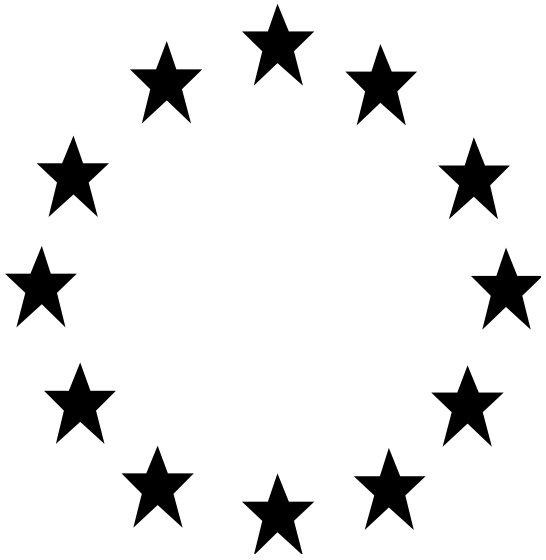


Draft Assessment Report (DAR)

- public version -

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

ETRIDIAZOLE

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

Volume 3, Annex B, part 2, B.6

August 2007

B.6 TOXICOLOGY AND METABOLISM

B.6.1 ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM (TOXICOKINETICS) (ANNEX IIA 5.1)

STUDY 1
Characteristics

reference	:	Markham P. (1994)	exposure	:	single (i.v.) & single or repeated by gavage
type of study	:	Absorption, distribution and excretion	doses	:	5 (single i.v., single oral and repeated oral) and 150 (single oral) mg/kg bw
year of execution	:	1993-1994	vehicle	:	1/1 v/v ethanol/water at 1 mL/kg bw (i.v.) and corn oil at 5 mL/kg bw (oral)
test substances	:	Non-labelled etridiazole, lot no. AC-1366-104A, 99.2% pure. [3-thiadiazole-ring^{14}C]-etridiazole: lot no. CSL-92-359-77-25, chemical purity not reported, radiochemical purity 93-97%.	GLP statement	:	yes
route	:	i.v. & oral	guideline	:	EPA 85-1
species	:	SD rat (8-10 weeks old)	acceptability	:	acceptable
group size	:	see table 6.1.1.1-01			

Study design

The absorption, distribution and excretion of ^{14}C-etridiazole was investigated after a single intravenous or oral dose of 5 mg/kg bw, a single oral dose of 150 mg/kg bw, and a single oral dose of 5 mg/kg bw after 14 daily pre-treatments with unlabeled etridiazole at the same dose. All animals received water and food *ad libitum* throughout the study. Exposure and sampling of urine, cage wash, faeces, radioactivity in expired air (using 2 serial 4-6M KOH traps), blood and tissues was as described in table 6.1.1.1-01.

Table 6.1.1.1-01 Experimental groups for each dose level

Group	No. of animals	Treatment[(B)]	Sampling times (h after dosing)	Sacrifice time (h after last dose)
1	5 M & 5 F	Single intravenous dose at 5 mg/kg [3-thiadiazole-ring-^{14}C]-etridiazole	0-4, 4-8, 8-12, 12-24, 24-32, 32-48, 48-72, 72-96, 96-120, 120-144, 144-168: urine, faeces; 168: cage wash	168[(A)]
2	7 M & 8 F	Single oral dose at 5 mg/kg [3-thiadiazole-ring-^{14}C]-etridiazole	As above.	168[(A)]
3	6 M & 6 F	Single oral dose at 150 mg/kg [3-thiadiazole-ring-^{14}C]-etridiazole	As above.	168[(A)]
4	5 M & 5 F	14 daily oral pre-treatment doses of unlabelled etridiazole (5 mg/kg bw) followed by one oral dose of 5 mg/kg bw [3-thiadiazole-ring-^{14}C]-etridiazole	As above.	168[(A)]

n.a. = not applicable
(A) At sacrifice, the following tissues and organs were taken from all animals: blood (separated into plasma and blood cells), liver, kidney, heart, lung, bone (femur), brain, adipose tissue (representative sample), skeletal muscle (representative sample), testes (male), ovaries (female) and spleen.
(B) All ^{14}C-labelled etridiazole was isotopically diluted with unlabelled etridiazole to the required specific activity.

