7	
-pale area	5	6	1	7	0	6	6	16	
-dark area	12	6	12	9	9	16	9	12	
-cyst	0	1	3	2	2	4	4	9	
<u>microscopy</u>									
<i>neoplastic lesions</i>									
Liver (n=50):									
-hepatocellular adenoma	1 (2%)	2 (4%)	0	1 (2%)	0	2 (4%)	8 (16%)	12 (24%)	
-hepatocellular carcinoma	1 (2%)	0	1 (2%)	0	2 (4%)	0	1 (2%)	12 (24%)	
-cholangiocarcinoma	0	0	1 (2%)	0	0	0	1 (2%)	11 (22%)	
Thyroid:									
-follicular cell adenoma	6/50 (12%)	0/50	6/48 (12.5%)	1/31 (3%)	7/49 (14%)	0/25	14/49 (29%)	4/50 (8%)	
-follicular cell carcinoma	0/50	1/50 (2%)	1/48 (2%)	1/31 (3%)	6/49 (12%)	0/25	4/49 (8%)	1/50 (2%)	
-follicular cell adenoma/carcinoma	6/50 (12%)	1/50 (2%)	7/48 (15%)	2/31 (6%)	13/49 (27%)	0/25	18/49 (37%)	5/50 (10%)	m
Testis (n=50):									
-interstitial cell tumors	0	-	4 (8%)	-	4 (8%)	-	10 (20%)	-	
Kidney (n=50):									
-Tubule cell adenoma	0	0	1 (2%)	0	0	0	1 (2%)	0	
-Tubule cell carcinoma	0	0	0	0	4 (8%)	0	1 (2%)	0	
Mammary gland (n=50)									
fibroadenoma		9 (18%)		18 (36%)		17 (34%)		23 (46%)	
<u>microscopy</u>									
<i>non-neoplastic lesions</i>									
Liver (n=50):									
-hepatocytomegaly	6	7	5	7	16	15	34	37	m,f
-area of cellular alteration	30	28	22	27	30	32	36	41	m,f
-centrilobular hepatocyte pigment	0	0	0	0	0	10	4	45	m,f
-sinusoidal cell pigment	15	26	17	23	9	18	21	36	
-bile duct hyperplasia	42	26	39	27	35	38	30	43	f
-bile duct fibrosis	37	23	37	17	27	26	28	35	f
-bile duct chronic inflammation	40	24	35	21	31	28	32	33	f
-cholangiectasis	20	8	24	6	17	15	17	18	f
-spongiosis hepatis	14	2	6	3	21	5	21	13	f
-focal/coagulative necrosis	5	6	9	9	3	10	5	11	f
-vacuolization	37	41	35	31	35	37	44	39	
Kidney (n=50):									
-tubular cell karyomegaly	0	0	31	39	50	50	49	50	m,f
Testis (n=50):									
-Interstitial cell hyperplasia	4	-	5	-	6	-	18	-	m

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative

+ / ++ Represent an increase in incidence (relative to concurrent controls) of ≤ 25% (+) or > 25% (++)

Pathology findings in bold represent relevant findings

Conclusions

No statistically significant effect on survival was noted after treatment with the test substance at 100, 640 and 1280 mg/kg food for 104 weeks. Body weight (gain) and food consumption were statistically significantly lower for mid and high dose females at the end of treatment. Most notably was a reduction of mean body weight of the high dose females by 26% compared to control. For high dose males body weight (gain) was also reduced compared to controls, although this difference did not reach statistical significance after 104 weeks. Consistent with these observations, it was noted that high dose animals were, in general, smaller in size. Haematology revealed changes in mid and high dose females at the end of treatment and comprised a 1.5 fold increase in white blood cell count (absolute and corrected), with an increased segmented neutrophil count (absolute and/or relative) and decreased lymphocyte count (relative). In addition erythrocyte count and haematocrit were decreased (82 to 86% of control values) in mid and high dose females, and haemoglobin was lower (90% of control value) in high dose females.

Absolute and relative (to body weight) liver weights were increased in both sexes in mid- and high dose groups. Relative liver weights were 127% of controls for mid dose groups and 138% and 242% of controls for high dose males and females, respectively. Macroscopic findings were recorded at an increased incidence in liver (enlarged, raised area, thickened lobe, discoloured area, and/or cysts) of high dose females and/or males. Of these changes, an enlarged liver and discoloured areas were also noted in mid dose males and females, respectively.

Treatment with the test substance resulted in neoplastic lesions liver, thyroid, testis and kidney.

The occurrence of hepatic adenomas and carcinomas at 100 and 640 ppm were within the concurrent controls and/or historical controls for both the conducting laboratory and the breeder:

1.1.1.1 Historical control data for hepatic adenomas and carcinomas in rats

	Male rats		Female rats	
	Adenoma	Carcinoma	Adenoma	Carcinoma
Concurrent control	2% (incidence 1/50)	2% (incidence 1/50)	4% (incidence 2/50)	0%
Conducting laboratory (1984-1989)	1.3% (range 1-4%)	1.9% (range 1-4%)	0.4% (range 0-1%)	0.2% (range 0-1%)
Charles River Breeder (1992 data)	4.21% (range 1.3-18.2%)	2.62% (range 1.1-9.1%)	2.22% (range 1-5.5%)	0.4% (range 1-4%)

An increased incidence in hepatocellular adenomas and/or carcinomas was noted in high dose males and females. Furthermore an increased incidence of the rare cholangiosarcoma was recorded for high dose females. It is noted that this rare type of tumour was also recorded in individual cases in low and high dose males, however, the incidences in low and high dose males were within the historical controls:

Historical control data for cholangiosarcoma in rats

	Cholangiosarcoma male rats	Cholangiosarcoma female rats
Concurrent control	0%	0%
Conducting laboratory (1984-1989)	0%	0%
Covance Rockville location	2% (incidence 1/50)	2% (incidence 1/50)

Charles River Breeder (1992 data)	0.16% (range 1-2%)	1.29% (range 1-6%)
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The conducting laboratory is Covance Virginia location and the historical control data from Covance Rockville location are derived from a study that was conducted in the Rockville facility and was conducted/reported in the 1984-1989 time frame. In principle, the historical control data from the Rockville location and from the breeder are not relevant and only the historical control data from the conducting laboratory are relevant. However, considering the fact that cholangiosarcoma is a rare tumour type, it is considered acceptable to take all the presented historical control data into account.

In the thyroid, follicular cell adenomas and/or carcinomas were noted at an increased incidence in mid- and high dose males and high dose females, and the incidences exceeded the historical control range.

Historical control data for thyroid follicular cell adenomas and carcinomas in rats

	Male rats		Female rats	
	Adenoma	Carcinoma	Adenoma	Carcinoma
Concurrent control	12% (incidence 6/50)	0%	0%	2% (incidence 1/50)
Conducting laboratory (1984-1989)	4.7% (range 2-7%)	1.9% (range 1-4%)	0.4% (range 0-1%)	1.1% (range 1-2%)
Charles River Breeder (1992 data)	5.55% (range 1.1-25.7%)	1.29% (range 1-6%)	2.58% (range 1-14.5%)	1.05% (range 1-5.8%)

Incidence in interstitial cell tumours in the testis was statistically significantly increased in high dose males only. The incidences in the low and mid dose groups were within the historical control range.

Historical control data for interstitial cell tumours in male rats

	Adenoma in male rats
Concurrent control	0%
Conducting laboratory (1984-1989)	4.7% (range 2.4-13.3%)
Charles River Breeder (1992 data)	4.68% (range 1.4-10%)

In the kidney tubular cell tumours were recorded for 1, 4 and 2 males of the low, mid and high dose groups, respectively. The historical control data are presented below.

Historical control data for kidney tubular cell tumours in male rats

	Adenoma male rats	Carcinoma male rats
Concurrent control	0%	0%
Conducting laboratory (1984-1989)	0.2% (range 0-1.2%)	0.3% (range 0-2.9%)
Charles River Breeder (1992 data)	0.24% (range 1.4-2.1%)	0.32% (range 1-2%)

The tubule cell adenoma (1/50=2%) in one male rat in the 100 ppm group and in the 1280 ppm group slightly exceeded the historical control range of the conducting laboratory, but was within the historical control range of the breeder, although this is less relevant. The tubule cell carcinoma in one male rat in the 1280 ppm dose group was within the historical control range. The incidence of 8% for the tubule cell carcinoma in male rats in the 640 ppm group exceeded the historical control range. However, there was no dose-response relationship for the observed carcinomas.

In addition, an increased incidence of mammary gland fibroadenoma was noted in all treated females. This increase might be due to a low control value. Furthermore, the incidences in all treated groups were within the historical control data.

Historical control data for fibroadenoma in female rats

	Fibroadenoma female rats
Concurrent control	18% (incidence 9/50)
Conducting laboratory (1984-1989)	39% (range 11.6-69.2%)
Charles River Breeder (1992 data)	31.44% (range 13.7-49%)

A variety of non-neoplastic lesions were noted in the liver of mid and high dose groups and comprised hepatocytomegaly, areas of cellular alterations, cellular pigmentation, bile duct changes (hyperplasia, fibrosis, chronic inflammation and cholangiectasis), spongiosis hepatic, focal or coagulative necrosis and vacuolization. A non-neoplastic lesion, tubular cell karyomegaly, was also observed in the kidney in all dose groups. The toxicological relevance of this effect is not known. Furthermore, in this study no clinical biochemistry and urinalysis were performed, and therefore it is not known whether the kidney function was affected or not.

Based on abovementioned changes in the low dose group (tubular cell karyomegaly in kidney), a NOAEL could not be established. The test substance had an oncogenic effect on rat liver, thyroid, testis and kidney. However, it should be noted that the highest dose level exceeded the MTD (based on reduced body weight in females and increased liver weight in both sexes).

STUDY 2

Characteristics

reference	: DAR, Annex IIA, 5.5/02 (2004)	exposure	: 79 weeks, diet
type of study	: 18-month carcinogenicity study	doses	: 0, 50, 900/1300, and 1800/2000/1600 mg/kg food ¹
year of execution	: 2002-2004	Vehicle	: not applicable
test substance	: Terrazole® Technical (etridiazole) Lot no. 1RC012320, purity 97.9% Lot no. CHCAES001, purity 99.9%	GLP statement	: yes
route	: oral	Guideline	: OECD guideline 451
species	: mouse, Crl:CD-1® (ICR) BR	Acceptability	: acceptable
group size	: 50/sex/dose	NOAEL	: 7.5 mg/kg bw/day, oncogenic for mouse liver

¹ equal to 0, 7.5, 184.7 and 263.3 mg/kg bw/day in males and 0, 9.1, 221.7, 312.1 mg/kg bw/day in females (see acceptability)

Study design

The study was performed according to the OECD guideline 451. For the first week, dose levels in the diet were 0, 50, 900 and 1800 mg/kg food. After the first week of dosing the dietary concentration for the mid and high dose levels were increased to 1300 and 2000 mg/kg food. In Week 43, high dose level was decreased to 1600 mg/kg food due to excessive mortality in this group. Terminal sacrifices were performed after 79 weeks of treatment.

Considering the increased mortality in the high dose group, histopathology should have been extended to all organs and tissues of the mid dose group.

Diet analysis revealed low levels for accuracy: mean concentration ranged from 77 to 87% for the high dose group, 76 to 82% for the mid dose group and was 89% for the low dose group. This was considered related to binding of test article to food proteins and therefore no correction was made for the test article intake.

It is stated in the report that no historical control data were available for this study, as most mouse carcinogenicity studies were of 24 month duration. Therefore, liver tumours in mid and high dose groups were considered toxicologically relevant.

Results

The results are summarised in table 6.5.1.2.

