Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

etridiazole

EC number: 219-991-8
CAS number: 2593-15-9

CLH-O-0000002504-80-02/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

4 June 2013

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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CONTENTS

PART A. 5

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING 5

1.1 SUBSTANCE 5
1.2 HARMONISED CLASSIFICATION AND LABELLING PROPOSAL 5
1.3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING BASED ON CLP REGULATION AND/OR DSD CRITERIA 6

2 BACKGROUND TO THE CLH PROPOSAL 11

2.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 11
2.2 SHORT SUMMARY OF THE SCIENTIFIC JUSTIFICATION FOR THE CLH PROPOSAL 11
2.3 CURRENT HARMONISED CLASSIFICATION AND LABELLING 11
2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation 11
2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation 12
2.4 CURRENT SELF-CLASSIFICATION AND LABELLING 12
2.4.1 Current self-classification and labelling based on the CLP Regulation criteria 12
2.4.2 Current self-classification and labelling based on DSD criteria 12

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 12

PART B. 13

1 IDENTITY OF THE SUBSTANCE 13

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 13
1.2 COMPOSITION OF THE SUBSTANCE 14
1.3 PHYSICO-CHEMICAL PROPERTIES 15

2 MANUFACTURE AND USES 16

2.1 MANUFACTURE 16
2.2 IDENTIFIED USES 16

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES 17

4 HUMAN HEALTH HAZARD ASSESSMENT 18

4.1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 18
4.1.1 Non-human information 18
4.1.2 Human information 20
4.1.3 Summary and discussion on toxicokinetics 20
4.2 ACUTE TOXICITY 20
4.2.1 Non-human information 20
4.2.1.1 Acute toxicity: oral 20
4.2.1.2 Acute toxicity: inhalation 21
4.2.1.3 Acute toxicity: dermal 22
4.2.1.4 Acute toxicity: other routes 22
4.2.2 Human information 22
4.2.3 Summary and discussion of acute toxicity 22
4.2.4 Comparison with criteria 22
4.2.5 Conclusions on classification and labelling 22
4.3 SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE (STOT SE) 23
4.4 IRRITATION 25
4.4.1 Skin irritation 25
4.4.2 Eye irritation 25
4.4.3 Respiratory tract irritation 26
4.4.3.1 Non-human information 26
4.4.3.2 Human information 26
4.4.3.3 Summary and discussion of respiratory tract irritation 26

4.4.3.4 Comparison with criteria 26
4.4.3.5 Conclusions on classification and labelling 27
4.5 CORROSIVITY 27
4.6 SENSITISATION 27
4.6.1 Skin sensitisation 27
4.6.1.1 Non-human information 27
4.6.1.2 Human information 28
4.6.1.3 Summary and discussion of skin sensitisation 28
4.6.1.4 Comparison with criteria 28
4.6.1.5 Conclusions on classification and labelling 28
4.6.2 Respiratory sensitisation 29
4.7 REPEATED DOSE TOXICITY 29
4.8 SPECIFIC TARGET ORGAN TOXICITY (CLP REGULATION) – REPEATED EXPOSURE (STOT RE) 32
4.9 GERM CELL MUTAGENICITY (MUTAGENICITY) 36
4.10 CARCINOGENICITY 38
4.11 TOXICITY FOR REPRODUCTION 56
4.11.1 Effects on fertility 58
4.11.1.1 Non-human information 58
4.11.1.2 Human information 60
4.11.2 Developmental toxicity 60
4.11.2.1 Non-human information 60
4.11.2.2 Human information 65
4.11.3 Other relevant information 65
4.11.4 Summary and discussion of reproductive toxicity 65
4.11.5 Comparison with criteria 66
4.11.6 Conclusions on classification and labelling 66
4.12 OTHER EFFECTS 70
4.12.1 Non-human information 70
4.12.1.1 Neurotoxicity 70
4.12.1.2 Immunotoxicity 70
4.12.1.3 Specific investigations: other studies 70
4.12.1.4 Human information 70
4.12.2 Summary and discussion 70
4.12.3 Comparison with criteria 70
4.12.4 Conclusions on classification and labelling 70

5 ENVIRONMENTAL HAZARD ASSESSMENT 70

5.1 DEGRADATION 71
5.1.1 Summary and discussion of degradation 73
5.2 ENVIRONMENTAL DISTRIBUTION 73
5.3 AQUATIC BIOACCUMULATION 73
5.3.1 Aquatic bioaccumulation 73
5.3.1.1 Bioaccumulation estimation 73
5.3.1.2 Measured bioaccumulation data 73
5.3.2 Summary and discussion of aquatic bioaccumulation 73
5.4 AQUATIC TOXICITY 74
5.4.1 Fish 74
5.4.1.1 Short-term toxicity to fish 74
5.4.1.2 Long-term toxicity to fish 75
5.4.2 Aquatic invertebrates 75
5.4.2.1 Short-term toxicity to aquatic invertebrates 75
5.4.2.2 Long-term toxicity to aquatic invertebrates 77
5.4.3 Algae and aquatic plants 77
5.4.4 Other aquatic organisms (including sediment) 79
5.5 COMPARISON WITH CRITERIA FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.4) 79
5.6 CONCLUSIONS ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS (SECTIONS 5.1 – 5.4) 80

6 OTHER INFORMATION 85

7 REFERENCES 85

8 ANNEXES 85

8.1 ANNEX SUMMARY RECORDS ECBI/94/95, ECBI/45/96, ECBI/27/97 86

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

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

3.3.	Serious eye damage / eye irritation	-	-	-	Data conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	
3.4.	Skin sensitisation	Skin Sens 1B (H317)	-	-	
3.5.	Germ cell mutagenicity	-	-	-	Data conclusive but not sufficient for classification
3.6.	Carcinogenicity	Carc. 2 (H351)	-	Carc. 2 (H351)	
3.7.	Reproductive toxicity	-	-	-	Data conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	STOT SE 3 (H335)	-	-	
3.9.	Specific target organ toxicity – repeated exposure	-	-	-	Data conclusive but not sufficient for classification
3.10.	Aspiration hazard	-	-	-	Data conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)	Acute M-factor 1 Chronic M-factor 1	Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)	
5.1.	Hazardous to the ozone layer				Data conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Warning

Pictogram: GHS07, GHS08, GHS09

Hazard statements:
H351, Suspected of causing cancer
H302, Harmful if swallowed.
H335, May cause respiratory irritation
H317, May cause an allergic skin reaction.
H410, Very toxic to aquatic life with long lasting effects

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry:

A note is not proposed.

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	-	-	-	Data conclusive but not sufficient for classification
Oxidising properties	-	-	-	Data conclusive but not sufficient for classification
Flammability	-	-	-	Data conclusive but not sufficient for classification
Other physico-chemical properties	-	-	-	Data conclusive but not sufficient for classification
Thermal stability	-	-	-	Data conclusive but not sufficient for classification
Acute toxicity	Xn; R22	-	T; R23 Xn; R21/22	
Acute toxicity – irreversible damage after single exposure	-	-	-	Data conclusive but not sufficient for classification
Repeated dose toxicity	-	-	-	Data conclusive but not sufficient for classification
Irritation / Corrosion	Xi; R37	-	-	
Sensitisation	Xi; R43	-	-	
Carcinogenicity	Carc. Cat.3; R40	-	Carc. Cat.3; R40	
Mutagenicity – Genetic toxicity	-	-	-	Data conclusive but not sufficient for classification
Toxicity to reproduction – fertility	-	-	-	Data conclusive but not sufficient for classification
Toxicity to reproduction – development	-	-	-	Data conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	-	-	-	Data conclusive but not sufficient for classification
Environment	N, R50/53		N, R50/53	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger:

Xn	Harmful
N	Dangerous for the environment

R-phrases:

R22	Harmful if swallowed
R37	Irritating to respiratory system
R40	Limited evidence of a carcinogenic effect
R43	May cause sensitisation by skin contact
R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

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).

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 LD_{50} 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	Hazard statement	Pictogram, Signal	Hazard statement	Suppl. Hazard

Category Code(s)	Code(s)	Word Code(s)	Code(s)	statement Code(s)
Carc.2	H351	GHS06	H351	
Acute Tox 3*	H331	GHS07	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).

RAC general comment
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 and ECBI/27/97, respectively). Since the basis for the current classification is unknown, it is not possible to indicate in this CLH report which studies were or were not considered for the classification in the 25th ATP to Directive 67/548/EEC.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

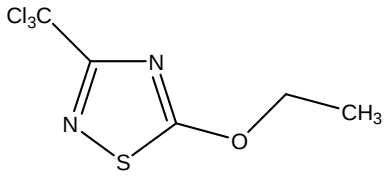
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	219-991-8
EC name:	etridiazole
CAS number (EC inventory):	2593-15-9
CAS number:	2593-15-9
CAS name:	5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole
IUPAC name:	ethyl-3-trichloromethyl-1,2,4-thiazol-5-yl ether
CLP Annex VI Index number:	613-133-00-X
Molecular formula:	C5H5Cl3N2OS
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 (H352), 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	22.0ºC	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 20ºC (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 ^{14}C-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 ½ 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}, ½ 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: 1141mg/kg bw LD_{50} female: 945 mg/kg bw	Rat: Sprague-Dawley Crl: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 Crl: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 LD_{50} 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 Crl: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 LC_{50} 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 LD_{50} 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 LD_{50} 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 (LD_{50} >5000 mg/kg bw and LC50 >5.7 mg/L respectively).

4.2.4 Comparison with criteria

Etridiazole has an oral LD_{50} in female rats of 945 mg/kg bw. In 67/548/EEC this is considered harmful ($200 < LD_{50} \leq 2\ 000$ 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 LD_{50} (>5000 mg/kg bw) is above the limits in 67/548/EEC ($400 < LD_{50} \leq 2\ 000$ 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 LC_{50} (>5.7 mg/L) is above the limits in 67/548/EEC for aerosols and particulates ($1 < LC_{50} \leq 5$ 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.

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal Based on the incongruity between the results of the acute toxicity studies in the DAR and the current classification, it was concluded that the studies in the DAR were not available for the TC-C&L in 1995-1997, even though these studies were conducted already in 1994.

Acute toxicity: oral

In an OECD TG 401 –compliant study on rats, doses of 700, 850, 1000 and 1400 mg/kg bw were tested. The acute oral LD_{50} of etridiazole was determined to be 1141 and 945 mg/kg bw for males and females, respectively. Therefore the current classification as acute toxicity 4*; H302 is proposed to be changed to acute toxicity 4; H302 by removing the *, indicating the removal of the minimum classification from this category.