Radioactivity in urine, plasma, cage washings and CO_2 trapping liquid was quantified by LSC and in faeces (homogenised in water) and tissues by combustion/LSC.

Results

Data on excretion and retention are presented in table 6.1.1.1-02. At the end of the study (168 h post-dosing), overall mean recovery for all groups was found to be 91-100% AR. There were no essential

differences between sexes for any of the dosing regimes. Ranges given below are for males and females together.

After a single oral dose of 5 and 150 mg/kg bw, or repeated oral dosing at 5 mg/kg bw, after 168 hours 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. Radioactivity in selected KOH traps was confirmed to be CO_2 by $BaCl_2$ precipitation. Excretion was essentially complete (≥93%, mean 96%) after 96 hours. Radioactivity in tissues (excluding the residual carcass, which was not analysed) at 168 hours post-dose represented 2.4-3.9% AR. Pre-treatment for 14 days did not affect the pattern of excretion and retention. 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%.

Table 6.1.1.1-02 Excretion and retention of radioactivity (% AR) in rats after single intravenous and oral or repeated oral exposure to ^{14}C-etridiazole

		5 mg/kg bw (single i.v.)		5 mg/kg bw (single oral)		150 mg/kg bw (single oral)		5 mg/kg bw (multiple oral)	
sample		M	F	M	F	M	F	M	F
urine	0-24 h	57	54	49	56	37	28	49	60
	0-48 h	62	63	53	61	54	45	56	65
	0-72 h	64	68	55	63	57	51	59	67
	0-96 h	66	70	58	64	59	54	60	71
	0-168	69	73	60	67	60	58	63	73
faeces	0-24 h	10	5.0	11	8.4	4.2	2.9	8.6	7.7
	0-48 h	13	8.1	15	13	12	9.6	12	11
	0-72 h	14	9.1	16	14	14	13	13	13
	0-96 h	14	9.3	16	15	15	14	14	14
	0-168	15	10	16	16	15	16	14	15
expired air	0-168	6.0	4.8	4.2	4.2	7.4	5.6	7.4	6.1
cage wash	168 h	5.7	2.9	8.3	3.8	4.8	8.8	9.9	3.1
tissues	168 h	2.8	2.5	2.8	2.4	3.9	2.8	2.9	2.8
recovery	168 h	99	93	92	94	92	91	98	100

The distribution of radioactivity in tissues at 168 hours post-dose is presented in table 6.1.1.1-03. There were no essential differences between sexes for any the dosing regimes, and ranges given here are for males and females together, unless indicated differently. 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 (0.45-0.47mg 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).

Table 6.1.1.1-03 Distribution of radioactivity in tissues and organs [mg etridiazole equivalents/kg and % AR(B)] at 168 post-dose of 14C-etridiazole.