Table 6.5.1.2

Dose (mg/kg food)	0		50		900/1300		1800/2000/1600		dr
	m	f	m	f	m	f	m	f	
Mortality (n=50)	12	14	9	14	22	22	30	34	
Clinical signs									
-decreased activity							++	++	
-inappetance							+	+	
-tremors							+	+	
-few or absent faeces							+	+	
-hunched posture							+		
-impaired limb function							+		
-skin cold to touch							+		
-pale skin							++	++	
-slow breathing							+		
-difficult breathing							+		
-shallow breathing							+	+	
-swollen appearance								+	
-distended abdomen	12/50	11/50	30/50	16/50	45/50	34/50	50/50	46/50	m,f
Body weight (gain)							dc		
Food consumption					dc		dc	dc	m,f
Haematology					No treatment-related findings				
Organ weights									
-liver					ic ^{ar}	ic ^{ar}	ic ^{ar}	ic ^{ar}	m,f
-kidney					dc ^{ar}	dc ^{ar}	dc ^{ar}	dc ^{ar}	m,f
-spleen							ic ^{ar}		
-uterus				d ^{ar}		dc ^{ar}		dc ^{ar}	f
Pathology									
<u>macroscopy (n=50)</u>									
Liver:									
-enlarged	0	1	0	0	4	6	8	15	m,f
-discoloured	0	0	0	0	4	6	2	3	
-irregular/granular surface	0	0	0	0	2	0	6	2	m
-masses	4	0	3	0	13	4	9	1	m,f
-nodules	1	0	1	0	8	7	11	14	m,f
Kidney:									
-irregular surface	1	2	2	3	1	17	2	20	f
Spleen:									
-enlarged	0	5	0	3	7	8	7	11	f

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Dose (mg/kg food)	0		50		900/1300		1800/2000/1600		dr
	m	f	m	f	m	f	m	f	
<u>microscopy (n=50)</u> <i>neoplastic lesions</i> Liver: -hepatocellular adenoma	4	0	3	0	12	5	11	11	f
-hepatocellular carcinoma	0	0	0	0	4	0	0	3	
<u>microscopy</u> <i>non-neoplastic lesions</i> Liver (n=50): -bile duct hyperplasia	0	0	0	0	20	2	32	27	m,f
-regenerative hyperplasia	1	0	2	1	18	18	39	35	m,f
-hepatocyte hypertrophy,	0	0	0	1	13	9	21	23	m,f
-Kupffer cell hypertrophy/hyperplasia	0	0	0	1	11	3	20	24	m,f
-hepatocyte necrosis	0	1	0	1	8	3	18	9	m,f
-increased cellular pigment	1	3	1	10	21	29	40	28	m,f
-hepatocellular/diffuse vacuolation	0	0	0	0	4	13	23	20	m,f
Kidney: -Infarct	5/50	4/50	0/14	0/14	8/26	25/39	16/50	32/50	m,f
Spleen: -increased extramedullary haematopoiesis	2/50	6/49	1/9	5/17	13/25	12/23	27/49	35/49	m,f
Heart: -myofiber mineralization	0/50	0/50	0/9	0/14	5/22	4/22	13/50	13/50	m,f
-thrombus	0/50	0/50	0/9	1/14	4/22	3/22	8/50	17/50	m,f

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
+ / ++ finding observed in ≤ 15/50 (+) or >15/50 (++) animals
Pathology findings in bold represent relevant findings

Conclusions

Survival was decreased after treatment with the test substance at 900/1300 and 1800/2000/1600 mg/kg food. Because of high mortality the dose level for group 4 was reduced from 2000 to 1600 mg/kg food from week 43 onwards. A variety of clinical signs (a.o. decreased activity, tremors, inappetance, hunched posture, discoloured skin and/or breathing difficulties) were recorded in the high dose group, more often in males than in females. An increase in incidence of a distended abdomen was noted in a dose related fashion in all treated groups. In the absence of any corroborative findings, the toxicological relevance of the distended abdomens was doubted. High dose males showed statistically significantly lower body weights than controls from week 3 of treatment onwards (91% of controls at study termination). In addition, high dose males and females showed lower food consumption than controls, reaching statistical significance in 25/30 weeks for males and in 18/30 weeks for females. Furthermore, a reduced food intake was noted in mid dose males in 15/30 weeks.

Changes were noted in the following absolute and relative (to body weight)) organ weights: liver weight was increased, and kidney weight reduced in mid and high dose males and females; spleen weight was increased in high dose males and uterus weight was reduced in mid and high dose

groups. Relative organ weight changes were 166 to 219% of controls for liver, 82 to 89% of controls for kidneys, 159% of control for spleen and 45 to 81% of controls for uterus.

Macroscopic findings were recorded in mid and high dose groups and comprised findings in liver of both sexes (enlarged, discoloured, abnormal surface, nodules/masses), kidneys of females (irregular surface) and spleen of both sexes (enlarged).

Treatment with the test substance resulted in neoplastic lesions in the liver of mid and high dose males and females and comprised hepatocellular adenoma and carcinoma. The incidence of these neoplasms was comparable for mid and high dose groups. However, it should be noted that these dose levels clearly exceeded the MTD, considering the high increase in mortality and changes in organ weights. A variety of non-neoplastic lesions were also noted in mid and high dose animals in a dose related fashion. These lesions were recorded in liver (hepatocellular necrosis, bile duct- and regenerative hyperplasia, hypertrophy and/or hyperplasia of hepatocytes and Kupffer cells, increased cellular pigment, and vacuolation), kidney (infarct), spleen (increased extramedullary haematopoiesis) and heart (myofiber mineralization and thrombi).

Based on abovementioned changes in mid and high dose groups, the NOAEL was set at 50 mg/kg food (equal to 7.5 and 9.1 mg/kg body weight/day for males and females, respectively). The test substance had an oncogenic effect on mouse liver.

STUDY 3

A 104-week oral toxicity study in rats (non-GLP) was available (DAR, Annex IIA, 5.5/01, 1968). The test substance (Terrazole, etridiazole) was administered via the diet for 104 weeks at dose levels of 0, 10, 80 or 640 mg/kg food. The control and treated groups comprised 10 rats/sex and 30 rats/sex, respectively. After 6 months relative organ weight changes were noted in high dose males (spleen and testis) and females (kidney and liver). After 24 months the only significant difference was an increased liver to body weight ratio for high dose males. There were no other relevant findings noted. As this was a non-GLP study that lacked documentation on various issues (e.g. no information on statistics, no individual data, no details on study design, histopathology was only performed on 10 rats/dose group), this study was not fully summarized. The results of this study were within the range of results obtained in Study 1.

STUDY 4

A carcinogenicity study in rats (non-GLP) was available (DAR, Annex IIA, 5.5/02, 1983). The test substance (Echlomezol (F24-24), etridiazole) was administered via the diet for 81 weeks at dose levels of 0, 160, 320, 640 and 1280 mg/kg food. The study was terminated after 81 weeks of treatment due to a pneumonia infection. As this was a non-GLP study that lacked information on the test substance, that was terminated prematurely, and taking into account that results were confounded by the pneumonia infection, this study was considered to be of limited value for evaluating the carcinogenic potential of the test substance. The study was therefore not fully summarized.

STUDY 5

An 18 month oncogenicity in CD-1 mice was available (DAR, Annex IIA, 5.5/04, 1980). The test substance (Terrazole (etridiazole), Lot no. 76-19-B, purity 97.7%) was administered via the diet for 18 months at dose levels of 32, 640 and 1280 mg/kg food. A vehicle (corn oil) control and untreated control group were included. After 18 months of treatment various mice were allowed a treatment-free period of 3 months. Treatment for 18 month resulted in a reversible increase in liver weight and a reversible decrease in kidney weight in all treated groups. No further changes (e.g. histopathological) were noted in these (target) organs. It was concluded that Terrazole was not oncogenic as all neoplastic lesions were considered to be within historical control data or lacked a dose response relationship. It is noted that no historical control data for CD-1 mice were available and historical data of other strains of mice were used. Furthermore, it was noted that tumour incidence in untreated control group was 2-2.5 times higher than in the vehicle control group. This made interpretation of results complicated. Taking the above into account the study was not fully summarized.

MECHANISTIC STUDIES

A further discussion about the oncogenic effects observed in the carcinogenicity studies and the possible underlying mechanisms can also be found in the additional report (Volume 3, B.6.8.2). The overall conclusion (B.6.8.2.2) is copied below.

Several subjects will be presented:

1. an overall evaluation of the mechanistic studies
2. an evaluation of etridiazole carcinogenicity studies by an expert, submitted by the notifier
3. a summary of a position paper with regard to the kidney tumours, submitted by the notifier
4. a summary of an expert review and a literature search with regard to the hyaline droplets in the 13-week rat study, submitted by the notifier
5. comments with regard to the oncogenic properties of etridiazole by the RMS
6. conclusion

1. Overall evaluation of the mechanistic studies

To further evaluate the carcinogenic mechanism of action a number of *in vivo* mechanistic studies were conducted that investigated whether etridiazole possessed promotor and/or initiator activity.

The first 2 studies (DAR, Annex IIA, 5.5/05 and 06, 1995) were comparable in study design. Rats were treated for up to 28 days with etridiazole and various parameters indicative for promotor activity were evaluated. These parameters comprised metabolizing liver enzymes and connexin 32 levels in liver. In the first study, dose levels of etridiazole were 0, 640 and 1280 mg/kg food and in the second study dose levels were 0, 100, 200 and 400 mg/kg food. In the first study, a positive control (phenobarbital) was included.

Both etridiazole treatment (at 400, 640 and 1280 mg/kg food) and phenobarbital treatment for 28 days resulted in induction of liver enzymes and a reduction of connexin 32 levels in liver.

A discrepancy between the positive control group and etridiazole treated group(s) was recorded with regard to phase I metabolizing enzymes, whereas the induction of phase II enzymes was similar for etridiazole and phenobarbital treatment. These differences indicate etridiazole has a different biochemical profile than the known tumour promotor phenobarbital.

Connexin 32 is a protein of the gap junction, involved in intercellular communication. A reduction in gap junctions leads to reduced intercellular communication, and is known to be related to (the promotion stage of) carcinogenesis. Both etridiazole treatment (at 400 and 1280 mg/kg food) and

phenobarbital treatment resulted in reduced connexin 32 levels in liver. Etridiazole treatment at 400 mg/kg food led to a reduction of approximately 20%, phenobarbital treatment led to a reduction of approximately 40% and etridiazole treatment at 1280 mg/kg food resulted in a reduction of approximately 80%. No connexin effect was noted at 640 mg/kg food.

This all suggests that etridiazole may act as a tumour promotor, comparable to phenobarbital. However, based on the differences in induction of metabolizing enzymes, it is concluded that if etridiazole can act as promotor, it has another profile than phenobarbital. These effects were not noted at 100 or 200 mg/kg food, indicating that etridiazole has a threshold level for promotor activity.

The third study was designed to investigate whether etridiazole had promotor and/or initiator activity in liver tumour development. Animals were pretreated once, intraperitoneally, with N-nitrosodiethylamine (DEN) as an initiator (or saline as control). Two weeks later test diets were provided at dose levels of 100, 640 and 1280 mg/kg food, a phenobarbital group was also included as positive control for tumour promotion. After 1 and 6 weeks sections of liver lobes were stained immunohistochemically for GST-P. GST-P positive liver foci served as an endpoint marker for hepatocarcinogenicity. Etridiazole treatment at 640 and 1280 mg/kg food markedly increased both the number and size of GST-P positive liver foci. Treatment with the positive promotor control resulted in comparable effects, with the magnitude of changes between those seen at 640 or 1280 mg/kg food etridiazole. This third study confirmed that etridiazole possesses promotor activity at 640 and 1280 mg/kg food, but not at 100 mg/kg food for hepatocarcinogenicity. In addition, no initiation potential was noted with doses up to 1280 mg/kg food etridiazole.

In conclusion, etridiazole possesses promotor activity, with a threshold level for this promotor activity of 200 mg/kg food (18 mg/kg bw/day). No initiation potential was noted for doses up to 1280 mg/kg etridiazole (88 mg/kg bw/day).

2. Evaluation of etridiazole carcinogenicity studies by the notifier (DAR, Annex IIA, 5.5/08, 1997)

Summary of evaluation

Four carcinogenicity studies were evaluated by the notifier. These comprised one study in mice (DAR, Annex IIA, 5.5/04, 1981) and three studies in rats (DAR, Annex IIA, 5.5/01, 1968; DAR, Annex IIA, 5.5/02, 1983 and DAR, Annex IIA, 5.5/03, 1988), which were also evaluated by the RMS in the DAR (see 4.10 of this CLH report, study 5, 3, 4 and 1, respectively). Based on these studies it was concluded by the notifier, that etridiazole was not carcinogenic in mice. Also, it was concluded that a carcinogenic response was found in rats (mainly in liver, thyroid and testis), which only occurred at a dose level (1280 mg/kg food) that clearly exceeded the MTD.

A carcinogenic response in the rat was found in study 1 (1988). At 1280 mg/kg food, an increased incidence in the following tumours was noted: liver tumours (adenoma and/or carcinoma) in both sexes, cholangiosarcoma in females, interstitial cell tumours in the testis and thyroid tumours (adenoma and/or carcinoma) in both sexes.