Acute toxicity: inhalation

It is proposed to remove the current classification of acute toxicity 4*; H331 because in the OECD TG 403 rat study, the acute inhalation LC_{50} values of etridiazole were found to be greater than 5.7 mg/l for both males and females (aerosol exposure with mass median aerodynamic diameter (MMAD) 1.9-3.8 µm; geometric standard deviation (GSD) 1.60-1.81 µm).

Acute toxicity: dermal

It is proposed to remove the current classification of acute toxicity 4*; H312 because in an OECD TG 402 rabbit study no mortality occurred at the tested dose of 5000 mg/kg bw. The observed clinical signs consisted of loss of appetite and decreased defecation and/or no stools. 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 in 4/5 females. Thus, the acute dermal LD_{50} of etridiazole was found to be >5000 mg/kg bw in male and female rabbits.

Assessment and comparison with the classification criteria

The acute LD_{50} of etridiazole (1141 mg/kg bw in male rats and 945 mg/kg bw in female rats) via the oral route falls within the range of values for classification for acute toxicity 4; H302 (300 <LD_{50}> 2000 mg/kg bw) and Xn; R22 (200 <LD_{50}> 2000 mg/kg bw) in accordance with the CLP and DSD criteria, respectively. For the inhalation and dermal routes, all estimated LD/C_{50} values are above the criteria thresholdfor classification and labelling (CLP and DSD).

RAC supported the classification as acute toxicity 4; H302 (Xn, R22) and agreed with the dossier submitter (DS) to remove the classification as acute toxicity 3*, H331 (T, R23) and acute toxicity 4*; H312 (Xn, R21).

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).

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

The DS proposed to classify etridiazole for respiratory tract irritation (STOT SE3, H335) based on the results of an OECD TG 403 acute inhalation study in rats and an OECD TG 412 subacute repeated dose inhalation toxicity study in rats.

In the acute inhalation study, laboured breathing (M:1/5; F:5/5) was observed in the high-dose group (5.7 mg/l) within 1 day of exposure. After day 1, rapid respiration

(M:3/5; F:5/5) and laboured breathing (F:2/5) were observed, but these effects disappeared before the termination of the experiment. At 1.2 mg/l, rapid respiration was observed in all animals on the day of exposure. After day 1, one female still showed rapid respiration but the effect was reversed by the end of the experiment.

In the repeated dose inhalation toxicity study, no NOAEL could be derived for local effects, because squamous metaplasia of the larynx mucosa was observed at all dose levels (LOAEL 15 mg/m^3 = 0.015 mg/l).

According to the DS, the observed findings (breathing difficulties and squamous metaplasia) were signs of reversible respiratory tract irritation. Therefore, for these effects, classification for specific target organ toxicity after a single exposure (STOT SE 3; H335 and Xi; R37) was considered as more appropriate than classification for specific target organ toxicity after repeated exposure.

Comments received during public consultation
The German MSCA did not support the proposed classification as STOT SE 3 because etridiazole had been tested with rather high dust concentrations (about 1000 and 5000 mg/m³). According to the MSCA, it was normal that animals tried to prevent inhalation of such high dust concentrations. Therefore, clinical observations such as laboured breathing and/or rapid respiration would be an expected general reaction in animals exposed to such high concentrations of dust rather than indicating a potential for chemical-specific primary respiratory irritation.

|Two MSCAs supported the DS proposal to classify etridiazole as STOT SE 3; H335 and Xi; R37.

Assessment and comparison with the classification criteria
Since human data for respiratory irritation was not available, information obtained from single and repeated inhalation toxicity tests was used in accordance with the CLP Guidance Annex 1:3.8.2.2.1.

The acute inhalation study reported abnormal gait, tremors, decreased activity, increased salivation and labored breathing in exposed rats. While no mortality was seen up to 5.7 mg/l, laboured breathing was seen immediately after exposure and rapid respiration was noted after day 1 in the acute inhalation test. At 1.2 mg/l rapid respiration was observed immediately after exposure in males and females. The effect was reversible on day 1 in all animals apart from one female. At the end of the observation period no treatment-related macroscopic observations were seen. Histopathology data were not available in the CLH report or in the draft assessment report (DAR). Neither nasal discharge nor rhinitis was reported, although it was recognised that the reporting was not very detailed and there was no information on effects during the exposure. Given the dusty nature of the chemical, RAC does not interpret these observations as clear evidence of a chemical-specific, respiratory irritant response.

Rats repeatedly exposed to etridiazole at concentrations of 0.015, 0.075 and 0.2 mg/l showed red nasal discharge with a dose-related trend. However, in the single exposure study, with the higher concentrations of 1.2 and 5.7 mg/l etridiazole, no nasal discharge was reported. RAC concluded that the effect was likely to occur only after repeated exposure and does not support classification for STOT SE.

As the metaplasia of the larynx reported in rats after repeated exposure was only of minimal severity and as it was unclear whether the effect was a result of single or repeated exposure the effect was not considered as sufficient to support classification for STOT SE.

Overall, RAC concluded that the available evidence did not warrant classification for STOT SE 3 – H335 (Xi, R37).

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.

RAC evaluation of skin corrosion/irritation
Summary of the Dossier submitter's proposal No skin irritation was observed in an OECD TG 404 skin irritation study in rabbits. The dossier submitter did not propose classification for skin corrosion/irritation. **Comments received during public consultation** No specific comments were received on this hazard class. **Assessment and comparison with the classification criteria** As no skin irritation was observed in the rabbit study, RAC agreed with the dossier submitter's conclusion that no classification for skin corrosion/irritation is warranted.

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.

RAC evaluation of eye corrosion/irritation
Summary of the Dossier submitter's proposal No classification for eye corrosion/irritation was proposed by the dossier submitter as only slight eye irritation was observed in an OECD TG 405 eye irritation study in rabbits and no human data were available. **Comments received during public consultation** No specific comments were received for this hazard class. **Assessment and comparison with the classification criteria** Mean scores (24/48/72 h) on eye irritation were reported in the DAR. Due to the low scores for eye irritation RAC agreed with the dossier submitter's conclusion that no classification for eye corrosion/irritation was warranted.

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 is 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 observation 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/m^3 = 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 genotoxicity[1]. CIGA binding to α_{2u}-globulin is reversible and not

[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 α2u-globulin, Hyaline Droplet Neuropathy, and Tubule Neoplasia in the Kidneys of Male Rats; Environmental Health Perspectives, Vol. 99, pp. 313-349, 1993.

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.

RAC evaluation of respiratory sensitisation
Summary of the Dossier submitter's proposal No data on respiratory sensitisation was available for the dossier submitter. Accordingly, no classification was proposed for this hazard class. **Comments received during public consultation** No comments were received for this hazard class. **Assessment and comparison with the classification criteria** RAC could not conclude on the classification for respiratory sensitisation due to the lack of data and this should be considered as 'not evaluated'.

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, Magnussen & 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 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.

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 Magnussen & 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.

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal In an OECD TG 406 (Magnusson and Kligman) study on 20 control and 20 test female guinea pigs within a group, the following concentrations of etridiazole were tested: 20% intradermal induction, 100% topical induction, 100 or 50% first challenge and 20, 10, 5 or 1% second challenge. After topical challenge, none to moderate erythema and none to well-defined oedema were observed in the treated animals. Also thickening, dryness and sloughing of the epidermis were noted. In comparison, to the 50% concentration, the effects were more marked after challenge with undiluted etridiazole. All treated animals were sensitised. After topical rechallenge with 20, 10, 5, or 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 reactions. The results of the positive control were not reported. According to the dossier submitter, etridiazole showed skin reactions in 100% of the animals in an adjuvant type guinea pig test method for skin sensitisation. The dossier submitter proposed to classify etridiazole for skin sensitisation 1B; H317 (CLP) and Xi; R43(DSD) because over 30% of the animals were considered positive after >1% induction concentration. **Comments received during public consultation** Three MSCAs supported the dossier submitter's proposal to classify etridiazole for skin

sensitisation1B; H317 because skin reactions were observed in ≥30% of the animals treated with >1% etridiazole.

Assessment and comparison with the classification criteria

100% positive responders were observed in a Guinea Pig Maximization Test using 20% etridiazole for the intradermal induction dose.

The CLP criteria for skin sensitisation as amended by the 2nd ATP state that "Skin sensitisers shall be classified in category 1 where data are not sufficient for sub-categorisation" (3.4.2.2.1.1.). In the draft CLP guidance it is stated that:

> *"Classification into sub-categories is only allowed if data are sufficient. Therefore care should be taken when classifying substances into category 1B when category 1A cannot be excluded. In such cases classification into category 1 should be considered. This is particularly important if only data are available from certain tests showing a high response after exposure to a high concentration but where lower concentrations which could show the presence of such effects at lower doses are absent (in line with some test protocols where a maximised dose should be used)".*

RAC agreed with the dossier submitter that etridiazole should be classified as a skin sensitiser because skin reactions were seen in ≥30% of test animals at >1% induction concentrations. RAC did not, however, agree with the dossier submitter's original proposal to classify for skin sensitisation 1B because the data did not allow the exclusion of category 1A. RAC concluded therefore that etridiazole should be classified as skin sensitisation 1; H317 (Xi; R43).

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 $NOAEL_{syst}$: 20 mg/kg bw/d	Rats, Sprague Dawley Crl:CD(SD)IGS BR	DAR, Annex IIA, 2002
OECD 412: 28-d inhalation toxicity	$NOAEC_{local}$: <15 mg/m^3 $NOAEC_{syst}$: 15 mg/m^3	Rats, Sprague Dawley Crl: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 Crl: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 is 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) Crl: CD (SD)IGS BR	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL local** **NOAEL systemic**	:	<15 mg/m^3 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/m^3. 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/m^3 and minimal effects at 100 mg/m^3 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							ic		
- potassium					ic		ic		
- calcium							ic		
Organ weights									
- liver			$ic^{a,r}$	i^{a},ic^{r}	i^{a},ic^{r}	i^{a},ic^{r}	i^{r}	i^{r}	
Pathology									
Macroscopy	No treatment-related findings								
Microscopy									
- 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/m^3 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/m^3 (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/m^3 (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/m^3 in males (102 and 104% of control, resp.). Potassium was significantly increased at 75 and 200 mg/m^3 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/m^3 in males (113% of control) and increased at 75 mg/m^3 in males (107% of control) and at 15 and 75 mg/m^3 in females (106-107% of control). Relative liver weight was significantly increased at 15 and 75 mg/m^3 in both sexes (109-111% of control) and increased at 200 mg/m^3 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/m^3 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 genotoxicity[2]. 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/m^3 (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) .