Dose (mg/kg bw)		5 (single i.v.)		5 (single oral)		150 (single oral)		5 multiple oral)	
sex		M	F	M	F	M	F	M	F
blood	mg eq./kg			0.29	0.36				
	% AR			*0.43*	*0.57*				
plasma	mg eq./kg	0.06	0.06	0.11(A)	0.10	4.5	3.4	0.09	0.08
	% AR	*0.05*	*0.05*	*0.08(A)*	*0.10*	*0.10*	*0.08*	*0.06*	*0.06*
blood cells	mg eq./kg	0.44	0.52	0.68(A)	0.70	20	17	0.46	0.59
	% AR	*0.37*	*0.41*	*0.51(A)*	*0.5*	*0.43*	*0.37*	*0.31*	*0.42*
liver	mg eq./kg	0.76	0.71	0.97	0.83	41.12	27.8	1.0	0.82
	% AR	*0.66*	*0.55*	*0.68*	*0.58*	*0.78*	*0.55*	*0.65*	*0.58*
kidney	mg eq./kg	0.55	0.68	0.77	0.86	24	24	0.66	0.79
	% AR	*0.1*	*0.11*	*0.13*	*0.12*	*0.12*	*0.12*	*0.11*	*0.12*
heart	mg eq./kg	0.17	0.17	0.22	0.23	8.9	6.5	0.23	0.20
	% AR	*0.02*	*0.01*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*	*0.02*
lung	mg eq./kg	0.72	0.84	0.45	0.47	9.9	8.3	0.46	0.35
	% AR	*0.08*	*0.11*	*0.06*	*0.06*	*0.03*	*0.03*	*0.05*	*0.04*
brain	mg eq./kg	0.06	0.06	0.08	0.09	3.0	2.4	0.08	0.07
	% AR	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*
fat	mg eq./kg	0.06	0.09	0.05	0.06	2.5	3.4	0.04	0.07
	% AR	*0.13*	*0.19*	*0.11*	*0.15*	*0.14*	*0.20*	*0.07*	*0.13*
skeletal muscle	mg eq./kg	0.10	0.09	0.14	0.10	6.1	3.9	0.16	0.13
	% AR	*1.0*	*0.87*	*1.3*	*0.92*	*1.7*	*1.1*	*1.4*	*1.2*
spleen	mg eq./kg	0.23	0.26	0.24	0.21	9.0	6.7	0.25	0.23
	% AR	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*
testes/ovaries	mg eq./kg	0.07	0.16(A)	0.10	0.18	4.2	5.6	0.09	0.16
	% AR	*0.02*	*0.00(A)*	*0.02*	*0.00*	*0.03*	*0.00*	*0.02*	*0.00*
bone	mg eq./kg	0.13	0.11	0.20	0.16	9.2	5.9	0.21	0.16
	% AR	*0.26*	*0.22*	*0.38*	*0.29*	*0.50*	*0.32*	*0.36*	*0.28*

(A) Data from 1 rat.
(B) Percentages were calculated from organ weights and by assuming that plasma = 4%, blood cells = 4%, fat = 11%, skeletal muscle = 50% and bone = 10% of the body weight.

Conclusions

The absorption, distribution and excretion of ^{14}C-etridiazole was investigated after a single intravenous or oral dose of 5 mg/kg bw, a single oral dose of 150 mg/kg bw, and a single oral dose of 5 mg/kg bw after 14 daily pre-treatments with unlabeled etridiazole at the same dose. There were no essential differences between sexes for any the dosing regimes, and ranges given below are for males and females together.

After a single oral dose of 5 and 150 mg/kg bw, or repeated oral dosing at 5 mg/kg bw, after 168 hours 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. Radioactivity in tissues (excluding the residual carcass, which was not analysed) at 168 hours post-dose represented 2.4-3.9% AR. Pre-treatment for 14 days did not affect the pattern of excretion and retention. Excretion and retention after single intravenous dose administration was comparable to that for oral dosing. From the results of this study, oral absorption is estimated to be 100%.

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 (0.45-0.47mg 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).

Acceptability

(1) The residual carcass was not analysed. The mean overall recovery per dose group was however acceptable. There were minor reporting deficiencies. Raw data were not available to verify: trapping efficiency of CO_2 traps; combustion efficiency; replication and variation of combustion analysis; the radiochemical purity of each separate dosing formulation (only the range for all formulations was given: 93-97%). It was not reported whether any clinical signs of intoxication were observed.

(2) The radiochemical purity in the dosing formulations was reported to be 93-97%. The radiochemical purity of individual dosing formulations was however not reported. Chromatograms were not shown and no information was supplied on the identity of impurities. The radioactivity excreted with expired air represented 4.2-7.4% AR. Since impurities accounted for up to 7% AR in dose formulations, the radioactivity in expired air may have represented CO_2 formed from degradation of impurities in the radioactive test material. However, the metabolic pathway of etridiazole in rat was elucidated in the next study and ring opening and cleavage occurred, forming small molecules such as oxalic acid, ethyl (aminocarbamyl) carbamate and N-carbethoxy oxamic acid. CO_2 is therefore likely to represent an ultimate breakdown product of etridiazole in rat.

(3) Certain tissues/organs were not analysed (e.g. eyes, thymus, thyroid, adrenals, pancreas, intestine, lymph nodes, mammary glands, pituitary, skin, spleen, uterus). The next study however investigated retention in these tissues/organs.