Furthermore the author continues to discuss why findings at lower doses can be discounted:

1. Increased incidence in mammary gland fibroadenoma at 100, 640 and 1280 mg/kg food. The incidences were considered to be within the laboratories historical control data and the incidence in control group was low.
2. Thyroid follicular carcinoma incidence was increased at 640 (statistically significant) and 1280 mg/kg food (not statistically significant). Thyroid follicular adenoma combined with carcinoma was increased at 640 (not statistically significant) and 1280 mg/kg food (statistically significant). This leads the author to conclude that a carcinogenic response was only seen at 1280 mg/kg food.

3. Very uncommon tubular cell tumours in the kidney were noted at 100 (1/50), 640 (4/50), and 1280 mg/kg food (2/50) in males. The author refers to a previous 3 month study in which hyaline droplets were noted in males at all dose levels tested (50, 600 and 1250 mg/kg food) and postulates that the observed tumours are the result of alpha-2-microglobulin accumulation. As this is not relevant for humans, the kidney tumours are considered to be of no relevance.

To further evaluate the carcinogenic mechanism of action a number of *in vivo* mechanistic studies were conducted. The results from these studies indicate that etridiazole acts as a promoter and not as an initiator of carcinogenicity, suggesting a threshold level exists for the oncogenic potential.

3. Additional position paper with regard to kidney tumours

The notifier submitted an additional position paper (DAR, Annex IIA, 5.5/09, 2006b) with regard to the occurrence of kidney tumours in male rats in the 2-year study.

Summary of position paper:

Etridiazole is a promoter of carcinogenicity with a demonstrable threshold level of effect. At levels below the threshold for promotion, there is no increase in the incidence of male kidney tumours. In addition, the kidney tumours seen in the male rats were not statistically significant. The small increase in kidney tumours was at a level that clearly exceeded the MTD. The fact that etridiazole is a promoter combined with excessive cellular damage caused by treatment above the MTD increases the possibility for an initiating event to be promoted. Finally, the proposed mechanism of action for male specific kidney tumours is alpha-2-microglobulin, male rat specific mechanism and not relevant to humans. The overall pathobiological weight of evidence strongly suggests that the alpha-2-microglobulin mode of action is responsible for the kidney tumours in male rats (note from the RMS: in 2009 more information became available, see the expert review below). The evidence includes specific pathobiology common to the mechanism of action: ongoing cell death, regenerative hyperplasia, protein droplet accumulation and the opportunity for spontaneous errors in replication during repair. Although the lack of the additional testing to confirm the presence of the alpha-2-microglobulin and binding precludes definitive confirmation of the mode of action, it is a reasonable assumption.

The presence of tubular cell karyomegaly is not a unique or unexpected finding in chronic nephrotoxicity or in aged rats with age-related tubular changes. These cytopathological changes are associated with chronic interstitial nephritis, and are not considered a biomarker for kidney tumour initiation.

4. Expert review and literature search with regard to the hyaline droplets

The notifier submitted an expert review (DAR, Annex IIA, 5.3.2/05, 2009) and the results of a literature search with regard to the hyaline droplets in male rats in the 13-week rat study.

Summary of expert review:

There has been a regulatory request by the rapporteur member state for etridiazole, to investigate and characterize the hyaline droplets reported in the 13-week rat oral study with etridiazole. This work has been completed by Covance.

Hyaline droplets (occasionally referred to as eosinophilic droplets/inclusions) are a well established background feature in the renal tubules of male rats and the incidence and severity of this feature generally increases with the age of the animal. It is the experience of the author of this report and others, that the incidence and severity of these hyaline droplets can be either increased or decreased after administration of a wide range of pharmaceuticals and chemicals (Greaves, P., 2007¹ and Kurata et al., 1994²).

In the 13-week rat study there was a dose related increase in the incidence and severity of hyaline droplet accumulation in intermediate and high dose animals killed at termination. See table below:

Incidence and severity of selected microscopic findings in kidney from animals killed at the end of the treatment period.

		Incidence of hyaline droplets in kidney from terminal kill animals:								
Tissue and finding	Level (mg/kg/day)	Males				Females				
		1M	2M	3M	4M	1F	2F	3F	4F	
Kidney hyaline droplets	No. examined:	10	10	10	10	10	10	10	10	
	Grade -	1	4	3	5	1	0	0	0	0
		2	2	1	3	7	0	0	0	0
		3	0	1	1	2	0	0	0	0
		4	0	0	0	0	0	0	0	0
		5	0	0	0	0	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe, 5 = severe

In the recovery animals it was reported that the level of hyaline droplets in high dose males had reduced but was not completely reversed. Special procedures to further characterise the hyaline droplets were not performed at the time of reporting of this study.

In response to the regulatory request to investigate and characterise the hyaline droplets, a new study was initiated to generate additional sections from selected paraffin blocks from the original study. The objectives this study (DAR, Annex IIA, 5.3.2/04, 2009) were to stain the droplets with standard haematoxylin and eosin (HE), chromotrope 2R-aniline blue (de Rijk E.P., et al 2003³) and also demonstrate positive immunostaining with a commercially available goat anti-rat polyclonal antibody against alpha-2-microglobulin.

The additional procedures (HE, chromotrope 2R-aniline blue and immunostaining) have clearly shown that the hyaline droplets in control and high dose males show identical staining properties. This confirms the findings of the original study in which a dose dependent exacerbation of this common background feature was reported with etridiazole.

The additional staining with chromotrope 2R-aniline blue and goat anti-rat polyclonal antibody against α_2 -globulin also clearly shows an association with the identity known as "rat hydrocarbon nephropathy". Thus, in the 13 week rat study etridiazole caused a treatment related increase in alpha-2-microglobulin in male animals which accounted for the increase in hyaline droplets that was reported. As stated previously this is a fairly common response following administration of a wide range of drugs and chemicals.

The change is considered to be male rat specific and of no consequence for administration to man (Khan, K.N.M. and Alden, C.L. 2002⁴). This statement is supported by the fact that other mammals, including female rats and both sexes of mice, dogs and monkeys do not show similar findings when dosed with compounds which cause this change (Alden C.L. 1986⁵).

¹ Greaves, P., 2007. Histopathology of Preclinical Toxicity Studies Third Edition p.600-605. Academic Press.

² Kurata, Y. Diwan, B.A, Lehmanmckeeman, Rice J.M and Ward J.M. 1994. Comparative hyaline droplet nephropathy in male F344Ncr rats induced by sodium barbital and diethylacetylurea, a breakdown product of sodium barbital. Toxicol and Appl. Pharmacol. 126: 224-232.

³ deRijk, E.P. Ravestloot, W.T. Wijnands, Y and van Esch, E., 2003. A fast histochemical staining method to identify hyaline droplets in the rat kidney. Toxicologic Pathology. 31(4): 462-464.

⁴ Khan, K.N.M. and Alden, C.L. 2002. Handbook of Toxicologic Pathology (Haschek, W.M., Rousseaux C.G. and Wallig, M.A. Eds.) p.294-298. Academic Press Inc.

⁵ Alden, C.L., 1986. A review of unique male rat hydrocarbon nephropathy. Toxicol.Pathol. 14, 109-111.

Summary of literature search:

The notifier conducted an intensive literature search and the argument is put forth below. There are many references related to hazard evaluation of chemicals that cause accumulation of alpha-2-microglobulin, hyaline droplet neuropathy, and tubule neoplasia in the kidneys of male rats¹. All invoked a specific type of protein droplet neuropathy in male rats but not in other sexes or species tested. It has been proposed that such renal tumors are the end product in the following sequence of functional changes in the epithelial cells of proximal tubules.

- Excessive accumulation of hyaline droplets in proximal tubules, representing lysosomal overload, leads to tubule cell degeneration, cell loss, and regenerative cellular proliferation.
- Cell debris in the form of granular casts accumulates at the “corticomedullary” junction with associated dilation of the affected tubule segment and more distally, mineralization of tubules within the renal medulla.
- Single-cell necrosis accompanied by compensatory cell proliferation and exacerbation of the chronic progressive nephropathy characteristically found in aging rats occurs.
- Renal tubule hyperplasia and neoplasia develop subsequently.

This line of reasoning leads to conclude that acute and chronic renal effects induced in male rats by these chemicals will be unlikely to occur in any species not producing alpha-2-microglobulin, or a very closely related protein, in large quantities typically seen in the male rat.

Hyaline droplets in the proximal tubules of normal male rats contain alpha-2-microglobulin and their occurrence appears to parallel the variable synthesis of the protein.

Protein accumulation in the proximal tubule can reach pathogenic levels resulting in excessive hyaline droplet formation for several reasons:

- a) the rate of protein delivery to the tubule cells is abnormally high
- b) the proteins delivered are difficult to hydrolyze, or
- c) the lysosomal hydrolysis capacity is sufficiently reduced.

The combination of difficult hydrolysis of the protein, as suggested by its long half-life, coupled with high rate of protein delivery to tubule cells in the sexually mature male rat, also appears to be a factor in the accumulation of alpha-2-microglobulin in the renal tubules of male rats.

¹ Gordon C. Hard, Imogene Sevin Rodgers, Karl P. Baetcke, William L. Richards, Robert E. McGaughy, and Lawrence R. Valcovic; Hazard Evaluation of Chemicals that cause Accumulation of α 2u-globulin, Hyaline Droplet Neuropathy, and Tubule Neoplasia in the Kidneys of Male Rats; Environmental Health Perspectives, Vol. 99, pp. 313-349, 1993.

5. Comments with regard to the oncogenic properties of etridiazole by RMS:

The RMS discussed the possible mechanisms and relevance of the tumours observed in the 2-year rat study and 18-month mouse study, on the basis of the study results, the mechanistic studies and the evaluation by the notifier, for each tumour type.

1.1.1.2 Liver tumours rat

The liver tumours were observed at a dose level exceeding the MTD, which means that the toxicological relevance is equivocal. The mechanistic studies showed that etridiazole possesses promoter activity for hepatocarcinogenicity, and this probably played an important role in the observed increased incidence of liver tumours at the highest dose in the 2-year rat study. The mechanistic studies also showed that there is a threshold dose for the promoter activity of etridiazole.

1.1.1.3 Liver tumours mouse

Etridiazole had an oncogenic effect on mouse liver, at dose levels clearly exceeding the MTD, and the toxicological relevance is therefore equivocal. Furthermore, since etridiazole is an enzyme inducer (see mechanistic studies), liver tumours in mice can be expected.

1.1.1.4 Thyroid tumours rat

The fact that the increased incidence in thyroid tumours does not always reach a statistically significant level, does not automatically mean it is not biologically relevant. In the mechanistic studies, an increase in UDP-GT was observed, but there was no corresponding increase in TSH in blood or changes in T₃ or T₄ in blood. The mechanism of the thyroid tumour formation is therefore not known. It should be noted that humans are considerably less sensitive to the development of follicular thyroid tumours as a result of long-term stimulation than rodents (especially rats).

1.1.1.5 Testis tumours rat

The testis tumours were observed at a dose level exceeding the MTD, meaning that, here too, the toxicological relevance is equivocal (the observed tumours at the low and mid dose, without a dose-response relationship, were within the historical control range). The incidence of interstitial cell tumours (Leydig cell tumours) in humans is extremely rare, while these tumours occur frequently in rats. Depending on the underlying mechanism, these tumours may not be relevant for human risk assessment. However, since the mechanism is not known for etridiazole, it should be assumed that humans are potentially susceptible.

1.1.1.6 Kidney tumours rat

In the 2-year rat study, the kidney tumours were only observed in males. There was no dose-response relationship. In the 13-week rat study, hyaline droplets were found, only in males. Finally, kidney tumours were not observed in the mouse study.

Based on the recent results of the staining of new kidney slides from the 13-week rat study showing the presence of alpha-2-microglobulin in the hyaline droplets the mode of action has sufficiently been proven. It can be concluded that the kidney tumours in the rat are alpha-2-microglobulin associated, and therefore not relevant for human risk assessment.

6. Conclusion

To further evaluate the carcinogenic mechanism of action a number of *in vivo* mechanistic studies were conducted that investigated whether etridiazole possessed promotor and/or initiator activity. In conclusion, etridiazole possesses promotor activity, with a threshold level for this promotor activity of 200 mg/kg food (18 mg/kg bw/day). No initiation potential was noted for doses up to 1280 mg/kg etridiazole (88 mg/kg bw/day).