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)
Summary of the Dossier submitter's proposal
The dossier submitter presented a summary table on the available repeated dose studies. Three rat studies (a 28-day dermal, a 28-day inhalation and a 90-day oral) and a 1-year oral study in dogs were referenced in the CLH report. The detailed study results were given only for the 28-day inhalation study. For other studies only the NOAEL/C was reported. All studies followed OECD TG test guidelines. More information

[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 α2u-globulin, Hyaline Droplet Neuropathy, and Tubule Neoplasia in the Kidneys of Male Rats; Environmental Health Perspectives, Vol. 99, pp. 313-349, 1993.

on the studies from the DAR has therefore been added below.

The dossier submitter did not propose to classification for STOT RE.

Comments received during public consultation

According to the one MSCA, the increased platelet counts in male and female rats at ≥64.7 mg/kg and reduced prothrombin time (PTT) and activated thromboplastin time (APTT) at ≥29.5 mg/kg in the oral 90-day study (Richards, 1994) indicated a hypercoagulable status. Thrombus formation in the mouse heart at ≥184.7 mg/kg in the 18-month carcinogenicity study (Goldenthal, 2004) supported their interpretation.. The same MSCA concluded that these effects may be significant (meaning changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant) but not serious (meaning more profound or serious effects and indicating changes that are of a considerably adverse nature with a significant impact on health) and questioned whether this evidence would be sufficient for STOT RE 2 classification. The DS responded that the increase in platelet counts was small (126% of the control) and was only statistically significant in females. The effects on PTT and APTT were only minor and observed only in males. Furthermore, no increased thrombus formation was observed at these dose levels in the 13-week study.

No other specific comments were received on this endpoint.

Additional key elements
No full documentation on the results from studies other than the 28-day inhalation study were originally included in the CLH report, but supplementary summary information from the DAR are inserted below:

OECD TG 410: 28-day dermal toxicity study:

Four weeks of dermal exposure of rats to 0, 20, 400 and 1000 mg/kg bw/d resulted in increased liver weights and concomitant centrilobular hypertrophy at 400 mg/kg bw/d (7/10 males and 0/10 females) and at 1000 mg/kg bw/d (10/10 males, 8/10 females). Heart cardiomyopathy was observed in 1/10 males at 1000 mg/kg bw/d compared to none in controls or high dose females. Also at 1000 mg/kg a statistically significant decrease in mean hindlimb splay was noted in males and the reticulocyte count was significantly increased in both sexes (f: 133%, m: 125% of control). Chronic progressive nephropathy was observed in one animal of both sexes in the control group and in four males at 1000 mg/kg bw/d. The NOAEL for systemic effects was set at 20 mg/kg bw/d. As no local effects were observed, the NOAEL for local effects was established at 1000 mg/kg bw/d.

OECD TG 408: 90-day oral toxicity study:

Dietary exposure of rats to 0, 50, 600 and 1250 mg/kg food/d of etridiazole (equal to 0, 2.7, 29.5 and 64.7 mg/kg bw/d for males and 0, 3.3, 35.2 and 73.6 mg/kg bw/d for females) for 13 weeks resulted in decreased body weight gain at 600 mg/kg food in females (90% of control) and 1250 mg/kg food in both sexes (78% (m) and 64% (f) of controls). Food consumption was decreased at 1250 mg/kg food. Reticulocytes and platelet count were increased at 1250 mg/kg food. Prothrombin time and APTT were decreased at 600 and 1250 mg/kg food in males (92% and 84%-86% of control). Sodium and chloride were slightly reduced and potassium was increased in males at 1250 mg/kg food. Glucose was decreased at 1250 mg/kg food in both sexes. Bilirubin was increased at 1250 mg/kg food in males. T3 levels were decreased at 1250 mg/kg food in females. Cholesterol was increased in males and liver weights were increased in both sexes with concomitant centrilobular hypertrophy at 600 and 1250 mg/kg food. The changes in sodium, chloride and potassium were reported to correlate with hyaline

droplets observed in the kidneys of male animals, but according to the rapporteur this correlation was not observed in mid dose males. The incidence was slightly increased at 600 and 1250 mg/kg food, which in the DAR was considered to be due to α2μ-globulin. According to the DAR new slides had been stained and the correlation of the staining results and the patterns strongly suggested that the hyaline droplets seen in the male rats were of α2u-globulin in origin. The NOAEL was established at 50 mg/kg food/d (equal to 2.7 mg/kg bw/d for males and 3.3 mg/kg bw/d for females), based on effects on liver and kidneys.

OECD TG 452: 1-year oral toxicity study (dog)

Dietary exposure of dogs to 0, 160, 500 and 1000 mg/kg food/d of etridiazole technical (equal to 0, 3.11, 8.07 and 22.4 mg/kg bw/d for males and 0, 4.27, 9.33 and 24.0 mg/kg bw/d for females) for 12 months resulted in reduced body weight gain at 1000 mg/kg food. No treatment-related findings on food consumption were observed. An increased APTT were noted at 1000 mg/kg food. Urea nitrogen and creatinine were decreased at 1000 mg/kg food. Total protein, albumin and A/G ratio were decreased at 1000 mg/kg food. Alkaline phosphatase was increased at 500 and 1000 mg/kg food. Cholesterol was increased at 1000 mg/kg food. According to the DAR increased alkaline phosphatase and cholesterol, and decreased protein parameters indicated a disturbance in liver function. Concomitantly, liver weights were increased at 500 and 1000 mg/kg food. No macroscopic or microscopic findings were noted. Therefore, the NOAEL was set at 160 mg/kg food/d (equal to 3.1 mg/kg bw/d for males and 4.3 mg/kg bw/d for females).

Assessment and comparison with the classification criteria

1. Inhalation route:

Effects occurring below the guidance value for STOT RE 2 (600 mg/m³/6 h/d for the 28-day inhalation study as extrapolated from the guidance value for a 90-day inhalation study according to the Haber's rule Table 3.9.2.2, CLP guidance) were considered:

A. Systemic effects

The observed systemic effects included decreased body weight gain (75-78% of control), increased liver weight and liver cell hypertrophy, reduced forelimb grip strengths in males (83% of control) and squamous metaplasia of the larynx. Liver effects were not considered relevant for classification because they were not corroborated by other significant findings indicating significant morphological or functional damage. With regard to the reduced forelimb grip, the DAR did not consider this as a relevant toxic effect, since no effect was observed on hindlimb grip strength in males and no effect on grip strength in females. RAC did not agree with the reasoning in the DAR regarding the irrelevance of the reduced forelimb grip in males because neuronal discords may occur in segments of the spinal cord. However, the significance of the finding is unclear because no indications of associated clinical neuro-functional disorders or histopathological findings were reported. Impaired limb function or ataxia was also observed in other repeated dose studies (mouse carcinogenicity study, developmental toxicity study on rats), but the effects were only seen at maximum tolerated dose (MTD) levels.

B. Local effects on the respiratory tract

Larynx metaplasia was reported as minimal in the CLH report and it was interpreted as a non-adverse lesion. Minimal metaplasia in the ventral area of the larynx is commonly observed in non-treated rats (Kaufmann et al., 2009). As no evidence was seen in control rats, the effect could be attributed to the exposure. However, RAC agrees with the dossier submitter's interpretation of the non-adverse nature of the lesion at this minimal grade and concludes that classification for STOT RE is not warranted for the reported effect.

2. Oral route:

Relevant effects occurring below the guidance value for STOT RE 2 (100 mg/kg bw/d for the 90-day oral study, and 25 mg/kg bw/d for the 1-year oral study as extrapolated from the guidance value for a 90-day oral study according to the Haber's rule Table 3.9.2.2, CLP guidance) were considered.

Increased reticulocyte counts (in both sexes at 1250 mg/kg food) observed in the 90-day oral study in rats as an isolated effect without other relevant findings on red blood cell parameters indicating anaemic conditions (see the CLP guidance Section 3.9.2.5.2 on haemotoxicity) is not relevant for classification. Decreased PTT and APTT times in males at ≥600 mg/kg food (equal to 29.5 mg/kg bw/d for males and 35.2 mg/kg bw/d for females) were considered as indicative of a hyper-coagulable status by one MSCA. RAC accepted the dossier submitter's argument that the effects are not severe enough to warrant classification. In the case of a biologically significant hypercoagulative status, thrombi should be seen in circulation of major organs and in small peripheral vessels. The 18-month mouse carcinogenicity study reported increased incidences of kidney infarcts and thrombi in the myocardium in mid and high dose (at above 900/1300 mg/kg food, equal to 184.7 and 221.7 mg/kg bw/d in males and females, respectively) after 18 months of administration in diet, which is far above guidance values for classification.

In the 90-day oral study, increased bilirubin in high dose males and increased cholesterol in mid and high dose males indicate disturbances of liver function. However, no corresponding morphological abnormalities were reported in the liver or bile system in the study. It is to be noted that bile duct hyperplasia was observed after chronic administration in rats and mice at doses above guidance values for classification when corrected for duration, and cholangiocarcinomas were increased in rats. In the 90-day oral study, liver weight increases and liver cell hypertrophy were seen in both sexes at ≥600 mg/kg food, being reversible at the end of recovery period. RAC considers these liver effects to be adaptive responses that do not warrant classification.

Increased incidence of hyaline droplets in the kidneys was noted at 600 and 1250 mg/kg food in 9/10 and 10/10 males, respectively. As the incidence was already high in the control males (6/10) and the α2u-globulin-specific staining was positive, these kidney effects are interpreted as a male rat-specific finding and the increase in its incidence is of questionable toxicological significance.

Increased alkaline phosphatase, APTT and cholesterol were noted in dogs in the 1-year dog study. Increased cholesterol levels and increased activity of alkaline phosphatase may indicate disturbed liver/biliary function, but because no corresponding morphological abnormalities (except increase in liver weight) were reported, RAC does not consider these effects as sufficient to warrant classification for STOT RE. RAC concludes that the high mortality rates seen at 45 mg/kg bw in the rabbit teratogenicity study do not either support classification for STOT RE, as it was not known at which time-point during the 13-day treatment the dams died and because no serious adverse effects occurred below the guidance values in the oral 90-day rat study or in the inhalation 28-day rat study.

Overall RAC considered that the observed findings after the exposure via the oral route were not sufficiently serious to justify classification.

3. Dermal route:

Effects occurring below the guidance value for STOT RE 2 (600 mg/kg bw/d for the 28-day dermal study as extrapolated from the guidance value for a 90-day dermal study according to the Haber's rule Table 3.9.2.2, CLP guidance) were liver cell hypertrophy and liver weight increases at ≥400 mg/kg bw/d. As no signs of degenerative lesions or indications of significant functional disorders were observed, RAC considered the liver effects as adaptive and not relevant for classification.