(4) The study is **acceptable, with the annotations** that the results were determined in rats not deprived of food prior to dose administration, and that retention was only investigated for selected tissues/organs.

STUDY 2
Characteristics

reference	:	McManus J.P. (1995)	exposure	:	single (i.v.) & single or repeated by gavage
type of study	:	Metabolism	doses	:	5 (single i.v., single oral and repeated oral) and 150 (single oral) mg/kg bw
year of execution	:	1993-1995	vehicle	:	1/1 v/v ethanol/water at 1 mL/kg bw (i.v.) and corn oil at 5 mL/kg bw (oral)
test substances	:	Non-labelled etridiazole, lot no. AC-1366-104A, 99.2% pure. [3-thiadiazole-ring^{14}C]-etridiazole: lot no. CSL-92-359-77-25, chemical purity not reported, radiochemical purity 93-97%.	GLP statement	:	yes
route	:	i.v. & oral	guideline	:	EPA 85-1
species	:	SD rat (8-10 weeks old)	acceptability	:	**acceptable with limitations**
group size	:	see table 6.1.1.1-01			

Study design

The metabolism of ^{14}C-etridiazole was investigated in urine (0-4, 4-8, 8-12 and 12-24 hour samples), collected from rats of all four dosing regimes in the previous study (see Table 6.1.1.1-01), which had

received a single intravenous or oral dose of 5 mg/kg bw, a single oral dose of 150 mg/kg bw, or a single oral dose of 5 mg/kg bw after 14 daily pre-treatments with unlabeled etridiazole at the same dose. A faeces sample was also extracted and chromatographed, but the dose group, sex of the rats and collection period were not reported for this faeces sample.

Urine was analysed by LSC, filtered and analysed by reverse phase HPLC using different solvent systems and/or HPLC on an anion exchange column. The polar metabolites were also analysed by HPLC using a Zorbax amino column. All seven metabolite fractions identified during reverse phase HPLC were isolated by ion exchange HPLC and analysed by mass spectrometry and co-chromatography with reference standards, where possible. A faeces sample was extracted two times with methanol and the residue was rinsed with acetone. The combined liquid fractions were partitioned between water and ethylacetate, and the aqueous phase (containing 90% of the extracted radioactivity) was analysed by reverse phase HPLC and by HPLC on an anion exchange column.

Results

Column recovery for the reverse phase column used for metabolite quantification in urine was adequate (>90%).

Urine

Results of the metabolite identification in urine are presented in Table 6.1.1.1-04 (% AR) and 6.1.1.1-05 (% TRR). Radioactivity in the 0-24 hour urine samples ranged between 28 and 60% AR. Parent compound was not identified in urine. The main metabolite was etridiazole carboxylic acid (20-36% AR, 53-71% TRR). 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.

Faeces

Results were reported for "a sample of faeces from treated rats" (not further identified). About 78% of the TRR in the sample was extracted into methanol, and about 90% of the extracted radioactivity (hence about 70% TRR) did not partition into ethylacetate but remained in the aqueous phase. The radiochromatogram of the aqueous phase showed that etridiazole carboxylic acid was also the main component in faeces. Other compounds could not be identified in the chromatogram, which however had a low resolution.

Table 6.1.1.1-04 Metabolite identification (% AR) in urine of male and female rats after single intravenous and oral or repeated oral exposure to ^{14}C-etridiazole

	% AR in urine								
	5 mg/kg bw i.v.		5 mg/kg bw single oral		150 mg/kg bw single oral		5 mg/kg bw multiple oral		
Fraction	M	F	M	F	M	F	M	F	Identity
metabolite 1	12.7	5.2	7.6	11	4.3	1.8	8.1	7.5	multitude of minor metabolites(A)
metabolite 2	-	3.8	1.1	1.3	4.4	1.8	-	-	mixture (≥5 fractions)
metabolite 3	-	-	-	-	-	-	-	-	oxalic acid(B)
metabolite 4	1.7	1.3	2.3	3.1	1.8	-	0.5	4.3	ethyl (aminocarbamyl) carbamate
metabolite 5	8.3	8.6	7.4	8.4	5.2	4.1	7.9	12	N-carbethoxy oxamic acid
metabolite 6	-	-	1.7	2	0.3	0.5	-	-	N-acetyl cysteinyl conjugate of etridiazole
metabolite 7	35	36	29	30	21	20	32	35	etridiazole carboxylic acid
total identified	57.7	54.9	49.1	55.8	37.0	28.2	48.5	58.8	