Based on the observations in the 2-year rat study (liver tumours, and the potentially relevant thyroid and testis tumours), the 18-month mouse study, the mechanistic studies and further argumentation in the additional report, it is concluded that classification of etridiazole as Carc. Cat. 3, Xn, R40 (67/548/EEC), and Carc. Cat 2: H351 (EC 1272/2008) in line with the current ECB classification, is warranted.

4.11 Toxicity for reproduction

In PRAPeR 79 the foetal toxicity observed in the developmental studies in rat and rabbits was discussed. The experts discussed whether the findings (missing sternbrae) seen at the highest dose levels in rabbit trigger classification. A majority of the PRAPeR experts agreed that classification is not required. Classification issue to be flagged and further considered by ECHA based on the findings observed in the rabbit study.

For completeness, the conclusion of the 2-generation study in rats and the full evaluation of both developmental studies (rats and rabbits) are copied from the additional report (Volume 3, B.6.6).

For the current classification, etridiazole was discussed in the TC-C&L in November 1995, November 1996 and May 1997 (Summary records ECBI/94/95, ECBI/45/96, ECBI/27/97: see Annex). Since the basis for the current classification is unknown due to a lack of information on the studies used, it is not possible to indicate in this CLH report which studies are new information compared to the studies used for the classification in the 25th ATP to Directive 67/548/EEC.

Table 13: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
OECD 416: 2-generation reproduction	NOAEL _{par} : 5.3 mg/kg bw/d NOAEL _{dev} : 5.3 mg/kg bw/d NOAEL _{repro} males: >53.3 mg/kg bw/d NOAEL _{repro} females: >42.7 mg/kg bw/d	Rat, Sprague-Dawley CrI:CD®(SD)IGS BR	DAR, Annex IIA, 5.6.1/01, 2003
OECD 414: teratogenicity	NOAEL _{mat} : 30 mg/kg bw/d NOAEL _{dev} : 30 mg/kg bw/d NOAEL _{terato} : >75 mg/kg bw/d	Rat, Charles River COBS® CD®	DAR, Annex IIA, 5.6.2/02, 1982
OECD 414: teratogenicity	NOAEL _{mat} : 15 mg/kg bw/d NOAEL _{dev} : 15 mg/kg bw/d NOAEL _{terato} : 15 mg/kg bw/d	Rabbit, Dutch-Beltd	DAR, Annex IIA, 5.6.2/023, 1979

In an oral 2-generation reproduction study in rats (0, 80, 320 and 800 mg/kg food for males and 0, 80, 320 and 640 mg/kg food for females), a decrease in body weight and food consumption was noted among males and females from the F₀-generation at 800/640 mg/kg food and for the F₁-generation at 320 and 800/640 mg/kg food. An increase in T₃ concentration in males and a decrease in females at 320 and 800/640 mg/kg food (F₀-generation measured only) was noted. In addition, decreased pituitary weight at 320 mg/kg in F₀-males food and at 800 mg/kg food F₀- and F₁-males, changes in kidney weight at 320 and 800/640 mg/kg food in F₀ and F₁-animals, increases in liver weight at 320 and 800/640 mg/kg food in F₀ and F₁-animals, and increased seminal vesicle weight at 320 and 800 mg/kg food in F₁-males were observed. There were no changes detected between parental animals of the treated and control groups in mating indices, pregnancy rates, fertility, oestrus cycle and macroscopic findings.

Examination of the F₀ and F₁-offspring revealed decreased body weights of pups at 320 (F₀ only) and 800/640 mg/kg food. In addition, changes in thyroid weight were noted at both levels in the F₀-generation and in the F₁- high dosed group. T₃ concentration was only measured the F₁-offspring and was decreased in males at 800 mg/kg food and females at 320 and 640 mg/kg food. No treatment-related changes were detected in litter size, sex ratio, litter survival or macroscopic observations of the F₀ and F₁-offspring. Based on the data presented in this study, the NOAEL for parental toxicity was 80 mg/kg food (equivalent to 5.3 mg/kg bw/day). The NOAEL for developmental toxicity was 80 mg/kg food (equivalent to 5.3 mg/kg bw/day). The NOAEL for reproductive toxicity was considered to exceed 800/640 mg/kg food (equivalent to ≥ 53.3 mg/kg bw/day for males and ≥ 42.7 mg/kg bw/day for females).

In a teratogenicity study in rats (0, 10, 30 or 75 mg/kg bw/day) the NOAEL for maternal effects was 30 mg/kg bw/day, based on an increased mortality, clinical signs, and decreased body weight.

The NOAEL for developmental effects was set at 30 mg/kg bw/day, based on decreased mean foetal weight, anasarca in 2 foetuses, and retarded ossification of various bones. No treatment-related effects were observed on the number of corpora lutea or implantations, the number or percentage of live foetuses, and the sex ratio. There were no morphological changes observed in foetuses that could be attributed to treatment. Therefore, the NOAEL for teratogenicity was considered to exceed 75 mg/kg bw/day.

In a teratogenicity study in rabbits (0, 1.7, 5, 15 or 45 mg/kg bw/day), a NOAEL for maternal effects of 15 mg/kg bw/day was derived, based on mortality and decreased body weight. Potential critical effects (liver, kidneys, thyroid) were not studied and therefore, the derived maternal NOAEL from this study might not be accurate. The NOAEL for developmental effects was set at 15 mg/kg bw/day, based on decreased mean foetal weight, a reduction of live foetuses per dam and an increase of dams with resorptions at 45 mg/kg bw. No treatment-related effects were observed on the number of corpora lutea or implantation sites, and sex ratio.

Skeletal examination revealed an increased incidence of missing sternbrae, tail defects and underdeveloped hind limbs. Soft tissue examination showed an increased incidence of crossed hind legs and open eyes. Therefore, the NOAEL for teratogenicity was set at 15 mg/kg bw/day.

Potential critical effects of the test substance (liver, kidneys, thyroid) were not studied in the teratogenicity studies in rats and rabbits. Consequently, the observed NOAEL for maternal effects might not be accurate. Based on this consideration and based on the effect levels for foetal changes, classification for developmental toxicity is not considered necessary.

4.11.1 Effects on fertility

4.11.1.1 Non-human information

The 2-generation study in rats did not provide any indications for classification for reproductive toxicity. For completeness the conclusion from the additional report (Volume 3, B.6.6.1) is copied below:

Conclusions

In the 2-generation reproduction study, F₀ animals showed no treatment-related mortality or clinical signs. Two males (day 20 and day 91) and one female (day 68) at 80 mg/kg food were found dead. These spontaneous deaths at the lowest treatment level were not considered to be treatment-related. Body weight gain was significantly decreased at 800 mg/kg food in males (89% of control) and 640 mg/kg food in females (92% of control) during the pre-mating period, while during lactation body weight gain was increased relative to control (body weight was still decreased). Food consumption was significantly reduced during pre-mating and post-mating in males at 800 mg/kg food and during gestation and lactation in females at 640 mg/kg food.

There were no changes detected between parental animals of the treated and control groups in mating indices, pregnancy rates, male fertility, sperm evaluations and oestrus cycle. No changes in post-implantation loss were observed.

For T₃ a significant increase was noted at 320 and 800 mg/kg food in males (132% of control). T₃ was significantly decreased at 320 and 640 mg/kg food in females (77 and 73% of control, resp.). T₄ and TSH were also reduced at 640 mg/kg food in females (81 and 75% of control, resp.). Changes in T₃ in both males and females at 320 mg/kg food and males at 800 mg/kg food are of equivocal toxicological significance, in absence of a correlating finding in T₄ and TSH. In F₀ females

decreased T_3 , T_4 and TSH values were noted at 640 mg/kg food at termination. As the changes did not correspond biologically, and in the absence of clear histopathological changes in thyroid, pituitary or liver, the toxicological significance of these findings is unclear.

The increased relative testes weight and decreased absolute adrenal weight are considered to be due to lower body weight. Increased absolute and relative liver weight was noted in males at 800 mg/kg food (114 and 123% of control) and increased relative liver weight was observed in females at 640 mg/kg food (115% of control). Relative kidney weight was increased in males at 800 mg/kg food (108% of control) and absolute kidney weight was increased in females at 640 mg/kg food (107% of control) and relative kidney weight was increased at 320 and 640 mg/kg food (107-113% of control). Absolute pituitary weight was decreased in males at 320 and 800 mg/kg food (94% of control; not dose-related).

At necropsy no treatment-related abnormalities were observed. Histopathological examination of the kidney showed a chronic progressive nephropathy in 7/8 males at 800 mg/kg food; the effect was not present in the female at 640 mg/kg food nor in three control males examined.

No treatment-related changes were detected in litter size, live birth index, viability index, lactation index, sex ratio or clinical signs of the F_0 offspring (F_1 pups). A slight delay in vaginal opening was noted at 640 mg/kg food in F_1 female pups.

F_1 pup weights were significantly decreased on day 4, 7, 14 and 21 post partum at 800/640 mg/kg food (91, 85, 84 and 86% of control for males, resp. and 90, 84, 84 and 86% of control for females, resp.). F_1 pups weights were significantly decreased on days 7, 14 and 21 post partum at 320 mg/kg food (93, 94 and 93% of control for males, resp. and 91, 93 and 93% of control for females, resp.).

Blood analysis of F_1 foetuses on gestation day 20 showed no treatment-related findings on T_3 , T_4 or TSH. T_3 was significantly decreased at 800 mg/kg food in male pups and 640 mg/kg food in female pups on day 4 and day 21 of lactation (70-77% of control for males and 79-80% for females). In addition T_3 was decreased at 320 mg/kg food in female pups (81% of control). No change in T_4 or TSH was observed. In the absence of clear histopathological changes in thyroid, pituitary or liver, the toxicological significance of these findings is unclear.

Absolute brain weight of F_1 pups was significantly decreased at 640 mg/kg food in females (96% of control) and relative brain weight was increased at 800 mg/kg food in males (115% of control) and 640 mg/kg food in females (113% of control). Absolute spleen weight was decreased in F_1 male pups at 800 mg/kg food (74% of control) and in F_1 female pups at 640 mg/kg food (73% of control). Relative spleen weight was decreased in F_1 female pups at 640 mg/kg food (89% of control). Absolute thymus weight was decreased in F_1 female pups at 640 mg/kg food (84% of control). Changes in brain, thymus and spleen weight might be due to lower pup weights. Absolute thyroid weight was increased in F_1 female pups at 320 and 640 mg/kg food (117-118% of control). Relative thyroid weight was increased in F_1 male pups at 320 and 800 mg/kg food (118 and 130% of control, resp.).

At necropsy no treatment-related abnormalities were found.

F_1 parental animals showed no treatment-related mortality and clinical signs. Body weight was decreased during the whole treatment period in both sexes at 320 and 800/640 mg/kg food. Body weight gain was significantly decreased during the pre-mating period at 320 and 800/640 mg/kg food in males (90 and 86% of control, resp.) and females (90 and 84% of control, resp.). During the pre-mating period, food consumption was decreased in males and females at 320 and 800/640 mg/kg food. During gestation and lactation food consumption was decreased in females at 640 mg/kg food.

No changes were noted between parental animals of the treated and control groups in mating indices, pregnancy rates, male fertility, sperm evaluations and oestrus cycle. Copulatory interval was increased at 320 mg/kg food in females, which was not dose-related and not considered toxicologically relevant. There were no changes in post implantation loss for F₁ females.

Changes in brain, adrenals, epididymides and ovaries were probably due to a lower body weight. Absolute and relative liver weight was increased in males at 800 mg/kg food (111 and 128% of control, resp.) and females at 640 mg/kg food (109 and 129% of control, resp.). Relative liver weight was also significantly increased at 80 and 320 mg/kg food in females (106-115% of control). Absolute pituitary weight was decreased in males at 800 mg/kg food (88% of control). Relative kidney weight was increased in males at 320 and 800 mg/kg food (107 and 112% of control, resp.). Absolute kidney weight was decreased in females at 320 and 640 mg/kg food (98 and 94% of control, resp.). Absolute seminal vesicle weight was increased at 320 mg/kg food (108% of control) and relative seminal vesicle weight was increased at 320 and 800 mg/kg food in males (116 and 121% of control, resp.).

At necropsy no treatment-related abnormalities were observed, including histopathological examination.