In conclusion RAC supported the dossier submitter's proposal that no classification is warranted for STOT RE. **1**

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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal
The dossier submitter did not propose classification for mutagenicity, because in the genotoxicity studies there were no indications for classification.

Comments received during public consultation
No specific comments were received on this hazard class.

Additional key elements
The study summaries of the mutagenicity studies were not included in the CLH report. Additional information and summary tables on the results of the in vitro and in vivo genotoxicity studies from the DAR are given below (The references are also available in the DAR):

***In vitro* genotoxicity studies**

Test substance	**2 TYPE OF STUDY**		**Result**		**3**
	Indicator cells	**5 ENDPOINT**	**without activation**	**with activation**	**4 REFERENCE**
Terrazole, batch nr. 79-02-B, liquid, red/orange, 99.0%	B: *S. typh.* TA 98 TA 100	 point mut. point mut.	 - -	 - -	Loveday, K.S., Seixas, G.S.A., 1981
Terrazole (technical grade), batch nr. 7.506, drum #4, purity 93.7%.	B: *S. typh.* TA 98 TA 100 TA 1535 TA 1537 TA 1538 B*. E.coli* WP2 WP2uvrA WP67 WP6 CM611 CM571	. point mut. point mut. point mut. point mut. point mut point mut point mut point mut point mut point mut point mut	 - - - - - - - - - - -	 - - - - - 	Ercegovich, C.D., Rashid, K.A., 1977
Terrazole, Batch 79-02-	Chinese hamster ovary cell line	gene mutations (HPRT)	-	-	Loveday, K.S., Gorodecki, J., 1981

B, liquid, red-orange, 99.0%	CHO				
Terrazole, Batch 10626-1, liquid .	Chinese hamster ovary cell line CHO	Sister chromatid exchange	-/+	-	Loveday, K.S., Donahue, B.A., 1982
Terrazole, Batch 79-02-B, liquid, 99.0%	Chinese hamster ovary (CHO) cells	-Sister chromatid exchange -Chromosome aberration	+ 1) + 1)	+ 1)	Loveday, K.S., 1982
Etridiazole technical, Batch SI 1281, clear liquid, 98.1%	*Saccharomyces cerevisiae*, D6	Mitotic aneuploidy	-	-	Edwards, C.N., McSheehy, T.W., 1987

1) The results of this study should not be considered for the overall evaluation.

***In vivo* genotoxicity studies**

6 **7 TEST SUBSTANCE**	**8 TYPE OF STUDY** **13 SPECIES**	**14 ENDPOINT**	**9** **10 RESULT**	**11** **12 REFERENCE**
Terrazole technical, Batch CM-8561 S045017, liquid, min. 95%	mouse, NMRI, KFM 15	16 17 MICRONUCLEI (BONE MARROW)	-	Banduhn, N., 1985
Etridiazole (Technical), Batch 206S048 (SI 3429), brown liquid, 99.3%	rat, Sprague-Dawley	18 Chromosome aberration (bone marrow)	-	McEnaney, S. 1995
Terrazole (Technical), batch nr. 306S052, reddish-brown liquid, purity 98.7%.	rat, male Sprague-Dawley	Unscheduled DNA synthesis (liver hepatocytes)	-	San, R. H. C., Sly, J. E., 1995
Etridiazole, batch nr. 306S052, purity 98.7%.	rat, male F344/DuCrJ	replicative DNA synthesis (liver hepatocytes)	+	Miyagawa, M., 1995

Etridiazole did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA 1538, with or without metabolic activation, or in different *E. coli* tester strains without S9-mix. In the mitotic recombination assay with the tester strain *S. cerevisiae,* D6 etridiazole did not induce mitotic aneuploidy. Etridiazole was negative in a gene mutation test using CHO cells. In a chromosome aberration test with CHO cells, etridiazole was positive with and without S9-mix. However, these results were obtained

at very toxic dose levels and the historical control data were not available, and therefore this study was not considered acceptable. Etridiazole was also positive in two Sister Chromatid Exchange (SCE) studies, although the other study was not considered acceptable. Furthermore, results of both SCE studies were considered as biologically not relevant despite statistical significance, because only a two-fold increase in sister chromatid exchange was observed.

Etridiazole was negative in an in vivo micronucleus test and in an in vivo rat chromosome aberration test. In addition, etridiazole was negative in a UDS test. These studies fulfil the requirements of the corresponding guidance.

Etridiazole was positive in an in vivo-in vitro replicative DNA synthesis test with rat hepatocytes. The test did not include positive controls.

Assessment and comparison with the classification criteria

According to the dossier submitter's proposal, many of the in vitro tests were not in accordance with the corresponding guidelines, but based on the three well performed negative in vivo studies (micronucleus test, chromosomal aberration test, UDS test) etridiazole was considered to be non-mutagenic in vivo.

RAC also concluded that the result of the replicative DNA synthesis test did not support the assumption that etridiazole possesses promotor activity with a threshold level.

RAC agreed with the dossier submitter that in vitro and in vivo testing indicates that etridiazole had no genotoxic potential and that classification for germ cell mutagenicity was therefore not warranted.

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.

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, Crl: 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%						dc		dc	f
WBC (absolute and corrected)						ic		ic	
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									
macroscopy (n=50)									
Liver:									
-enlarged	3	1	5	1	**11**	2	**9**	3	
-raised area	1	2	5	1	1	1	2	**6**	
-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**	
microscopy									
neoplastic lesions									
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%)**	-	

Dose (mg/kg food)	**0**		**100**		**640**		**1280**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
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%)	
microscopy									
non-neoplastic lesions									
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:

18..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%)

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% (indicence 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
<u>microscopy (n=50)</u> *neoplastic lesions*									
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> *non-neoplastic lesions*									
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 hyper-trophy/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

Dose (mg/kg food)	0		50		900/1300		1800/2000/1600		dr
	m	f	m	f	m	f	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 notifer
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 notifer
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 promoter 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., 2007[1] and Kurata et al., 1994[2]).
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:

		Males				Females			
Tissue and finding	Level (mg/kg/day)	1M	2M	3M	4M	1F	2F	3F	4F
Kidney	No. examined:	10	10	10	10	10	10	10	10
hyaline droplets	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 2003[3]) 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 a2,-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. 2002[4]). 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. 1986[5]).

[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. Ravesloot, 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. l4, 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 rats[1]. 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 α2u-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.

18..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.

18..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.

18..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 T_3 or T_4 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).

18..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.

18..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), the18-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.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The dossier submitter proposed classification for Carc. 2; H351 (CLP, DSD: Carc. Cat. 3; R40), in accordance with the current classification, as it was proposed by TC-C&L in November 1995, November 1996 and May 1997 (summary records ECBI/94/95, ECBI/45/96 and ECBI/27/97, respectively).

The proposal was based on the observations in a 2-year rat study (liver tumours, and potentially relevant thyroid and testis tumours), an 18-month mouse study, mechanistic studies and further argumentation in the DAR. Both oral carcinogenicity studies were performed according to the OECD TG guidelines. No studies via inhalation or dermal routes were available.

Comments received during public consultation

During the first PC, comments on this hazard class were received from one MSCA, which agreed with the proposal of the dossier submitter based on a review of the carcinogenicity studies in rat and mouse and of the in vivo mechanistic studies in rat.

During the development of this opinion, carcinogenicity was re-opened for a second (targeted) public consultation in order to strengthen the information base and because this hazard class was not specifically included in the list for which comments had been requested during the first public consultation.

During the targeted public consultation one MSCA supported the conclusion that the mid and high doses in the mouse carcinogenicity study exceeded the MTD. Furthermore, the observations that a number of deaths were related to liver failure and that liver tumours were not the cause of increased mortality were found to be consistent with the interpretation that the maximum tolerated concentration (MTC) was exceeded for the mid

and high doses in the mouse study. The classification as Carc. 2 (CLP) was supported by three commenting MSCAs and by industry.

Assessment and comparison with the classification criteria
In total, three rat studies and two mouse studies on carcinogenicity were available. Only the 2-year rat study and the 18-month mouse study, which were predominantly performed in accordance with test guidelines, were considered relevant for the RAC evaluation. The other studies (from 1968 to 1983) were incomplete due to lack of investigated parameters/animals and early termination because of infections or unusually high tumour incidences in controls.

There was an indication of treatment-related increases in tumour rates in the two valid carcinogenicity studies. Only incidences were reported in the study summaries, but the statistics and information about the severities of the effects were lacking.

Rat carcinogenicity study (study 1 in the CLH-report):

In the 109-week carcinogenicity study, rats received etridiazole at 0, 100, 640, or 1280 mg/kg food (equivalent to 0, 5, 30, or 63 mg/kg bw/d for males and 0, 6, 38, or 84 mg/kg bw/d for females). The tumour rates in treated animals as compared to controls were increased in the liver, thyroid, testes, kidneys and mammary glands. Survival rates were not affected by the treatment. A reduction in body weight gain was reported in mid and high dose females. The reduced growth can be assumed to be a consequence of reduced food consumption. The absence of clinical signs and effects on survival support the assumption. However, the food efficiency calculations in the original study report did not reveal marked differences between the control and test groups, although the mean body weight gains of the high dose females relative to their feed intake tended to fall somewhat consistently below control until week 46. With regard to the selected doses, RAC considers that the MTD was not exceeded. The non-neoplastic effects in kidneys and liver are thought to be related to specific toxic/pre-neoplastic effects rather than to non-specific general toxicity.
Compared to controls, the incidences in hepatocellular adenomas were increased in male and female rats at the high dose. In addition, high dose females had increased incidences in hepatocellular carcinomas and cholangiocarcinomas, a tumour type that is rarely found in rats. In general, female rats were more sensitive than male rats. Liver tumours in male rats were mainly benign. The incidences of the liver tumours in concurrent controls were largely in the range of tumour incidences of the conducting laboratory. In conclusion, the liver adenomas/adenocarcinomas and cholangiocarcinomas were considered to be caused by etridiazole treatment.

With respect to non-neoplastic lesions in the liver, there were dose-related increases in incidences of hepatocytomegaly in male and female rats of the mid and high dose groups that was interpreted by RAC to be a precursor lesion in the development of hepatocellular adenomas and carcinomas. Bile duct hyperplasia, bile duct fibrosis, and chronic inflammation of bile ducts may be considered as possible precursor lesions of cholangiocarcinomas and occurred in rats of control and dose groups. Incidences appeared to be higher in high dose females. However, the incidences in controls were already high. Data on severity and distribution of the lesions would have been informative to enable conclusions to be drawn on the dose-response relationship of these lesions conclude on dose-responsiveness. These data were absent in the CLH report and DAR.