(A) Each individual fraction accounted for ≤1.2% AR; very polar molecules showing similar chromatographic behaviour as natural urinary compounds (e.g., urea, hippuric acid, uric acid, aspartic acid etc).
(B) Peak was present in reverse phase chromatogram (used for quantification) as a rider on that of metabolite 2 and metabolite 3 was not quantified in report. In the reported chromatograms, the levels of metabolite 3 were always much lower than those of metabolite 2. The identification is tentative (not confirmed by MS).

Table 6.1.1.1-05 Metabolite identification (% TRR) in urine of male and female rats after single intravenous and oral or repeated oral exposure to ^{14}C-etridiazole

	% TRR in urine								
	5 mg/kg bw i.v.		5 mg/kg bw single oral		150 mg/kg bw single oral		5 mg/kg bw multiple oral		
Fraction	M	F	M	F	M	F	M	F	Identity
metabolite 1	22	10	16	20	12	6.4	16	13	multitude of minor metabolites
metabolite 2	-	7.0	2.3	2.3	12	6.4	-	-	mixture (≥5 fractions)
metabolite 3	-	-	-	-	-	-	-	-	oxalic acid
metabolite 4	3.0	2.4	4.7	5.5	4.9	-	1.0	7.2	ethyl (aminocarbamyl) carbamate
metabolite 5	15	16	15	15	14	15	16	20	N-carbethoxy oxamic acid
metabolite 6	-	-	3	4	0.9	1.7	-	-	N-acetyl cysteinyl conjugate of etridiazole
metabolite 7	61.6	66.5	59.4	53.4	56.9	71.2	64.8	58.3	etridiazole carboxylic acid
total identified	102	101	101	99	100	100	98	98	

Conclusions

In 0-24 hour urine of rats treated with ^{14}C-etridiazole, containing 28-60% AR, the metabolite pattern was essentially the same for sexes and dosing regimes, and ranges given below are for males and females together, and for 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 compounds could not be identified in the chromatogram. 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.

Acceptability

The radiochemical purity in the dosing formulations was reported to be 93-97%. The radiochemical purity of individual dosing formulations was however not reported. Chromatograms of dosing formulations were not shown and no information was supplied on the identity of impurities in the starting material. Metabolites found at low levels in urine (metabolites 2, 4, 6) may have represented impurities already present in the radioactive test material. **Metabolite identification in urine is**

therefore acceptable for the major fractions (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.

STUDY 3
Characteristics

reference	:	Thalacker F.W. (1997)	exposure	:	single by gavage
type of study	:	Absorption, distribution and excretion	doses	:	5 and 150 mg/kg bw
year of execution	:	1996	vehicle	:	corn oil at 5 mL/kg bw
test substances	:	Non-labelled etridiazole, lot no. AC-1322-17, 97.8% pure. [3-thiadiazole-ring^{14}C]-etridiazole: lot no. CSL-93-452-14-23, chemical purity not reported, radiochemical purity 99.3%.	GLP statement	:	yes
route	:	oral	guideline	:	EPA 85-1, OECD 417
species	:	Crl:CD(SD)BR rat (≥8 weeks old, 125-225 g)	acceptability	:	acceptable
group size	:	see table 6.1.1.1-06			

Study design

The absorption, tissue distribution and excretion of ^{14}C-etridiazole was investigated after a single oral dose of 5 and 150 mg/kg bw. All animals received water and food *ad libitum* throughout the study, except for an overnight fast prior to treatment until 4 hours post-dose. Exposure and sampling of urine, cage wash and cage wipe, faeces, blood and tissues was as described in table 6.1.1.1-06.