No treatment-related changes were observed in litter size, live birth index, viability index, lactation index, sex ratio or clinical signs of the F₁ offspring (F₂ pups). Pup weights were decreased at 800/640 mg/kg food from birth till weaning. Changes in brain, spleen and thymus weight at 800/640 mg/kg food were considered to be due to the lower body weight at this dose level. Relative thyroid weight was increased in male F₂ pups at 800 mg/kg food (130% of control). Absolute pituitary weight was decreased in female F₂ pups at 640 mg/kg food (67% of control). No treatment-related effects were observed at necropsy.

No effects on fertility were noted in the present study. No treatment related changes were noted in oestrus cycle, sperm parameters, mating behaviour, conception and gestation. Therefore, the NOAEL for reproductive effects is set at ≥ 800 mg/kg food for males (equivalent to ≥ 53.3 mg/kg bw/d) and ≥ 640 mg/kg food for females (equivalent to ≥ 42.7 mg/kg bw/d). Based on decreased body weight and food consumption, changes in T₃ and changes in weight of seminal vesicles, pituitary, kidneys and liver, the NOAEL for parental effects is set at 80 mg/kg food (equivalent to 5.3 mg/kg bw/day).

The NOAEL for development toxicity is set at 5.3 mg/kg food (equivalent to 5.3 mg/kg bw/day), based on the decreased body weight, increased absolute thyroid weight and decreased T₃ concentration.

4.11.1.2 Human information

No information.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In PRAPeR 79 it was concluded that the classification issue regarding reproductive toxicity to be flagged and further considered by ECHA based on the findings observed in the rabbit study. Therefore the evaluation of the rat and the rabbit developmental studies are copied from the additional report (Volume 3, B.6.6.2):

STUDY 1

Characteristics

reference	: DAR, Annex IIA, 5.6.2/02	exposure	: days 6-19 of gestation, gavage (10.9 ml/kg)
type of study	: teratogenicity study	doses	: 0, 10, 30 or 75 mg/kg bw/day
year of execution	: 1981	vehicle	: corn oil
test substance	: Terrazole techn. (etridiazole), Lot 79-02-B, purity min. 95%	GLP statement	: Yes
route	: oral	guideline	: OECD guideline 414 (1981 and 2001)
species	: Rat, Charles River COBS® CD®	acceptability	: acceptable
group size	: 25 females/dose	NOAEL _{mat}	: 30 mg/kg bw/day
		NOAEL _{dev}	: 30 mg/kg bw/day
		teratogenic effects	: ≥ 75 mg/kg bw/day

Study design

The study was generally performed in accordance with OECD guideline 414 (1981 and 2001). Dose levels were selected based on a teratology range-finding study (Laughlin, 1981), in which female rats were administered 10, 30, 100, 200 and 300 mg/kg bw/day Terrazole techn. in corn oil (10 ml/kg) by gavage. All rats at 200 and 300 mg/kg bw died or were killed in extremis between gestation day 12 and 14. Clinical signs at 100, 200 and 300 mg/kg bw included dry red matter in the nasal, limbs and/or ocular region, eyes crusted completely or partially closed, yellow and/or wet matting of the haircoat in the anogenital region, emaciation, ataxia, ill-kept and/or oily and matted haircoat. Inability to move or obtain food or water, tremors, loss of righting reflex and/or red or green mucoid discharge in the vaginal area were observed at 300 mg/kg bw only. Body weight loss was observed at 100, 200 and 300 mg/kg bw during the whole exposure period or till death. At necropsy pale kidneys were noted at 200 mg/kg bw and pale livers were observed at 300 mg/kg bw. The small intestine contained yellow, tan or white fluid, mucoid or caceous material at 200 and 300 mg/kg bw. The stomach and/or large intestine contained yellow fluid at 200 mg/kg bw. In one female at 300 mg/kg bw mucosa of the stomach, duodenum and ileum regions were severely reddened, and red and black mucoid material was found in the duodenum and ileum, and caecal contents were extremely dry. Uterine examination of females at 100 mg/kg bw revealed a high post-implantation loss relative to (historic) control caused primarily by one dam with resorptions only.

Results

The results are summarised in table 6.6.2.1.

Table 6.6.2.1

Dose (mg/kg bw/day)	0	10	30	75	dr
Maternal effects					
Mortality	0/25	0/25	0/25	5/25	
Clinical signs	see text				
Pregnant animals	22	25	24	25	
Body weight (gain)¹ Day 6-20				d	
Food consumption	Not performed				

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Dose (mg/kg bw/day)	0	10	30	75	dr
Uterus weight	No treatment-related findings				
Pathology	No treatment-related findings				
<u>macroscopy</u>	No treatment-related findings				
<u>Litter response</u>					
Number of dams examined	25	25	25	20	
Corpora lutea/dam	No treatment-related findings				
Dams with resorptions only	0	0	1	2	
Dams with live fetuses	22	25	23	18	
Live fetuses/dam	13.4	12.9	12.7	12.5	
Foetal weight		dc*		dc**	
Post- implantation loss/dam	0.7	0.5	0.8	1.6	
Sex ratio	No treatment-related findings				
<u>Examination of the fetuses</u>					
External observations					
- anasarca	0	0	0	2	
Skeletal findings²					
Variations:					
- sternebrae #5 and/or #6 unossified	11.7	22.4	17.1	28.0	
- other sternebrae unossified	0.7	3.7	-	4.0	
- entire sternum unossified	-	-	-	3.2	
- vertebrae reduced ossification	-	-	-	4.8	
- pubis unossified	-	0.6	-	4.8	
- ischium reduced ossification	-	-	-	4.8	
Visceral findings	No treatment-related findings				

- dr dose related
- dc/ic statistically significantly decreased/increased compared to the controls
- d/i decreased/increased, but not statistically significantly compared to the controls
- a/r absolute/relative organ weight
- * p<0.05
- ** p<0.01
- 1 no statistics performed
- 2 % fetuses

Conclusions

Five dams at 75 mg/kg bw/d died between day 16 and 20 of gestation. At necropsy in four of these animals red fluid in the vagina and/or cervix was noted, the other animals had erosions in the stomach and mucoid material in the intestines. Treatment-related clinical signs at 75 mg/kg bw/d included vaginal opening containing red fluid, and dry, red material in anogenital area, around nose and/or mouth and/or forelimbs, facial and/or anogenital haircoat stained red, entire haircoat feeling oily, feeling cool to touch, mucoid discharge from anus. Wet or dry anogenital matting was noted in all groups including control with an increased incidence in the treated groups. Body weight loss was observed initially, day 6-9 at 10 and 30 mg/kg bw and day 6-12 at 75 mg/kg bw/d. Body weight

gain was markedly reduced at 75 mg/kg bw/d during the remaining gestation period. No macroscopic abnormalities were noted in sacrificed animals.

There were no significant differences in the number of corpora lutea or implantations, the number or percentage of live foetuses or sex ratio. The increased post-implantation loss at 75 mg/kg bw/d was still within the historical control range and not considered to be related to treatment. Mean foetal weight was significantly decreased at 75 mg/kg bw/d (86% of control). At 10 mg/kg bw mean foetal body weight was slightly decreased (94% of control). Foetus examination revealed that 2 foetuses at 75 mg/kg bw/d had anasarca (oedema). The latter is within the historical control value. The increased incidences of unossified sternbrae #5 and/or #6, other unossified sternbrae, unossified entire sternum, reduced ossification in vertebrae, unossified pubis, and reduced ossification of ischium at 75 mg/kg bw/d were outside the historical range. It cannot be excluded that the increase of these skeletal variations is a consequence of treatment with the test substance. The incidence of unossified other sternbrae at 10 mg/kg bw/d was outside the historical control range, however, in absence of this finding at 30 mg/kg bw/d, it was not considered toxicologically relevant. No significant deviations in the incidence of soft tissue anomalies were noted.

Based on mortality and the decrease in body weight in maternal females, the NOAEL for maternal toxicity was set at 30 mg/kg bw/day. Based on decreased mean foetal weight and anasarca in 2 foetuses and retarded ossification of various bones the NOAEL for developmental toxicity was set at 30 mg/kg bw/day. Since no irreversible structural effects were reported, the NOAEL for teratogenic effects was set at ≥ 75 mg/kg bw/day. Potential critical effects of the test substance on thyroid, liver and kidneys were not studied. Accordingly, the NOAEL for maternal effects might not be accurate.

STUDY 2

Characteristics

reference	: DAR, Annex IIA, 5.6.2/03,	exposure	: days 6-18 of gestation, gavage (1 ml/kg)
type of study	: teratogenicity study	doses	: 0, 1.7, 5, 15 or 45 mg/kg bw/day and a positive control (6-aminonicotinamide in water on day 9 only)
year of execution	: 1978/79	vehicle	: corn oil
test substance	: Terrazole techn. (etridiazole), Lot 76-08-A, purity 97.7%	GLP statement	: No
route	: oral	guideline	: OECD guideline 414 (1981)
species	: Rabbit, Dutch-Belted	acceptability	: acceptable
group size	: minimal 15 pregnant females/dose	NOAEL _{mat}	: 15 mg/kg bw/day
		NOAEL _{dev}	: 15 mg/kg bw/day
		teratogenic effects	: 15 mg/kg bw/day

Study design

The study was predominantly in accordance with OECD guideline 414 (1981). However, food consumption, uterine weight, and macroscopy were not included in the study protocol. Furthermore, clinical signs in dams were not reported, external observation of the foetuses was not reported and the number of pregnant animals in the high dose group was slightly low (according to OECD 414, 2001: at least 16 dams/group). A positive control was used (2.5 mg/kg bw 6-aminonicotinamide). Dose levels were selected based on a teratology range-finding study, in which female rabbits were administered 30, 100 and 300 mg/kg bw/day etridiazole in corn oil (2 ml/kg) by gavage. All rabbits

at 100 and 300 mg/kg bw died. At 30 mg/kg bw one female died and another resorbed its entire litter.

Results

The results are summarised in table 6.6.2.2.

Table 6.6.2.2

Dose (mg/kg bw/day)	0	Positive control	1.7	5	15	45	dr
Maternal effects							
Mortality	0/17	0/17	0/15	1/16	0/17	3/14	
Clinical signs	Not reported						
Pregnant animals	17	17	15	16	17	14	
Body weight day 18 of gestation						dc	
Food consumption	Not performed						
Uterus weight	Not determined						
Pathology							
macroscopy	Not performed						
Litter response							
Number of dams examined	17	17	15	16	17	14	
Corpora lutea/dam	No treatment-related findings						
Dams with resorptions	5	14*	4	3	6	8*	
Dams with live foetuses	17	12	15	15	16	9	
Live foetuses/dam	5.3	2.5*	5.1	5.8	4.6	3.5*	
Dams with dead foetuses	0	1	0	0	0	0	
Post-implantation loss ¹	0.4	2.7	0.2	0.3	0.5	2.2	
% Viability ²	99	23*	96	97	97	80*	
Foetal weight	41.7	33.2*	39.9	38.1	38.4	32.8*	
Sex ratio	No treatment-related findings						
Examination of the foetuses							
External observations	Not reported						
Skeletal findings ³ - missing sternbrae - tail defects - hind limbs underdeveloped		2/2 39/10*				3/3* 5/2* 4/1	
Visceral findings ³ - hind legs crossed - Open eyes		14/6*				7/2* 6/2*	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

* p<0.05

¹ as calculated by reviewer; no statistical analysis performed

² after 24 hour in incubator

³ number of foetuses/ number of litters affected

Conclusions

Females received 0, 1.7, 5, 15 or 45 mg/kg bw etridiazole or 2.5 mg/kg bw 6-aminonicotinamide from day 6 to day 18 of gestation. Three dams at 45 mg/kg bw and 1 dam at 5 mg/kg bw died before day 29 of gestation. Clinical signs were not reported. Body weight gain was statistically decreased at 45 mg/kg bw etridiazole at day 18 of gestation. A non-statistical decrease in body weight compared to control was noted from day 6 through 30 of gestation.

There were no significant differences in the number of corpora lutea or implantation sites, and sex ratio. The number of dams with resorptions was significantly increased at 45 mg/kg bw and the positive control. The number of live foetuses per dam was significantly decreased at 45 mg/kg bw (66% of control) and in the positive control. Mean foetal weight was significantly decreased at 45 mg/kg bw (79% of control) and in the positive control group.

Foetus examination revealed a significant increase in missing sternebrae and tail defects (not further specified) at 45 mg/kg bw and the positive control. Under-developed hind limbs were noted in 4 foetuses from one litter at 45 mg/kg bw. Soft tissue examination showed a significantly increased incidence of crossed hind legs at 45 mg/kg bw Terrazole and in the positive control, and open eyes at 45 mg/kg bw. Historical control data were not included. It cannot be excluded that these increases are a consequence of treatment with the test substance.