In comparison to control incidences, increased incidences of follicular cell adenomas were seen in high dose males and females, but male rats were more sensitive than female rats. Higher incidences of follicular cell adenocarcinomas were only found in males at mid and high dose. In general, the tumour response in females was weaker and predominantly benign. Concurrent control incidences of adenomas in males (12%) were slightly above the laboratory control incidences (range 2-7% for the period 1984-1989,

mean 4.7%). There were no adenomas in concurrent female controls, which was consistent with the laboratory historical control values (range 0-1%, mean 0.4% 1984-1989). It was concluded by RAC that thyroid tumours in high dose males and females and mid dose males were caused by the etridiazole treatment.

In testis, interstitial tumour incidences were markedly increased at the high dose (20%). While in the concurrent control this tumour was not found, the historical incidences of the conducting laboratories were 4.7% (range 2.4-13.3 % for the period 1984-1989). A relationship to treatment can be assumed from the increased incidence at the high dose. Tumour findings were in line with increased incidence of the putative precursor lesion, the interstitial cell hyperplasia.

In the kidney, 1 adenoma was observed at 100 and 1280 mg/kg food whereas 4 and 1 adenocarcinomas were observed at 640 and 1280 mg/kg food, respectively, in males. Tubular cell tumours of the kidney are rare tumours in rats. There were no tumours in concurrent controls, which is consistent with the range of laboratory historical control values (range 0-1.2% for adenomas, 0-2.9% for carcinomas in 1984-1989). Although no clear dose-response relationship was given, since this tumour type is rare, this finding could be of some concern. Moreover, the high incidences of tubular cell karyomegaly in the dose groups only, a finding that can be seen as an early response to renal carcinogens, should be taken into account. Karyomegaly means that the cell nucleus is enlarged and often hyperchromatic, possibly due to polyploidy and abnormal DNA distribution.

The dossier submitter noted that kidney tumours may have resulted from alpha-2-microglobulin accumulation, which is a male rat specific mechanism and not relevant to humans. Neither alpha-2-microglobulin, hyaline droplets, intra-tubular protein casts nor any other nephropathy or related regenerative cellular proliferation were observed in the 109-week study in the rat (neither in the controls of the same age nor in the treated animals). RAC has noted that alpha-2-microglobulin and related effects should not be expected at the end of a chronic study and that RAC should reconsider the conclusion on the human relevance of the kidney tumours. RAC noted that the DAR on the 13-week study did report increased incidences of hyaline droplets at 600 and 1250 mg/kg food at the end of the 90-day treatment period, but no other effects were observed that may have indicated associated cell damage/cell death and secondarily increased (regenerative) cell proliferative activity. After recovery, alpha-2-microglobulin was found in all males of control and dosed groups. No information was available on the severity of hyaline droplets. In the original study results, chronic nephropathy was reported at similar prevalence among the study groups. Evidence of hyaline droplets and positive staining for alpha-2-microglobulin in control and treated rats at the end of the 13-week oral treatment alone does not give sufficient evidence that tumours can be related to this male rat- specific lesion. Furthermore it can not be excluded that the increases in karyomegaly in dosed males and females are precursors to proliferative lesions. Overall, there is some evidence for kidney carcinogenicity. However, there are uncertainties due to these findings being observed at low incidences, only in males and because there was no dose-response relationship. RAC concludes that the mode of action leading to the kidney tumours is unknown but it could also be related to etridiazole treatment.

In mammary glands, increased incidences of fibroadenomas were observed in all treatment groups with highest incidence in high dose females. Control incidence was 18% and the historical control range was 11.6%-69.25% (mean 39%) demonstrating that this tumour type was quite common over 1984-1989 in this rat strain. Due to the high spontaneous incidences RAC considers treatment relationship to be questionable.

Mouse carcinogenicity study (study 2 in the CLH report):

In the 18-month carcinogenicity study, mice received etridiazole in diet at 0, 50, 900 or 1800 mg/kg food. After the first week of dosing, the dietary concentrations were

increased to 1300 and 2000 mg/kg food for the mid and high dose groups, respectively, but at week 43, the high dose level was decreased to 1600 mg/kg food due to excessive mortality in this group. Dose levels corresponded to 0, 7.5, 184.7 and 263.3 mg/kg bw/d in males and 0, 9.1, 221.7 and 312.1 mg/kg bw/d in females.

Mortality at high dose groups was 60% and 68% in males and females, respectively, which is still below the percentage (survival 25%, OECD TG Guidance Document 116) when a study should be terminated due to low survival. Mortality at mid-dose was 44% both in males and in females. At high dose, clinical signs such as decreased activity, breathing difficulties, lack of appetite, tremors, hunched posture, impaired limb function and pale and cold skin indicated severely affected general health status. High dose males showed significantly lower body weights than controls from week 3 onwards (91% of the controls) and high dose males and females and mid dose males showed significantly lower food consumption. No numerical values were given either in the CLH report or in the DAR to allow concluding on whether lower body weight was related to the food consumption. Although no clinical signs of disturbed general health status (except lower food intake) were reported for the mid dose group, RAC agreed that also the mid dose was above the MTD due to high mortality rates.

No statistical information and information on the severity and distribution of non-neoplastic lesions were found in the CLH-report.

Increased liver adenoma incidences were at the same level in mid and high dose males, while increases appeared to be dose-related in mid and high dose females. However, tumour responses may have been influenced by mortality rates, but tumour data were not corrected for life-span. Tumour incidences in controls were low in males and absent in females. Thus tumour responses in dose groups were not confounded by high spontaneous tumour rates. Hepatocellular carcinomas were observed in mid dose males and high dose females. Corresponding findings accompanying the liver tumour development were increased liver weights, enlarged livers, livers with nodules and masses and several degenerative-regenerative hepatocellular lesions were more frequently found in mid and high dose males and females. Like in rats, in comparison to the controls bile duct hyperplasia occurred at higher incidences in mid and high dose males and high dose females, but in contrast to rats, mice did not develop cholangiocarcinomas.

Mode of action consideration

From mutagenicity testing, etridiazole was considered to have no genotoxic potential but other mechanisms may be relevant.

For the liver, the dossier submitter assumed similarities in tumour promoting activities of etridiazole and phenobarbital and analysed liver enzyme activities in mechanistic studies (see Etridiazole additional report, 05, Vol. 3, B6). Etridiazole treatment resulted in liver cell hypertrophy due to increased smooth endoplasmatic reticulum, in a decrease in phase 1 enzymes (CYP450, MCD and ECD), and a slight increase in CYP2B1 and CYP2C13. The conclusion that metabolic enzyme responses were different to phenobarbital was copied from the DAR in the CLH report.

Promoter activity was reported to be separately investigated in initiator and promoter studies using N-nitrosodiethylamine (DEN) as initiator. RAC did not agree with the interpretation of the dossier submitter that increased replicative DNA synthesis supports the assumption that etridiazole possesses promoter activity with a threshold level. It should be noted that promoter activity as such is of minor relevance for classification. Chronic administration of etridiazole alone in the diet induced liver tumours in rats and mice. Finally, the mode of action of etridiazole to induce liver adenomas/carcinomas and cholangiocarcinomas remains unknown.

To conclude on whether one or two species are clearly positive, the tumour data in the

mouse are critical. Although no clinical signs of disturbed general health (except lower food intake) were reported, RAC considered the mid and high dose groups in the mouse carcinogenicity study to be above the MTD due to the high mortality rates observed. Therefore, the tumour responses at the mid and high dose were of questionable relevance for the assessment of etridiazole-related carcinogenicity.

The mode of action for the rat thyroid tumours remains unknown. The UDP-gamma transferase activity was increased in the mechanistic study, but there were no effects on rat TSH or changes in T3 or T4. Furthermore, no consistent effects were seen in other studies. RAC concludes that the available data do not support for a rat-specific phenomenon.

RAC did not agree with the dossier submitter's interpretation that the increased tumour rate in rat testes was above the MTD. Also, the observation that Leydig cell tumours in humans are rare does not support the non-relevance of this tumour type for humans. As the mode of action is unknown, the default assumption is that the tumour is of potential relevance for humans.

For the rat kidney tumours, the proposed mode of action (involving alpha-2 microglobulin) does not appear to be plausible. However, the evidence for the kidney tumours resulting from treatment with Etridiazole is weak, because these findings were observed at low incidences, only in males and because there was no dose-response relationship.

Comparison with criteria

In accordance with the criteria in CLP Regulation EC/1272/2008 classification in category 1A for carcinogenicity is not justified (DSD category 1) given that there is no evidence of etridiazole having caused cancer in humans. It is therefore necessary to decide whether to classify etridiazole in CLP category 1B or category 2.

The RAC based its conclusions on carcinogenicity on two available studies, a 104-week study in rats and an 18-month study in mice. The neoplastic lesions observed in the liver at the mid and high dose groups in the mouse study were not considered to be relevant for classification, as both these doses appeared to exceed the maximum tolerable dose (MTD), based on high mortality rates. Regarding the rat study, the RAC concludes that the MTD was not exceeded at any dose. Evident increases in tumour incidences were observed in the liver, thyroid and testes. However, female rats were more prone to developing liver tumours than male rats and liver tumours in male rats were mainly benign. The thyroid tumours were markedly increased in males of the mid and high dose groups, whereas the tumour response in females was weaker and predominantly benign. Because one tumour type was more prevalent in one sex in the rat and because the neoplastic lesions were observed at above the MTD in the mouse study, the RAC concluded that the carcinogenic potential of etridiazole is not sufficient for classification as Carc. 1B. The existing classification of etridiazole as Carc. 2; H351 (DSD: Carc. Cat 3, R40) is therefore appropriate.