Table 6.1.1.1-06 Experimental groups for each dose level

Group no.	No. of animals	Treatment[B]	Sampling times (h after dosing)	Sacrifice time (h after last dose)
1	4 M & 4 F	Single oral dose at 5 mg/kg [3-thiadiazole-ring-^{14}C]-etridiazole	0.25, 0.5, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 168 hours: blood, plasma	n.a.
2	4 M & 4 F	Single oral dose at 150 mg/kg [3-thiadiazole-ring-^{14}C]-etridiazole	As above.	n.a.
3	9 M & 9 F	Single oral dose at 5 mg/kg [3-thiadiazole-ring-^{14}C]-etridiazole	168 hour animals only: 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168: urine, faeces; 168: cage wash & cage wipe	M & F: 4, 14 & 168[A]
4	9 M & 9 F	Single oral dose at 150 mg/kg [3-thiadiazole-ring-^{14}C]-etridiazole	As above.	M: 8, 36 & 168; F: 24, 60 & 168 [A]

n.a. = not applicable
(A) At plasma t_{max}, ½ t_{max} and at 168 hours post-dose, the following tissues and organs were taken from three animals: blood, plasma, liver, kidney, heart, lung, bone (femur), brain, fat (reproductive and renal), skeletal muscle, testes (male), ovaries (female), spleen, adrenal glands, urinary bladder, bone marrow (femur), residual carcass, eyes, intestinal tract, intestinal tract contents, mesenteric lymph nodes, mammary glands, pancreas, pituitary gland, skin, stomach, stomach contents, thymus, thyroid, uterus (female).
(B) All ^{14}C-labelled etridiazole was isotopically diluted with unlabelled etridiazole to the required specific activity.

Radioactivity in urine, plasma, cage washings, cage wipes (after extraction), fat (after dissolving in LSC cocktail), pancreas, stomach, skin and thymus (after digestion in 1N NaOH) was quantified by LSC. Radioactivity in faeces (homogenised in ethanol/water), remaining tissues, intestinal tract contents and residual carcass (after homogenisation) and blood was quantified by combustion/LSC.

Results

All rats behaved normally during the study.

Plasma pharmacokinetic parameters

The pharmacokinetic parameters are presented in Table 6.1.1.1-07. Peak ^{14}C-concentrations in blood were observed after 4 hours in both sexes after a dose of 5 mg/kg bw, but only after 8 and 21 hours in males and females, respectively, dosed with 150 mg/kg bw. The elimination half-life at 5 mg/kg bw (14 hours for both sexes) was much shorter than at 150 mg/kg bw (36 and 60 hours in males and females, respectively).

Table 6.1.1.1-07 Pharmacokinetic parameters in blood of male and female rats after single oral exposure to 14C-etridiazole at 5 and 150 mg/kg bw

	single oral, 5 mg/kg bw		single oral, 150 mg/kg bw	
	M	F	M	F
C_{max} (mg eq./kg)	1.39	1.93	20	16.4
T_{max} (h)	4	4	8	21
$T_{1/2}$ (h)	14	14	36	60
AUC_{0-168}	36	48	1008	1100
$AUC_{0-\infty}$	38	53	1087	1140

Excretion and retention (mass balance study)

Data on excretion and retention for the animals sacrificed at 168 hours post-dose are presented in table 6.1.1.1-08. At the end of the study, overall mean recovery for all groups was found to be 88-97% AR. There were no essential differences between sexes for the two dosing regimes except where noted. Ranges given below are for males and females together.

After a single oral dose of 5 and 150 mg/kg bw, after 168 hours 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). Excretion was essentially complete (mean 97%) after 72 hours. Radioactivity in tissues (including the residual carcass) at 168 hours post-dose represented 3.2-4.7% AR.