Based on mortality and the decrease in body weight in maternal females, the NOAEL for maternal toxicity was set at 15 mg/kg bw/day. Based on decreased mean foetal weight, a reduction of live foetuses per dam and an increase of dams with resorptions at 45 mg/kg bw the NOAEL for developmental toxicity was set at 15 mg/kg bw/day. Since irreversible structural effects were reported, the NOAEL for teratogenic effects was set at 15 mg/kg bw/day. Potential critical effects of the test substance (liver, kidneys, thyroid) were not studied. Consequently, the observed NOAEL for maternal effects might not be accurate.

4.11.2.2 Human information

No information.

4.11.3 Other relevant information

No information.

4.11.4 Summary and discussion of reproductive toxicity

In an oral 2-generation reproduction study in rats, the NOAEL for parental toxicity was 80 mg/kg food (equivalent to 5.3 mg/kg bw/day) based on body weight, food consumption, T3 concentration, and weights of several organs. The NOAEL for developmental toxicity was 80 mg/kg food (equivalent to 5.3 mg/kg bw/day) based on based on body weight, T3 concentration, and weights of thyroid. The NOAEL for reproductive toxicity was considered to exceed 800/640 mg/kg food (equivalent to 53.3 mg/kg bw/day for males and 42.7 mg/kg bw/day for females) since no effects on sexual function and fertility were observed.

In a teratogenicity study in rats (0, 10, 30 or 75 mg/kg bw/day) the NOAEL for maternal effects was 30 mg/kg bw/day, based on an increased mortality, clinical signs, and decreased body weight. The NOAEL for developmental effects was set at 30 mg/kg bw/day, based on decreased mean foetal weight, anasarca in 2 foetuses, and retarded ossification of various bones. No treatment-related effects were observed on the number of corpora lutea or implantations, the number or percentage of live foetuses, and the sex ratio. There were no morphological changes observed in foetuses that could be attributed to treatment. Therefore, the NOAEL for teratogenicity was considered to exceed 75 mg/kg bw/day.

In a teratogenicity study in rabbits (0, 1.7, 5, 15 or 45 mg/kg bw/day), a NOAEL for maternal effects of 15 mg/kg bw/day was derived, based on mortality and decreased body weight. Potential critical effects (liver, kidneys, thyroid) were not studied and therefore, the derived maternal NOAEL from this study might not be accurate. The NOAEL for developmental effects was set at 15 mg/kg bw/day, based on decreased mean foetal weight, a reduction of live foetuses per dam and an increase of dams with resorptions at 45 mg/kg bw. No treatment-related effects were observed on the number of corpora lutea or implantation sites, and sex ratio.

Skeletal examination revealed an increased incidence of missing sternbrae, tail defects and underdeveloped hind limbs. Soft tissue examination showed an increased incidence of crossed hind legs and open eyes. Therefore, the NOAEL for teratogenicity was set at 15 mg/kg bw/day.

4.11.5 Comparison with criteria

In EC 1272/2008 it is stated that a maternal mortality of over 10% is considered excessive and the data for that dose level shall not normally be considered for further evaluation. In both the rat and rabbit developmental studies the maternal mortality was 20% at the high dose.

The foetal toxicity observed in the rat study was based on retarded ossification. This findings was observed at a dose level causing severe maternal toxicity, including mortality. The foetal toxicity is considered to be secondary to the maternal toxicity and therefore not relevant for classification.

In the rabbit developmental study, there were no Historical Control Data. Missing sternbrae, tail defects, and underdeveloped hind limbs were observed in the highest dose group, and soft tissue examination showed an increased incidence of crossed hind legs and open eyes in the high dose group. There were mortalities observed in the dams (3/14) indicating high maternal toxicity. The dams which did not die were not examined for other critical signs of toxicity e.g. liver, thyroid and kidney. Moreover, the mortality at a slightly lower dose level (30 mg/kg bw/d) in the preliminary study also indicates that the maternal toxicity at the high dose (45 mg/kg bw/d) is considered excessive and the data for that dose level shall not normally be considered for further evaluation. Therefore, these effects are not considered to be relevant for classification. At all other doses there was no incidence of this effect.

4.11.6 Conclusions on classification and labelling

According to 67/548/EEC and EC 1272/2008, etridiazole does not need to be classified for reproductive toxicity. This is in accordance with the current classification.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No studies were submitted. However, clinical observations, FOB and pathology results from the subacute and (semi)chronic toxicity studies with rats, mice and dogs gave no indication for neurotoxicity of the test substance.

4.12.1.2 Immunotoxicity

No studies were submitted. However, clinical observations, hematology, clinical chemistry, and pathology results from the subacute and (semi)chronic toxicity studies with rats, mice and dogs gave no indication for immunotoxicity of the test substance.

4.12.1.3 Specific investigations: other studies

To further evaluate the carcinogenic mechanism of action a number of in vivo mechanistic studies were conducted that investigated whether etridiazole possessed promotor and/or initiator activity (see 4.10).

4.12.1.4 Human information

No information.

4.12.2 Summary and discussion

Not relevant.

4.12.3 Comparison with criteria

Not relevant.

4.12.4 Conclusions on classification and labelling

According to 67/548/EEC and EC 1272/2008, etridiazole does not need to be classified for neurotoxicity or immunotoxicity.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate and ecotoxicological properties of etridiazole were assessed in the Draft Assessment Report and additional report prepared in the context of the possible inclusion of etridiazole in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, April 2007 and additional report, May 2010, RMS the Netherlands).

Based on a review of the available data on aquatic toxicity, no change in the environmental classification is needed. However, a proposal for chronic aquatic toxicity and M-factors is made.

The summaries included in this proposal are partly copied from the DAR. Details of some of the summaries were not included when not considered important for a decision on the classification and labelling of this substance. Information on degradation studies are available from Volume 3 Annex B.8 of the DAR specifically section B.8.1.1.1 (soil studies) and B.8.4.3.2 (water-sediment studies).

To provide an overview of the substance, we have also included brief information on degradation and bioaccumulation. However, no changes are proposed to the conclusions on degradation and bioaccumulation.

5.1 Degradation

The available data show that the hydrolysis half-life of etridiazole at 25°C in buffered solutions of pH 5.2, 7.1, 8.9 and distilled water is 88 to 98 days. Data on hydrolysis in soil are not available from the DAR.

Etridiazole does not appreciably absorb light in wavelengths relevant to natural sunlight (maximum ϵ over the range 290 to 800 nm: 2.13 L/(mol \times cm) at 290 nm). Consequently, photochemical degradation was not determined.

Etridiazole was not readily biodegradable in a closed bottle test according to OECD 301D and EC C.4-E (1% biodegradation after 28 days) (Seyfried B., 2001). No inhibitory effects of the test substance were observed (>25% degradation within 14 days).

In two water/sediment systems, treated with [3-¹⁴C]-etridiazole at a concentration of 839 μ g/L and incubated at 20°C in the dark, etridiazole degraded in the total water/sediment system with half-lives of 1.78 d (r2 0.977) and 1.92 d (r2 0.981) (Schanné C., 1998). The half-lives for dissipation from the water layer were 1.33 d (r2 0.994) and 1.29 d (r2 0.996). CO₂ production was low (\leq 3.1% after 104 days).

Etridiazole acid was the most important metabolite in both water/sediment systems: maximum 13/8.3/<0.1% AR in water/sediment/volatiles on day 62/30/104. Other metabolites exceeding 5% AR in any compartment were dichloro-etridiazole (maximum 9.5/1.4/3.2% AR in water/sediment/volatiles on day 2/2/104) and M5 (maximum 4.8/2.9/1.6% AR in water/sediment/volatiles on day 7/7-14/14). Dichloro-etridiazole dissipated in the total water/sediment system with half-lives of 1.55 d (r2 0.995) and 2.99 d (r2 0.995). M5 dissipated in the total water/sediment system with a half-life of 17.2 d (r2 0.933).

No half-life for the sediment could be calculated.

In one soil treated with [3-¹⁴C] etridiazole in the laboratory, unextractable residues increased from 1.2% AR on day 0 to a maximum of 6.0% AR on day 90 and decreased to 5.2% AR on day 180 (Nag J.K. and Yu W.C., ((1994)). CO₂ was evolved from soil to a maximum of 22% AR on day 120 (90 day value: 8.2% AR; 180 day value: 21% AR). Volatile organic compounds, consisting of etridiazole (max. 50% AR) and dichloro-etridiazole (max. 6.1% AR), increased to a maximum of 60% AR on day 180. Etridiazole dissipated (volatilisation and degradation) with a FOMC half-life of 12.9 days (25°C) and degraded with a FOMC half-life of 45.5 days (25°C).

Dichloro-etridiazole and etridiazole acid exceeded 5% AR at two consecutive time points and reached a maximum in soil of 7.0% AR after 21 days and 6.7% AR after 90 days, respectively. The maximum level of dichloro-etridiazole formed at any time point (soil + traps) was 10.2% AR. The first-order half-life for dissipation (ignoring formation and including degradation and volatilisation) of dichloro-etridiazole was 119 days (25°C).

In two other soils treated with [3-¹⁴C] etridiazole (3.7 mg/kg, 20°C) in the laboratory, unextractable residues increased from 0.9/0.2% AR on day 0 to 39/30% AR on day 8 and thereafter remained between 29-40/23-33% AR (Nag J.K. and Regis R.R., (1998)). CO₂ was evolved from the soil to 4.8/4.7% AR on day 100. Volatile organic compounds increased to 19/26% AR on day 16/32 and thereafter remained between 16-21/26-36% AR, consisting of etridiazole (12-33% AR) and dichloro-etrydiazole (~3% AR). Etridiazole dissipated (volatilisation and degradation) with SFO half-lives of 1.9 and 3.6 days (20°C) and degraded with a SFO half-life of 2.2 and 4.8 days (20°C).

Dichloro-etrydiazole and etridiazole acid were the most important soil metabolites with a maximum of 13.3/12.9% AR (including volatiles) on day 4/8 and 31/20% AR on day 32/64, respectively. The first-order half-lives for dissipation (ignoring formation and including degradation and volatilisation) of dichloro-etrydiazole were 4.7 and 7.8 days (20°C).

A summary of laboratory data on biodegradation in soil is presented in Tables 14a-c.

Table 14a Summary of the laboratory data on biodegradation of etridiazole in soil

Parent	Aerobic conditions - persistence endpoints						
Soil type	X ¹	pH	t. °C / % MWHC	DT ₅₀ /DT ₉₀ (d)	DT ₅₀ (d) 20°C	St. (r ²)	Method of calculation
Sandy loam		6.6	25°C / 75% FC	45.5/194	67.9	0.98	FOMC
loam		7.4	20°C / pF 2.5	2.22/7.37	2.22	0.98	SFO
sandy loam		6.0	20°C / pF 2.5	4.80/16.0	4.80	0.96	SFO
Geometric mean/median/mean					8.98/4.80/25.0		

¹ X This column is reserved for any other property that is considered to have a particular impact on the degradation rate

Table 14b Summary of the laboratory data on biodegradation of dichloro-etrydiazole in soil

Dichloro-etrydiazole	Aerobic conditions - persistence endpoints							
Soil type	X ¹	pH	t. °C / % MWHC	DT ₅₀ /DT ₉₀ (d)	f. f. k _{dp} /k _f	DT ₅₀ (d) 20°C	St. (r ²)	Method of calculation
Sandy loam		6.6	25°C/75% FC	119/395	-	178 ²	0.93	SFO
loam		7.4	20°C/pF 2.5	7.76/47.7	-	7.76 ²	0.96	FOMC
sandy loam		6.0	20°C/pF 2.5	4.66/33.3	-	4.66 ²	0.99	FOMC
Geometric mean/median/mean						18.6/7.76/63.5		

¹ X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

² worst-case half-life for *dissipation*

Table 14c Summary of the laboratory data on biodegradation of etridiazole acid in soil

Etridiazole acid	Aerobic conditions - persistence endpoints							
Soil type	X ¹	pH	t. °C / % MWHC	DT ₅₀ /DT ₉₀ (d)	f. f. k _{dp} /k _f	DT ₅₀ (d) 20°C	St. (r ²)	Method of calculation
Sandy loam		6.0	20°C/45%	36.0/120	-	36.0	0.99	SFO

loam		7.4	20°C/45%	36.5/121	-	36.5	0.99	SFO
sandy loam		5.1	20°C/45%	7.64/25.4	-	7.64	0.99	SFO
Geometric mean/median/mean						21.6/36.0/26.7		

¹X This column is reserved for any other property that is considered to have a particular impact on the degradation rate.