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 sternebrae) 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 Crl: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-Belted	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 F_0-generation at 800/640 mg/kg food and for the F_1-generation at 320 and 800/640 mg/kg food. An increase in T_3 concentration in males and a decrease in females at 320 and 800/640 mg/kg food (F_0-generation measured only) was noted. In addition, decreased pituitary weight at 320 mg/kg in F_0-males food and at 800 mg/kg food F_0- and F_1-males, changes in kidney weight at 320 and 800/640 mg/kg food in F_0 and F_1-animals, increases in liver weight at 320 and 800/640 mg/kg food in F_0 and F_1-animals, and increased seminal vesicle weight at 320 and 800 mg/kg food in F_1-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 F_0 and F_1-offspring revealed decreased body weights of pups at 320 (F_0 only) and 800/640 mg/kg food. In addition, changes in thyroid weight were noted at both levels in the F_0-generation and in the F_1- high dosed group. T_3 concentration was only measured the F_1-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 F_0 and F_1-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 sternebrae, 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, F_0 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 premating period, while during lactation body weight gain was increased relative to control (body weight was still decreased). Food consumption was significantly reduced during premating and postmating 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 T_3 a significant increase was noted at 320 and 800 mg/kg food in males (132% of control). T_3 was significantly decreased at 320 and 640 mg/kg food in females (77 and 73% of control, resp.). T_4 and TSH were also reduced at 640 mg/kg food in females (81 and 75% of control, resp.). Changes in T_3 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 T_4 and TSH. In F_0 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 F1 female pups at 640 mg/kg food (89% of control). Absolute thymus weight was decreased in F1 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 F1 female pups at 320 and 640 mg/kg food (117-118% of control). Relative thyroid weight was increased in F1 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 premating 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 premating 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 F_1 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 F_1 offspring (F_2 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 F_2 pups at 800 mg/kg food (130% of control). Absolute pituitary weight was decreased in female F_2 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 T_3 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 T_3 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
			NOAELdev	:	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				

Dose (mg/kg bw/day)	0	10	30	75	dr
Uterus weight	No treatment-related findings				
Pathology					
macroscopy	No treatment-related findings				
Litter response					
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 foetuses	22	25	23	18	
Live foetuses/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				
Examination of the foetuses					
External observations					
- anasarca	0	0	0	2	
Skeletal findings[2]					
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 % foetuses

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 form 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 sternebrae #5 and/or #6, other unossified sternebrae, 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 sternebrae 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
			NOAELdev	:	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 sternebrae		2/2				3/3*	
- tail defects		39/10*				5/2*	
- hind limbs underdeveloped						4/1	
Visceral findings[3]							
- hind legs crossed		14/6*				7/2*	
- Open eyes						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 sternebrae, 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 sternebrae, 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.

RAC evaluation of reproductive toxicity
Summary of the Dossier submitter's proposal Currently etridiazole is not classified for reproductive toxicity. The dossier submitter

clarified that etridiazole was discussed in the TC-C&L in November 1995, November 1996 and May 1997 (summary records ECBI/94/95, ECBI/45/96 and ECBI/27/97). 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 the CLH report which studies are new information compared to the studies used for the classification in the 25th ATP to Directive 67/548/EEC.

Sexual Function and Fertility

In a 2-generation oral reproductive toxicity study performed in accordance with OECD TG 416, rats were exposed to etridiazole at 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 for the F0-generation at 800/640 mg/kg food and for the F1-generation at 320 and 800/640 mg/kg food. No treatment related changes were observed in fertility, oestrus cycle, sperm parameters, mating behaviour, conception or gestation. The dossier submitter concluded that the 2-generation study in rats did not provide any indications for classification for reproductive toxicity.

Developmental toxicity

In a teratogenicity study performed in accordance with OECD TG 414 guideline, rats received etridiazole by gavage 0, 10, 30 or 75 mg/kg bw/d. The NOAEL for maternal effects was set at 30 mg/kg bw/d, based on increased mortality, clinical signs, and decreased body weight. The NOAEL for developmental effects was set at 30 mg/kg bw/d, based on decreased mean foetal weight, anasarca in 2 foetuses, and retarded ossification of various bones. No treatment-related effects were observed in the number of corpora lutea or implantations, the number or percentage of live foetuses, or the sex ratio. There were no morphological changes observed in fetuses that could be attributed to treatment.

In a teratogenicity study in rabbits receiving etridiazole by gavage 0, 1.7, 5, 15 or 45 mg/kg bw/d, the NOAEL for maternal effects was set at 15 mg/kg bw/d based on mortality and decreased body weight. Potential critical effects on e.g. liver, kidney and thyroid were not studied and therefore, the derived maternal NOAEL from this study might not have been accurate. The NOAEL for developmental effects was set at 15 mg/kg bw/d, based on decreased mean foetal weight, a reduction of live foetuses per dam and an increase in dams with resorptions at 45 mg/kg bw/d. No treatment-related effects were observed in the number of corpora lutea or implantation sites, or in the sex ratio. Skeletal examination revealed an increased incidence in missing sternebrae, tail defects and underdeveloped hind limbs. Soft tissue examination showed an increased incidence of crossed hind legs and open eyes. Developmental malformations were observed at doses causing high maternal mortality in rabbits.

The CLP regulation states that maternal mortality over 10% is considered excessive and the data for that dose level shall not normally be considered for further evaluation. In the rat and rabbit developmental studies the maternal mortality was about 20% at the high dose.

The foetal toxicity observed in the rat study was based on retarded ossification. This finding was observed at the dose level causing severe maternal toxicity, including mortality. The dossier submitter concluded that foetal toxicity was secondary to maternal toxicity and therefore not relevant for classification.

For the rabbit developmental study, no historical control data were provided. Missing sternebrae, tail defects, underdeveloped hind limbs, crossed hind legs and open eyes were reported 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. Moreover, the mortality at a slightly lower dose level (30 mg/kg bw/d) in the preliminary study also indicated that maternal toxicity at the high

dose (45 mg/kg bw/d) was excessive and the data for such dose levels should not normally be considered for further evaluation. Therefore, the dossier submitter concluded that the observed developmental effects were not relevant for classification.

The dossier submitter did not propose classification for reproductive toxicity.

Comments received during public consultation
During the first public consultation, the Two MSCAs agreed with the dossier submitter's proposal for non-classification for this endpoint. A third MSCA suggested that RAC should discuss whether classification for developmental toxicity category 2; H361 (Repr, 2; H361d) would be warranted, noting that the relevant question was whether there was sufficient evidence for classification in category 2 for developmental effects because the toxic dose in the rabbit teratogenicity study also caused serious toxicity in mothers., A fourth MSCA proposed classification as developmental toxicity category 2 and commented that since the historical control data was missing from the rabbit teratogenicity study, the developmental effects could have been a consequence of etridiazole treatment. Moreover, the NOAEL for teratogenic effects was set at 15 mg/kg bw/d based on irreversible structural effects.

During the opinion development process, reproductive toxicity was re-opened for a second (targeted) public consultation in order to strengthen the information base and because this hazard class was not specifically addressed in the list for which comments had been requested during the first public consultation. Industry did not agree with the proposal to classify for developmental toxicity and concluded that the foetal toxicity observed in the developmental studies was based on retarded ossification and that it was observed at dose levels causing severe maternal toxicity, including mortality. The foetal toxicity was considered to be secondary to the maternal toxicity and the retarded ossification of various bones and skeletal malformations observed was not considered relevant for classification for reproductive toxicity. Also, one MSCA supported non-classification for developmental toxicity.

Assessment and comparison with the classification criteria

Fertility

RAC agreed with the dossier submitter that in the absence of treatment-related effects on reproductive function in a 2-generation study, a classification could not be justified. (In this study histopathology was conducted on the kidney (no data on other organs) and chronic nephropathy was found in 7/8 F0-males at 800 mg/kg bw/d; this effect was not seen in other male and female groups.)

Developmental toxicity

The developmental study in rats revealed decreased mean foetal weight and anasarca in 2 foetuses and retarded ossification of various bones at 75 mg/kg bw/d. Retarded ossification was frequently observed in sternebrae #5 and #6 and some other bones. At the same dose mortalities (5/25 dams), reduced body weight gain and clinical signs of impaired general health were observed. Body weight loss was seen during the initial phase (GD 6-9 at ≥10 mg/kg bw/d and GD 6-12 at 75 mg/kg bw/d), but there was no data on the quantity of body weight loss in the CLH report. As foetal toxicity occurred only at a dose that induced severe maternal toxicity, the RAC considered these effects to be secondary to severe maternal toxicity.

At 45 mg/kg bw/d the number of live foetuses/dam and pub viability were reduced. Missing sternebrae (3 foetuses in 3 different litters), tail defects (5 foetuses in 2 different litters), underdeveloped hind limbs (4 foetuses in one litter), crossed hind limbs (7 foetuses in 2 different litters) and open eyes (6 foetuses in 2 different litters) were observed at 45 mg/kg bw/d. Mortalities in 3/14 dams at 45 mg/kg bw/d in the main

teratogenicity study appeared to be conclusive taking into account that in the range-finding study all rabbits that received 100 or 300 mg/kg bw/d during GD 6-18 died. At the lowest dose of 30 mg/kg bw/d in the range-finding study one rabbit died (the number of 5 dams/group was reported only in the original study report), one of the surviving dams resorbed its entire litter (6 implantation sites and 6 resorption sites), and one of them resorbed 1/8 implantations. The dead dam had also resorbed its entire litter (2 implantation sites and 2 resorption sites). In the negative control group none of the dams died or resorbed their litter.

In the main teratogenicity study, dam body weights were significantly lower than in the negative controls at 45 mg/kg bw/d on day 18 of gestation (absolute weight at GD 18 was 5% lower than on GD 0, while negative controls had 4% absolute weight gain at GD 18). At 45 mg/kg bw/d, a non-statistically significant decrease in body weight compared to controls was noted from day 6 to day 30 of gestation. Other clinical signs were not reported for the dams.

Original study reports indicated that malformed fetuses were not from dams that died prematurely. No viable pups originated from the dams that died (all implanted fetuses were resorbed.

RAC considers missing sternebrae as malformations on the basis of an international harmonisation activity on rat foetal skeletal terminology and classification (Solecki et al., 2001). Also, shortness or any other gross abnormality of the tail was considered to be a malformation by Solecki et al., 2001. Tail defects in combination with underdeveloped hind limbs may be indicative of a caudal dysplasia, which is defined as a severe reduction of caudal structures, including reduction or absence of hindlimbs, tail, and/or sacral area (Makris et al., 2009). However from the available report it cannot be clarified whether the reported effects occurred in combination or not. The RAC did not agree with industry's view that the observed effects can be interpreted as being caused by retarded ossification.

It is to be noted that the abnormalities occurred at a dose at which serious effects indicative of excessive maternal toxicity (21% mortalities, reduced body weight in survivors) had been observed. In survivors, 5% lower absolute body weight on GD 18 compared to GD 0 may be indicative of general toxicity. However no corrected body weight data are available to estimate the extent of the maternal toxic effect. No other signs of maternal toxicity were reported.

RAC concluded that classification for developmental effects at doses causing severe maternal toxicity was not consistent with the CLP criteria (3.7.2.4.4) which state that maternal mortality greater than 10% is considered excessive and that the data for that dose level shall not normally be considered for further evaluation. Accordingly, RAC concluded that the treatment-related effects on pup development at 45 mg/kg bw/d etridiazole co-occurring with maternal mortality greater than 10% do not justify the classification for this hazard class. However, taking into account the dose levels at which mortalities were observed in the range-finding study in rabbits, RAC points out that the highest dose of the main study was incorrectly chosen.

Overall, the observed malformations are considered to be a secondary non-specific consequence of severe maternal toxicity at the same dose. RAC concluded that no classification for reproductive toxicity is warranted.

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. References to individual studies are not included. For more details the reader is referred to the DAR and its addenda.

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/(molxcm) 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). 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 µg/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). 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 (≤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-^{14}C] 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. CO_2 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-^{14}C] 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. CO_2 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-etridiazole (~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-etridiazole 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-etridiazole 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℃ / 75% FC	45.5/194	67.9	0.98	FOMC
loam		7.4	20℃ / pF 2.5	2.22/7.37	2.22	0.98	SFO
sandy loam		6.0	20℃ / 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-etridiazole in soil

Dichloro-etridiazole	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℃/75% FC	119/395	-	178 [2]	0.93	SFO
loam		7.4	20℃/pF 2.5	7.76/47.7	-	7.76 [2]	0.96	FOMC
sandy loam		6.0	20℃/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℃/45%	36.0/120	-	36.0	0.99	SFO

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

[1] X 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 logKow 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.

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

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 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-etridiazole and etridiazole acid were identified as major metabolites. Acute aquatic toxicity data are available from the DAR for the metabolites dichloro-etridiazole 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	*Oncorhynchus mykiss*	Flow-through	2.4	mean measured
Etridiazole	97.9	*Cyprinodon variegatus*	Flow-through	4.0	mean measured
Etridiazole acid	99.8	*Lepomis macrochirus*	Static	> 100	nominal
Dichloro-etridiazole	99.75	*Oncorhynchus mykiss*	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. 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 LC_{50} 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 (etridiazole) 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. 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 LC_{50} 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. 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 LC_{50} 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. 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 LC_{50} 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). 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
Etridiazole	97.9	*Daphnia Magna*	48 h, flow-through	3.1	Mean measured
Etridiazole	97.92	Eastern oyster, *Crassostrea virginica*	96-h, flow-through	3.0	Mean measured
Etridiazole	97.92	Mysid shrimp, *Mysidopisis bahia*	96-h, flow-through	2.5	Mean measured

Etridiazole acid	100	*Daphnia Magna*	48 h, static	350	Mean measured
Dichloro-etridiazole	99.75	*Daphnia Magna*	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). 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 EC_{50} 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. 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 LC_{50} 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. 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 LC_{50} 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. 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 EC_{50} 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. 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 EC_{50} of 350 mg/L was determined in this study.

Conclusion: The lowest acute toxicity to aquatic invertebrates was obtained with *Mysidopisis bahia*, LC_{50} 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). 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_rC_{50} mg/L	NOEC mg/L
Etridiazole	97.92	*Selenastrum Capricornutum**	120 h, static	0.49	0.027#
Etridiazole	97.92	*Anabaena flos-aquae*	120 h, static	> 1.0	0.063
Etridiazole	97.92	Duckweed, *Lemna gibba*	14 d, static	14	5.7
Etridiazole acid	100	*Selenastrum Capricornutum**	72 h, static	29	12
Dichloro-etridiazole	92.6	*Selenastrum Capricornutum**	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 x 10^4 cells/mL at the start) was conducted with Terrazole Technical (etridiazole) at nominal test concentrations of 0.0024, 0.0081, 0.027, 0.090, 0.30 and 1.0 mg/L, with untreated and solvent-control. 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 µmhos/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 x 10^4 cells/mL at the start) was conducted with Terrazole Technical (etridiazole) at nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.0 mg/L, with untreated and solvent-control. 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 EbC50 and ErC50: 0.42 and >1.0 mg/L, respectively; 120-hour NOEbC and NOErC: 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 EC50 and NOEC values for growth inhibition and specific growth rate reduction were calculated by the RMS following the methods in OECD 202, based on the reported raw data and using Toxstat Release 3.5, 1996. Reported EC50 values (0.39, 0.31 and 0.072 mg/L after 72, 96 and 120 hours respectively) and the 120-hour NOEC value (0.0020 mg/L) were based on absolute values for cell density instead of the area under the growth curve and the specific growth rates as detailed by OECD 202. The 72-hour EC50 and NOEC values for growth inhibition and specific growth rate reduction were calculated by the RMS following the methods in OECD 202, based on the reported raw data and using Toxstat Release 3.5, 1996.

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per concentration, each containing 1.0 x 10^4 cells/mL at the start) was conducted with 5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03, metabolite of etridiazole, also referred to as dichloro-etridiazole) at nominal test concentrations of 0.010, 0.026, 0.064, 0.16, 0.40 and 1.0 mg/L, with untreated and solvent-control. Guidelines OECD 201 and EEC C3 were followed. 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 x 10^4 cells/mL at the start) was conducted with 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02, metabolite of etridiazole, also referred to as etridiazole acid) at nominal test concentrations of 6.4, 13, 25, 50 and 100 mg/L, with untreated control. Guideline OECD 201 was followed. 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 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 E_rC_{50} 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 E_rC_{50} 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 $L(E)C_{50}$ and NOEC values obtained in aquatic toxicity studies:

Acute toxicity

Etridiazole		
Fish	*Oncorhynchus mykiss*	96h-LC50 = 2.4 mg/L
Invertebrates	*Mysidopsis bahia*	96h-LC50 = 2.5 mg/L
Algae/aquatic plant	*Selenastrum capricornutum*	120 h-ErC50 = 0.49 mg/L
Etridiazole acid		
Fish	*Oncorhynchus mykiss*	96h-LC50 = >100 mg/L
Invertebrates	*Daphnia magna*	48h-EC50 = 350 mg/L
Algae/aquatic plant	*Selenastrum capricornutum*	72 h-ErC50 = 29 mg/L
Dichloro-etridiazole		
Fish	*Oncorhynchus mykiss*	96h-LC50 = 0.77 mg/L
Invertebrates	*Daphnia magna*	48h-EC50 = 1.1 mg/L
Algae/aquatic plant	*Selenastrum capricornutum*	72 h-ErC50 > 0.98 mg/L

Chronic toxicity

Etridiazole		
Fish	*Oncorhynchus mykiss*	90 d NOEC = 0.12 mg/L
Invertebrates	*Daphnia magna*	21 d NOEC = 0.37 mg/L
Algae/aquatic plant	*Selenastrum carpicornutum*	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 EC_{50} value ≤ 1 mg/L for algae.

Etridiazole produces acute aquatic EC_{50} 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 EC_{50} 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 aquaticNOEC 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)

6 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

7

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The current Annex VI entry for etridiazole has an environmental classification for both Aquatic Acute 1 and Aquatic Chronic 1. The DS proposed to add both acute and chronic M-factors of 1 to the existing Annex VI entry.

Degradation

The DS provided information on etridiazole's abiotic degradation via hydrolysis and photolysis, and they were not considered relevant routes of degradation for classification of etridiazole.

Information on biotic degradation in a screening test (ready degradation) and in two different water/sediment systems was provided. In a closed bottle test according to OECD TG 301D (EC C.4-E) only 1% biodegradation after 28 days was achieved. In the simulation studies the primary degradation half-lives of etridiazole in the total water/sediment systems were less than two days. Degradation data on etridiazole and its degradation products in soil was also presented in the CLH report. The dossier submitter concluded that etridiazole is not rapidly degradable.

Bioaccumulation

Etridiazole has a measured log K_{ow} of 3.37 (aqueous pH 7 buffer/n-octanol). There is also a study available that measured the bioconcentration factor of etridiazole in bluegill sunfish (*Lepomis macrochirus*) according to the guideline US EPA 165. The BCFs for etridiazole in edible tissue, non-edible tissue and whole fish are 92, 256 and 165 L/kg wet weight, respectively. These values were not corrected for lipid content.

Based on both measured log K_{ow} and BCF values in fish the dossier submitter concluded that etridiazole is not potentially bioaccumulative according to CLP (the BCF is below 500) but fulfils the criteria as a substance having a potential for bioaccumulation according to DSD (the BCF is above 100).

Acute (short-term) aquatic toxicity

Etridiazole's acute toxicity was tested in two fish studies (*Oncorhynchus mykiss*, OECD TG TG 203), three aquatic invertebrate studies (*Daphnia magna*, OECD TG TG 202; *Americamysis bahia* and *Crassostrea virginica*, EPA TG 72-3), and three algal or aquatic plant studies (*Pseudokirchneriella subcapitata* and *Anabaena flos-aquae*, EPA TGs 122-2 and 122-3; and *Lemna gibba*, OECD TG 221). In addition, two acute fish studies, two on invertebrates and two on aquatic algae were reported on etridiazole degradation products (i.e. etridiazole acid and dichloro-etridiazole). According to the DS the lowest reliable $L(E)C_{50}$ value for etridiazole was obtained in the test on *P. subcapitata,* i.e. E_rC_{50} (120 hours) = 0.49 mg/l (mean measured concentration). In studies with the degradation products, the lowest reliable $L(E)C_{50}$ value of 0.77 mg/l was obtained in fish using dichloro-etridiazole.

Based on the result of the toxicity test with algae, the DS concluded that etridiazole fulfils the CLP criteria for classification with Aquatic Acute 1 with an M-factor of 1.

Long-term aquatic hazard

The DS reported that there are adequate chronic toxicity data available for etridiazole on all three trophic levels (one study on fish, EPA TG 72-4; one on invertebrates, EPA TG 72-4 and two on algae, EPA TGs 122-2 and 122-3). No chronic toxicity data were reported for the degradation products, with the exception of the 72-h NOE_rC values for two degradation products in algae which were both higher than 0.1 mg/l. The reported sub-chronic early life stage study in fish resulted in a NOEC value of 0.12 mg/l and the chronic *Daphnia magna* study resulted in a (21-d) NOEC of 0.37 mg/l. The lowest reported NOEC value for etridiazole was in algae (*P. subcapitata*), NOE_rC (72 hours) =

0.027 mg/l. In addition, another NOE_rC (120 hours) of 0.063 mg/l in algae (*Anabaena flos-aquae)* was reported which is also below the threshold value of 0.1 mg/l for classification.

Classification according to CLP. The DS concluded that based on the NOE_rC for algae and information that etridiazole is not rapidly degradable, the substance fulfils the CLP criteria for classification as Aquatic Chronic 1. Since the lowest NOEC value for algae is between 0.01 and ≤ 0.1 mg/l (0.027 mg/l), a chronic M-factor of 1 is assigned to etridiazole.