Data on mobility in soil are not available from the DAR.

5.1.1 Summary and discussion of degradation

Etridiazole is considered to be not rapidly degradable.

5.2 Environmental distribution

Not applicable for this dossier.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

Based on experimentally determined partition coefficient (octanol water) etridiazole has a logK_{ow} value of 3.37 at 26 to 27.5°C.

5.3.1.1 Bioaccumulation estimation

No data available

5.3.1.2 Measured bioaccumulation data

One bioaccumulation study in Bluegill sunfish (*Lepomis macrochirus*) performed according to guideline EPA 165-4 is available (DAR, Volume 3 Annex B.9, section B.9.2.3).

The BCFs for etridiazole in edible tissue, non-edible tissue and whole fish are 92, 256 and 165 L/kg wet weight, respectively (Schocken M.J. (1994)). The depuration CT₅₀ for radioactivity in edible tissue, non-edible tissue and whole fish is <1 day, CT₉₀ values are >14 days.

5.3.2 Summary and discussion of aquatic bioaccumulation

Etridiazole has logK_{ow} of 3.37. Measured bioaccumulation data showed that the bioaccumulation potential of etridiazole is low to moderate, BCF values ranged from 92 to 256 L/kg ww.

The BCF value of 165 L/kg for whole fish is tested against the criteria for bioaccumulation. The BCF fulfils the criteria for bioaccumulating potential conform Directive 67/548/EEC, since it exceeds the value of 100 but not for Regulation EC 1272/2008, as it is not equal or does not exceed the value of 500.

5.4 Aquatic toxicity

A brief summary of the aquatic toxicity studies listed in the DAR for the three trophic levels fish, aquatic invertebrates and algae/aquatic plants are reported below. Only reliable and acceptable ecotoxicity tests from the Draft Assessment Report were used.

Aquatic toxicity data are available from Volume 3 Annex B.9 of the DAR. Details on the individual acute toxicity studies of the active substance and of metabolites can be found in sections B.9.2.1.1 and B.9.2.1.2, respectively. Additionally, details on the individual chronic toxicity studies of the active substance can be found in section B.9.2.2.1.

Aquatic toxicity studies of metabolites of etridiazole

Etridiazole acid was shown to be the only major (>10%) metabolite in the water phase of a water/sediment system. In soil, both dichloro-etrydiazole and etridiazole acid were identified as major metabolites. Acute aquatic toxicity data are available from the DAR for the metabolites dichloro-etrydiazole and etridiazole acid.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

A summary of acute toxicity data is presented in Table 15.

Table 15 Summary of the acute toxicity to fish

Substance	Purity (%)	Species	Method 96 hr	LC ₅₀ mg as/L	Concentration
Etridiazole	97.9	<i>Oncorhynchus mykiss</i>	Flow-through	2.4	mean measured
Etridiazole	97.9	<i>Cyprinodon variegatus</i>	Flow-through	4.0	mean measured
Etridiazole acid	99.8	<i>Lepomis macrochirus</i>	Static	> 100	nominal
Dichloro-etrydiazole	99.75	<i>Oncorhynchus mykiss</i>	Flow-through	0.77	mean measured

The acute toxicity of etridiazole to rainbow trout (*Oncorhynchus mykiss*) was tested at five concentrations (1.3-10 mg/L) for 96-h under flow trough test conditions following guidelines OECD 203, OPPTS 850.1075 and EEC C1 (Dionne E. (2002a)). 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). An LC₅₀ of 2.4 mg/L was determined in this study.

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 (etrydiazole) at nominal test concentrations of 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L, with untreated and solvent-control. Guideline EPA 72-3 was followed (Machado M.W. (1993a)). 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). An LC₅₀ of 4.0 mg/L was determined in this study.

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 (Sousa J.V. (1998)). Guidelines OECD 203 and EEC C1 were followed. 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).). An LC₅₀ of 0.77 mg/L was determined in this study.

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. Guideline OECD 203 was followed (Cafarella M.A. (2000a)). 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). An LC₅₀ of > 100 mg/L was determined in this study.

Conclusion: Acute toxicity values for etridiazole of 2.4 and 4.0 mg/L were observed. The lowest LC₅₀ of 2.4 mg/L was obtained with *Oncorhynchus mykiss*. For etridiazole acid one acute toxicity value of > 100 mg/L for *Oncorhynchus mykiss* was available. For dichloro-etridiazole the only acute toxicity value for fish was an LC₅₀ of 0.77 mg/L obtained with *Oncorhynchus mykiss*.

5.4.1.2 Long-term toxicity to fish

One study is available for chronic toxicity of etridiazole to fish. For the major metabolites etridiazole acid and dichloro-acid no chronic toxicity data for fish are available from the DAR.

A 90-day fish early life stage flow-through study was undertaken with rainbow trout (*Oncorhynchus mykiss*). The study was conducted in accordance with guideline EPA 72-4. 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) (Machado M.W. (1993c)). Mean measured concentrations were 0.032, 0.060, 0.12, 0.24 and 0.42 mg/L, representing 84 to 103% of nominal. 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. Based on reduced body weight, the NOEC was 0.12 mg/L and the LOEC was 0.24 mg/L.

Conclusion: In a chronic toxicity study for fish a NOEC value below 1 mg/L was obtained.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

A summary of acute toxicity data is presented in Table 16.

Table 16 Summary of the acute toxicity to aquatic invertebrates

Substance	Purity (%)	Species	Method	EC ₅₀ mg as/L	Concentration
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Etridiazole	97.9	<i>Daphnia Magna</i>	48 h, flow-through	3.1	Mean measured
Etridiazole	97.92	Eastern oyster, <i>Crassostrea virginica</i>	96-h, flow-through	3.0	Mean measured
Etridiazole	97.92	Mysid shrimp, <i>Mysidopsis bahia</i>	96-h, flow-through	2.5	Mean measured
Etridiazole acid	100	<i>Daphnia Magna</i>	48 h, static	350	Mean measured
Dichloro-etridiazole	99.75	<i>Daphnia Magna</i>	48 h, flow-through	1.1	Mean measured

The acute toxicity of etridiazole to *Daphnia magna* was tested according to EEC C2, OECD 202, OPPTS 850.1010 guidelines at five nominal concentrations (0.94-15 mg/L), with untreated and solvent-control (Dionne E. (2002b)). 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). An EC₅₀ of 3.1 mg/L was determined in this study.

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 (Machado M.W. (1993b)). Guideline EPA 72-3 was followed. 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). An LC₅₀ of 2.5 mg/L was determined in this study.

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 (Dionne E. (1993)). Guideline EPA 72-3 was followed. 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). An LC₅₀ of 3.0 mg/L was determined in this study.

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 (Putt A.E. (2001)). Guidelines EEC C2 and OECD 202 were followed. 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%. An EC₅₀ of 1.1 mg/L was determined in this study.

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 (Cafarella M.A. (2000b)). Guideline OECD 202 was followed. 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. An EC₅₀ of 350 mg/L was determined in this study.

Conclusion: The lowest acute toxicity to aquatic invertebrates was obtained with *Mysidopsis bahia*, LC₅₀ of 2.5 mg/L for etridiazole. For etridiazole acid one acute toxicity value of 350 mg/L for *Daphnia magna* was available. For dichloro-etridiazole the only acute toxicity value for aquatic invertebrates was an LC₅₀ of 1.1 mg/L obtained with *Daphnia magna*.

5.4.2.2 Long-term toxicity to aquatic invertebrates

One study is available for chronic toxicity of etridiazole to aquatic invertebrates. For the major metabolites etridiazole acid and dichloro-acid no chronic toxicity data for aquatic invertebrates are available from the DAR.

The chronic toxicity to *Daphnia magna* was tested in a flow-through test design for 21 days, in accordance to EPA guideline 72-4. *Daphnia* were exposed at five concentrations (0.37–3.6 mg/L, mean measured) (Putt A.E. (1993)). The NOEC (21-d) for *Daphnia magna* in this study was determined to be 0.37 mg/L.

Conclusion: In a chronic toxicity study for aquatic invertebrates a NOEC value below 1 mg/L was obtained.

5.4.3 Algae and aquatic plants

Algae

A summary of acute toxicity data is presented in Table 17.

Table 17 Summary of the acute toxicity to algae/aquatic plants

Substance	Purity (%)	Species	Method	E _r C ₅₀ mg/L	NOEC mg/L
Etridiazole	97.92	<i>Selenastrum Capricornutum</i> *	120 h, static	0.49	0.027#
Etridiazole	97.92	<i>Anabaena flos-aquae</i>	120 h, static	> 1.0	0.063
Etridiazole	97.92	Duckweed, <i>Lemna gibba</i>	14 d, static	14	5.7
Etridiazole acid	100	<i>Selenastrum Capricornutum</i> *	72 h, static	29	12
Dichloro-etridiazole	92.6	<i>Selenastrum Capricornutum</i> *	72 h, static	> 0.98	0.15

* *Pseudokirchneriella subcapitata* newer name for *Selenastrum capricornutum*.

72 h exposure

A 120-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per concentration, each containing 0.3×10^4 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 (Hoberg J.R. (1993a)). Guidelines EPA 122-2 and 122-3 were followed. 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 μ mhos/cm). 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; based on mean measured concentrations, 120-h E_bC_{50} and E_rC_{50} are 0.17 and 0.49 mg/L, respectively.

A 120-hour toxicity test on blue-green algae (*Anabaena flos-aquae*) (3 replicates per concentration, each containing 1.0×10^4 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 (Hoberg J.R. (1993b)). Guidelines EPA 122-2 and 122-3 were followed. 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). 120-hour E_bC_{50} and E_rC_{50} : 0.42 and >1.0 mg/L, respectively; 120-hour NOE_bC and NOE_rC : 0.063 mg/L; based on mean measured concentrations, 120-h E_bC_{50} and E_rC_{50} are 0.37 and >1.0 mg/L, respectively.

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 EC_{50} 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. Reported EC_{50} 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 EC_{50} 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.

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per concentration, each containing 1.0×10^4 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. Guidelines OECD 201 and EEC C3 were followed (Hoberg J.R. (1998)). 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). 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).

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per concentration, each containing 1.0×10^4 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. Guideline OECD 201 was followed (Hoberg J.R. (2000)). 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. 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.

Aquatic plants

The chronic toxicity of etridiazole to duckweed (*Lemma gibba*) was tested according to EPA-guidelines 122-2 and 123-2. A 14-day toxicity test (3 replicates per concentration, each containing five plants with three fronds each) was conducted with Terrazole Technical (etridiazole) at nominal (Hoberg J.R. (1993c)). 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). test concentrations of 1.6, 3.1, 6.3, 13, 25 and 50 mg/L, with untreated control. 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.

Conclusion: The lowest ErC50 for growth to aquatic algae/plants for etridiazole was >1 mg/L. For etridiazole acid one acute toxicity value of 29 mg/L for *Selenastrum capricornutum* was available. For dichloro-etridiazole the only acute toxicity value for aquatic algae/plants was an ErC50 of > 0.98 mg/L obtained with *Selenastrum capricornutum*.

5.4.4 Other aquatic organisms (including sediment)

Not relevant for this dossier.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Summary of the lowest L(E)C50 and NOEC values obtained in aquatic toxicity studies:

Acute toxicity

Etridiazole

Fish	<i>Oncorhynchus mykiss</i>	96h-LC50 = 2.4 mg/L
Invertebrates	<i>Mysidopsis bahia</i>	96h-LC50 = 2.5 mg/L
Algae/aquatic plant	<i>Selenastrum capricornutum</i>	120 h-ErC50 = 0.49 mg/L

Etridiazole acid

Fish	<i>Oncorhynchus mykiss</i>	96h-LC50 = >100 mg/L
Invertebrates	<i>Daphnia magna</i>	48h-EC50 = 350 mg/L
Algae/aquatic plant	<i>Selenastrum capricornutum</i>	72 h-ErC50 = 29 mg/L

Dichloro-etridiazole

Fish	<i>Oncorhynchus mykiss</i>	96h-LC50 = 0.77 mg/L
Invertebrates	<i>Daphnia magna</i>	48h-EC50 = 1.1 mg/L
Algae/aquatic plant	<i>Selenastrum capricornutum</i>	72 h-ErC50 > 0.98 mg/L

Chronic toxicity

Etridiazole

Fish	<i>Oncorhynchus mykiss</i>	90 d NOEC = 0.12 mg/L
Invertebrates	<i>Daphnia magna</i>	21 d NOEC = 0.37 mg/L
Algae/aquatic plant	<i>Selenastrum carpicornutum</i>	72 h NOEC = 0.027 mg/L

Degradation

Etridiazole is considered to be not rapidly degradable (see section 5.1.1). The main degradation product of etridiazole, etridiazole acid, does not fulfil the criteria for classification under CLP.

Bioaccumulation

Etridiazole fulfils the criteria for bioaccumulating potential conform Directive 67/548/EEC, since it exceeds the value of 100 but not for Regulation EC 1272/2008, since it does not exceed the value of 500 (see section 5.3.2).

CLP Acute aquatic hazard

Etridiazole produces an acute aquatic EC₅₀ value ≤ 1 mg/L for algae.

Etridiazole produces acute aquatic EC₅₀ values > 1 mg/L for fish and invertebrates.

Based on the result of the toxicity test with algae etridiazole fulfils the criteria for classification with Acute category 1.

Since the EC₅₀ value for algae is between 0.1 and ≤ 1 mg/L (0.49 mg/L), an M-factor of 1 is assigned to etridiazole. This classification results in labelling of etridiazole with H400.

CLP Chronic aquatic hazard

Etridiazole produces an aquatic NOEC value ≤ 0.1 mg/L for algae.

Etridiazole produces aquatic NOEC values between > 0.1 and ≤ 1 mg/L for fish and invertebrates.

Etridiazole is not considered rapidly degradable.

The log K_{ow} value of etridiazole is < 4 .

Based on the result of the toxicity test with algae etridiazole fulfils the criteria for classification with Chronic category 1.

Since the NOEC value for algae is between 0.01 and ≤ 0.1 mg/L (0.027 mg/L), an M-factor of 1 is assigned to etridiazole. This classification results in labelling of etridiazole with H410.

Directive 67/548/EEC

According to the criteria of Directive 67/548/EEC, a substance can be classified for acute or chronic hazards to the environment. If a substance has acute aquatic toxicity of < 100 mg/L and is not

rapidly degradable or has a log Kow of ≥ 3 , it is classified for long-term hazards to the environment. Assignment into division depends on the lowest acute aquatic toxicity value.

The lowest acute aquatic toxicity value for etridiazole is 0.49 mg/L in aquatic algae, respectively. Etridiazole is not rapidly degradable and fulfills the criteria for bioaccumulation. Etridiazole, therefore fulfils the criteria for classification with N;R50/53.

Based on the lowest acute aquatic toxicity value (0.49 mg/L) specific concentration limits are not proposed.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Table 18 Conclusion on environmental classification

	CLP Regulation	Directive 67/548/EEC (DSD)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410) M-factor Acute M-factor 1 Chronic M-factor 1	N; R50-53

6 OTHER INFORMATION

7 REFERENCES

European Food Safety Authority. Conclusion on the peer review of the pesticide risk assessment of the active substance etridiazole. EFSA journal 2010;8(10):1823

European Commission. Draft Assessment Report Etridiazole, Prepared by the Netherlands, April 2007.

European Commission. Additional report to the Draft Assessment Report Etridiazole, Prepared by the Netherlands, December 2009.

European Commission. Addenda to the Draft Assessment Report Etridiazole, Prepared by the Netherlands, May and June 2010.

8 ANNEXES

Etridiazole was discussed in the TC-C&L in November 1995, November 1996 and May 1997 (Summary records ECBI/94/95, ECBI/45/96, ECBI/27/97). The parts on etridiazole in these summary records are copied below.

8.1 Annex Summary records ECBI/94/95, ECBI/45/96, ECBI/27/97



EUROPEAN COMMISSION

DIRECTORATE GENERAL JRC

JOINT RESEARCH CENTRE
Environment Institute
European Chemicals Bureau

ECBI/94/95 - Rev. 1

SUMMARY RECORD
*Commission Working Group on the
Classification and Labelling of Dangerous Substances*

Pesticides

Meeting at ECB Ispra, 8 - 10 November 1995

Etridiazole (P547). EEC No: 219-991-8. CAS No: 2593-15-9.

Proposal: T; R23: Xn; R21/22: N; R51-53.

XI/502/93 - Add. 31

IRL, comments on pesticides covered by Italian list.

XI/502/93 - Add. 33

BE, Proposals for the classification and labelling of pesticides.

ECBI/71/95 - Add. 20

DE, Comments on pesticides, P521 to P590 and metaldehyde.

The **Group** agreed on the classification proposal. However, **NL** did ask that the manufacturers, Uniroyal, supply additional information on the algal data. Two other proposals were raised for reproductive toxicity and carcinogenicity. All delegates requested further information from Uniroyal on these additional health effects. **NL** stated that they would provide a short evaluation on the carcinogenicity of this substance.

Conclusion:

The **Group** agreed to the provisional classification as: T; R23: Xn; R21/22 : N; R51-53.

The issue of classification for additional health effects, such as reprotoxicity and carcinogenicity, would be addressed at the next meeting in November 1996. The producers, Uniroyal were asked to provide the **Group** with the additional information they required.

EUROPEAN COMMISSION

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European Chemicals Bureau

ECBI/45/96 - Rev. 2

SUMMARY RECORD
*Commission Working Group on the
Classification and Labelling of Dangerous Substances*

Pesticides

Meeting at ECB Ispra, 6 - 8 November 1996

Etridiazole (P547). EEC No: 219-991-8. CAS No: 2593-15-9.

Proposal: [Carc. Cat. 3; R40] : T; R23: Xn; R21/22: [N; R50-53].

XI/502/93 - Add. 31	IRL, comments on pesticides covered by Italian list
XI/502/93 - Add. 33	BE, proposals for the classification and labelling of pesticides
ECBI/71/95 a, b, c	ECB, classification proposals for P518 to P589
ECBI/71/95 - Add. 20	DE, comments on pesticides, P521 to P590 and metaldehyde
ECBI/71/95 - Add. 28	Uniroyal, additional information on Etridiazole (P547)
ECBI/71/95 - Add. 31	NL, evaluation of the carcinogenicity of Etridiazole (P547)
ECBI/71/95 - Add. 38	BE, comments on classification proposal for etridiazole (P547)
ECBI/71/95 - Add. 39	UK, comments on classification proposal for etridiazole (P547)
ECBI/71/95 - Add. 40	NL, evaluation of the environmental classification of Etridiazole (P547)
ECBI/71/95 - Add. 58	Uniroyal, additional comments on Etridiazole (P547)
ECBI/71/95 - Add. 65	DE, environmental classification of pesticides
ECBI/71/95 - Add. 69	Uniroyal, comments on classification of etridiazole (P547)

In November 1995, the **Group** agreed to the provisional classification as: T; R23: Xn; R21/22 : N; R51-53. Industry has provided additional data on the substance. **NL** has evaluated the carcinogenicity, and feels that the substance cannot yet be classified for this effect. **BE** feels that it is borderline to Category 3, whilst the **UK** feels that Category 3 is justified. **NL** has suggested classification as N; R50-53. Classification for reproductive toxicity is not supported.

The **UK** noted that there were several types of tumours observed in mice and rats. On the basis of the rat data, classification with Carc. Cat. 3; R40 was justified, an opinion shared by **ES**, **FR**, **FIN** and **S**. **NL** stated that it was first important to determine which tumour types were significant, as a number of types were observed in excess of the Maximum Tolerated Dose. Hence, **NL** did not accept that classification with Category 3 was justified. In reply, the **UK** asked if the **Group** were prepared to discount thyroid carcinogenesis in the male rat. **BE** stated that the substance was a borderline Category 3 or O, and they would welcome more information on kidney neoplasms. Industry were asked to provide additional information on the tumour types but not on the tumour promotion aspects.

It was noted that the concern with the thyroid tumours was similar to that of ethylene thiourea (W029), which was currently being discussed also for carcinogenic effects at the Main C/M/R Group meeting. The **Group** agreed that should ethylene thiourea be sent to the Specialised Experts

for their consideration of the relevance of the thyroid tumours, then etridiazole should also be sent. **FIN** remarked that should thyroid tumours be acceptable then this might reopen the discussion on other thyroid tumour producing substances in Annex I. **FIN** welcomed further mechanistic information.

With regard to the environment, agreement had been reached to classify the substance with N; R51-53. **NL** had subsequently sent in new information which suggested that classification with N; R50-53 was warranted. The **Group** agreed to classify the substance with N; R50-53.

Conclusion:

The **Group** agreed to the provisional classification as: [Carc. Cat. 3; R40]: T; R23: Xn; R21/22 : N; R50-53. The **Group** agreed to rediscuss the carcinogenic classification at the next meeting in May 1997, following the distribution of additional information from Industry.

EUROPEAN COMMISSION

DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Environment Institute
European Chemicals Bureau

ECBI/27/97 - Rev. 3

SUMMARY RECORD

*Commission Working Group on the
Classification and Labelling of Dangerous Substances*

Pesticides

Meeting at ECB Ispra, 28 - 30 May, 1997.

Etridiazole, P547. EEC No: 219-991-8, CAS No: 2593-15-9.

Proposal: [Carc. Cat. 3; R40] : T; R23 : Xn; R21/22 : [N; R50-53].

XI/502/93 - Add. 31	IRL, comments on pesticides covered by Italian list
XI/502/93 - Add. 33	BE, proposals for the classification and labelling of pesticides
ECBI/71/95 a, b, c	ECB, classification proposals for P518 to P589
ECBI/71/95 - Add. 20	DE, comments on pesticides, P521 to P590 and metaldehyde
ECBI/71/95 - Add. 28	Uniroyal, additional information on Etridiazole (P547)
ECBI/71/95 - Add. 31	NL, evaluation of the carcinogenicity of Etridiazole (P547)
ECBI/71/95 - Add. 38	BE, comments on classification proposal for etridiazole (P547)
ECBI/71/95 - Add. 39	UK, comments on classification proposal for etridiazole (P547)
ECBI/71/95 - Add. 40	NL, evaluation of the environmental classification of Etridiazole (P547)
ECBI/71/95 - Add. 58	Uniroyal, additional comments on Etridiazole (P547)
ECBI/71/95 - Add. 65	DE, environmental classification of pesticides
ECBI/71/95 - Add. 69	Uniroyal, comments on classification of etridiazole (P547)
ECBI/71/95 - Add. 78	Uniroyal, comments on classification of etridiazole (P547)
ECBI/71/95 - Add. 84	DE, environmental classification of etridiazole (P547)

In November 1995, the **Group** agreed to the provisional classification as: T; R23: Xn; R21/22 : N; R51-53. **NL** had evaluated the carcinogenicity prior to the 1996 meeting. In November 1996, the **Group** agreed to the provisional classification as: [Carc. Cat. 3; R40]: T; R23: Xn; R21/22: N; R50-53. The **Group** agreed to rediscuss the carcinogenic classification at the next meeting in May 1997, following the distribution of additional information from Industry (doc. ECBI/71/95 - Add. 71).

With regard to the environment, the **Group** confirmed their agreement to classify the substance with N; R50-53.

The **Group** recognised that classification as carcinogen category 3 was borderline, with several delegations prepared to accept no classification. The **Group** noted that this substance induces rat thyroid tumours similar to ethylene thiourea, although the exact mechanism was not known. **FIN** noted that **Industry** had concluded that the substance was a promoter. **IRL** raised the issue of kidney tumours. The **UK** noted that there was some evidence that hyaline droplets could be induced in the kidney by etridiazole. The **UK** had some concern about the liver tumours observed in rats even in the absence of an initiating agent and suggested that Carc. Cat. 3; R40 was appropriate. The **Group** agreed to classify the substance as a category 3 carcinogen. but recognised that this decision could be revised in the light of the discussions in IARC in November 1997 on mechanisms of cancer that are thought to be species-specific.

Conclusion: The **Group** agreed to classify the substance as: Carc. Cat. 3; R40: T; R23: Xn; R21/22: N; R50-53, with the symbols: T, N; R-phrases: 21/22-23-40-50/53 and S-phrases: (2-)36/37-38-45-60-61. The proposal will be sent to DG XI for possible inclusion in a future TPC. The proposal could however be rediscussed if necessary in the light of the discussions at IARC.