Classification according to DSD. The DS proposal was based on the lowest acute aquatic toxicity value (0.49 mg/l (120 hours) in aquatic algae) and its non-rapid degradation in the aquatic environment, the DS proposed that etridiazole be classified as N; R50/53 (no specific concentration limits proposed).

Comments received during public consultation
One MSCA raised several issues which were addressed in the RCOM by the DS. Most importantly, they questioned why the 120-h time point was used in defining the E_rC_{50} value rather than 72-h E_rC_{50} in defining acute toxicity to *P. subcapitata*. In the post public consultation response the DS pointed out that the coefficient of variation was too large for controls after 72 and 96-h and therefore it would be best to use the 48-h E_rC_{50} in classification for acute toxicity, since it is most in line with the current OECD guideline.

The DS informed also that the *P. subcapitata* study was revaluated. The coefficient of variation of the section by section specific growth rate of the control and solvent control is > 35%. This is mainly due to a reduced growth rate observed at 72h and 120 h. Therefore the EC_{50} and EC_{10} of the growth rate, based on linear regression, were calculated for the period between 0-48 h, resulting in a value of 0.80 and 0.33 mg/l (based on measured concentrations). These values are comparable to those calculated over the period between 0 -72 h (i.e. 0.80 and 0.22 mg/l, respectively). The DS suggested that the acute toxicity classification should be based on the 48-h EC_{50} in *P. subcapitata* as this value was considered most in line with the current OECD guideline. In addition the DS provided information that Henry's law constant for etridiazole is 3.02 Pa m^3 mol^{-1} at 25 °C. In a study (Dzialo, 1994) on the absorption/desorption of etridiazole, the K_{OC} (L/kg) values for etridiazole were 349 (sandy loam, 2.4 % OC), 195 (clay, 4.2 % OC) and 323 (silt loam, 1.6 % OC). Freundlich adsorption isotherm 1/n values were between 0.84 and 0.92.

Another MSCA agreed with the proposed classification of environmental hazards but had some editorial remarks, including proposing the removal of tables 14a and 14b from the CLH report, since they were withdrawn from the DAR by EFSA.

In response the DS noted that studies of Nag & Yu (1994), Nag & Regis (1998) and Völkel (2000) are considered valid in the DAR (see e.g. DAR addendum May 2010) although these studies have some limitations. The DS considered these results valid and did not support their withdrawal and stating unacceptability of the studies as the reason. However, the results of the soil studies are not used for classification and labelling since sufficient valid data for the aquatic environment is available. The added value of soil degradation data in the classification decision when adequate aquatic degradation studies are available is unclear and the DS proposed that additional guidance on this be sought from RAC.

Assessment and comparison with the classification criteria
<u>*Degradation*</u>
According to the Guidance on the Application of CLP Criteria, information on hydrolysis may indicate that a substance is rapidly degradable when the longest half-life (t½) determined within the pH range 4-9 is shorter than 16 days and the degradation

products are not classifiable. The available data show that the hydrolysis half-life of etridiazole at 25°C in buffered water solutions with various pH (5.2, 7.1 and 8.9) is 88 to 98 days. Thus all half-lives are well above 16 days, so etridiazole cannot be considered as rapidly degradable based on hydrolysis data.

In two water/sediment systems, treated with [3-^{14}C]-etridiazole at a concentration of 839 µg/l and incubated at 20°C in the dark, etridiazole had primary degradation in the total water/sediment system with half-lives of 1.78 d and 1.92 d. The main degradation products formed were etridiazole acid and dichloro-etridiazole. Etridiazole is primarily degraded with a half-life <16 days but the degradation product dichloro-etridiazole is classifiable (LC_{50} of 0.77 mg/l in fish). Furthermore, the CO_2 production was very low (≤3.1 % after 104 days) indicating that degradation was not ultimate. These data indicate that etridiazole undergoes fast primary degradation but with formation of a classifiable degradation product, and that the ultimate degradation is low over a 28-day period. Since the main degradation product has an acute LC_{50} below 1 mg/l, it is classifiable and therefore the parent substance cannot be considered to be rapidly degradable.

The reported screening study (a closed bottle test according to OECD TG 301D/EC C.4-E) can be considered as an adequate and conclusive study for classification. The degradation rate of etridiazole in the study after 28-days incubation was only 1 % and therefore it did not pass the CLP or DSD criteria for ready biodegradation. The reported water/sediment studies indicated very short half-life for etridiazole but with formation of classifiable metabolites and low ultimate degradation. Therefore, etridiazole should be considered as not rapidly degradable according CLP and not readily degradable according to DSD.

Bioaccumulation
The measured log K_{ow} value and the BCF value from the fish study are both considered as adequately performed and conclusive for classification although the BCF value has not been corrected for lipid content. Both the log K_{ow} of 3.37 and BCF of 165 L/kg are below the threshold values for potentially bioaccumulative substances according to CLP (i.e. log K_{ow} is below 4 and BCF is below 500) but meet the criteria for a potential bioaccumulative substance according to DSD (i.e. log K_{ow} was above 3 and BCF was above 100).

Acute aquatic hazard
Several acute toxicity studies according to different international test guidelines were included in the CLH report. Only one study on etridiazole, i.e. the test on *P. subcapitata* of E_rC_{50} (48 hours) = 0.80 mg/l, is considered as conclusive and meeting the criteria of $L(E)C_{50}$ < 1 mg/l threshold for aquatic acute hazard classification. This value was chosen after re-evaluation of the study by the DS and showed that the coefficient of variation (CV%) of the growth rate in the control cultures was more than 35% due to reduced growth rate at 72-h and 120-h. The 48-h E_rC_{50} in *P. subcapitata* is considered most in line with the current OECD guidelines. Since the EC_{50} value of acute toxicity is ≤ 1 mg/l the CLP classification criteria are met and RAC agrees that etridiazole should be classified as Aquatic Acute 1. Since the value of E_rC_{50} for algae was in the range of 0.1 – 1 mg/l, the value of M-factor should be 1.

Long-term aquatic hazard
CLP classification. Adequate and reliable chronic toxicity studies were reported for fish and crustaceans but the NOEC values in both studies were above the classification threshold value of 0.1 mg/l. RAC evaluated the validity of the reported algal studies and concluded that since both *P. subcapitata* and *A. flos-aquae* studies beyond durations of 48 hours did not meet the validity criteria of CV < 35% for section-by-section specific growth rates in controls, the studies cannot be used for direct conclusions on the classification of chronic toxicity (see details in section Supplemental information - In

depth analyses by RAC). Therefore, RAC applies the surrogate approach for long-term hazard classification based on Figure 4.1.1 and Table 4.1.0 (iii) in Annex I to CLP. The lowest acute toxicity value, for the trophic level for which no conclusive chronic toxicity value is available (i.e. algae), is the reported E_rC50 (48h) of 0.80 mg/l (*P. subcapitata*). Since this value was below 1 mg/l and etridiazole was not considered to be rapidly degradable, the RAC concluded that classification as Aquatic Chronic 1 with an M factor 1 was warranted.

DSD classification. Etridiazole has a BCF above 100 and the log K_{ow} above 3 and is not considered rapidly degradable according to DSD. Since the lowest conclusive acute toxicity value (algae E_rC_{50} (48 hours) = 0.80 mg/l) is below the 1 mg/l threshold value for classification, RAC agrees that etridiazole should be classified as N; R50-53 according to DSD with the specific concentration limits N; R50-53: C ≥ 25 % N; R51-53: 2,5 % ≤ C < 25 % R52-53: 0,25 % ≤ C < 2,5 %

Conclusion
The RAC agreed with the dossier submitter's proposal to add an acute M-factor of 1 and a chronic M-factor of 1 to the existing harmonised classification in Annex VI of CLP.

Supplemental information - In depth analyses by RAC
RAC evaluated the reliability the reported algal studies according to the validity criteria specified in OECD TG 201. In both studies (*P. subcapitata* and *A. flos-aquae*) the cell concentration in the control cultures increased by a factor of at least 16 within three days from the beginning of the experiment meeting the first validity criterion in the guideline. The second criterion, i.e. the sector specific coefficient of variation (CV) should be below 35% for a study to be considered as valid. For *P. subcapitata* this criterion was met only for the 0-48h period, however; for the *A. flos-aquae* study the CV was higher than 35% at all the time points (see the tables below). The third requirement for a valid study is that the CV of average specific growth rates during the whole testing period in replicate control cultures should be below 7%. For the *A. flos-aquae* test the CV was below 7% for all calculated testing periods (0-48h, 0-72h, 0-96h and 0-120h) and for the *P. subcapitata* it was below 7% in all the testing periods, the only exception was 0-48h where the CV of replicates was 8%. Despite this, the study was considered to be valid over the period 0-48h.

Sector specific average coefficient of variation for the growth rates in the reported Anabaena flos-aquae study.

	Average Coefficient of Variation (%)			
	0-120h	**0-96h**	**0-72h**	**0-48h**
Control	82.13	85.87	65.84	94.69
Solvent control	72.04	77.96	64.47	84.63

Sector specific average coefficient of variation for the growth rates in the reported Pseudokirchneriella subcapitata (formerly Selenastrum capricornutum) study.

	Average Coefficient of Variation (%)			
	0-120h	**0-96h**	**0-72h**	**0-48h**
Control	57.37	47.26	54.71	20.49
Solvent control	51.89	42.46	48.48	12.91

References
Dzialo, D.G. 1994. Adsoprtion/desorption of etridiazole. Report Uniroyal, Middlebury, U.S.A., No. 93127 TER – 196 GLP, Unpublished.

8 OTHER INFORMATION

9 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.

10 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.

10.1 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.

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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 Etridazole (P547)
ECBI/71/95 - Add. 31	NL, evaluation of the carcinogenicity of Etridazole (P547)
ECBI/71/95 - Add. 38	BE, comments on classification proposal for etridazole (P547)
ECBI/71/95 - Add. 39	UK, comments on classification proposal for etridazole (P547)
ECBI/71/95 - Add. 40	NL, evaluation of the environmental classification of Etridazole (P547)
ECBI/71/95 - Add. 58	Uniroyal, additional comments on Etridazole (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.

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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 Etridazole (P547)
ECBI/71/95 - Add. 31	NL, evaluation of the carcinogenicity of Etridazole (P547)
ECBI/71/95 - Add. 38	BE, comments on classification proposal for etridazole (P547)
ECBI/71/95 - Add. 39	UK, comments on classification proposal for etridazole (P547)
ECBI/71/95 - Add. 40	NL, evaluation of the environmental classification of Etridazole (P547)
ECBI/71/95 - Add. 58	Uniroyal, additional comments on Etridazole (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 etridazole (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.