Table 6.1.1.1-08 Excretion and retention of radioactivity (% AR) in rats after single oral exposure to ^{14}C-etridiazole at 5 and 150 mg/kg bw

		5 mg/kg bw		150 mg/kg bw	
sex	**time**	**M**	**F**	**M**	**F**
urine	0-24 h	71	61	50	33
	0-48 h	75	65	62	58
	0-72 h	76	66	64	60
	0-96 h	77	67	65	61
	0-168	78	68	66	62
faeces	0-24 h	12	17	11	12
	0-48 h	14	18	17	19
	0-72 h	14	18	18	21
	0-96 h	14	18	18	21
	0-168	14	18	18	21
cage wash	168 h	0.3	0.7	0.7	1.2
cage wipe	168 h	0.5	1.1	0.8	0.8
tissues	168 h	4.6	3.2	4.7	3.2
recovery	168 h	97	91	90	88

Distribution in tissues

The distribution of radioactivity in tissues of rats sacrificed at t_{max}, ½ t_{max} and 168 hours post-dose is presented in table 6.1.1.1-09 and -10. Ranges given below are for males and females together, unless indicated differently.

At the low dose, radioactivity concentrations ranged between 0.4-0.5 mg eq./kg (eyes) and 6.7-10 mg eq/kg (liver) at t_{max}, between 0.2-0.3 mg eq./kg (eyes & brain) and 4.9-5.3 mg eq/kg (liver) at ½ t_{max}, and between <0.005 mg eq./kg (5-6 tissues) and 0.9 mg eq/kg (liver) at 168 hours. At ½ t_{max} and 168 hours post-dose, radioactivity concentrations in tissues with quantifiable residues were on average 41-55% and 8-9% of those at t_{max}. At the high dose, radioactivity concentrations ranged between 5.9-8.4 mg eq./kg (eyes) and 546-567 mg eq/kg (fat) at t_{max}, between 2.9-3.9 mg eq./kg (eyes) and 75-96 mg eq/kg (liver) at ½ t_{max}, and between 1.3-1.4 mg eq./kg (plasma or eyes) and 19-24 mg eq/kg (liver) at 168 hours. At ½ t_{max} and 168 hours post-dose, radioactivity concentrations in tissues with quantifiable residues were on average 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.

Table 6.1.1.1-09 Distribution of radioactivity in tissues and organs [mg etridiazole equivalents/kg and % AR] at t_{max}, ½ t_{max} and 168 hours post-dose of 5 mg ^{14}C-etridiazole /kg bw.

	4 hours (t_{max})				14 hours (1/2 t_{max})				168 hours			
	male		female		male		female		male		female	
tissue	µg/g	% AR	µg/g	% AR	µg/g	% AR	µg/g	% AR	µg/g	% AR	µg/g	% AR
adrenals	2.1	*0.01*	2.3	*0.01*	0.7	*nq*	0.8	*nq*	0.1	*nq*	0.1	*nq*
urin. bladder	3.2	*0.02*	2.8	*0.02*	0.9	*0.01*	0.9	*0.01*	0.2	*nq*	0.1	*nq*
blood	2.1	*1.5*	1.5	*0.9*	0.9	*0.7*	0.9	*0.6*	0.2	*0.2*	0.3	*0.2*
bone	0.8	*0.04*	0.5	*0.02*	0.5	*0.02*	0.4	*0.02*	0.2	*0.01*	0.1	*0.01*
bone marrow	1.2	*nq*	1.0	*nq*	0.7	*nq*	0.7	*nq*	nq	*nq*	nq	*nq*
brain	0.6	*0.1*	0.5	*0.09*	0.2	*0.04*	0.3	*0.05*	0.04	*0.01*	0.05	*0.01*
carcass (res.)	1.5	*20*	1.5	*20*	0.6	*8.1*	0.5	*7.3*	0.2	*3.0*	0.1	*1.9*
eyes	0.5	*0.01*	0.4	*0.01*	0.2	*nq*	0.3	*0.01*	0.05	*nq*	0.05	*nq*
fat	6.8	*1.2*	8.7	*1.4*	0.9	*0.2*	2.2	*0.5*	0.03	*0.01*	0.04	*0.01*
heart	1.2	*0.08*	0.9	*0.06*	0.5	*0.04*	0.5	*0.03*	0.1	*0.01*	0.1	*0.01*
intest. content	3.9	*19*	3.5	*19*	1.9	*5.6*	2.0	*7.2*	nq	*nq*	nq	*nq*
intest. tract	3.0	*2.0*	3.3	*2.0*	0.8	*0.6*	0.9	*0.7*	0.06	*0.05*	0.05	*0.04*
kidneys	7.9	*1.4*	6.5	*1.1*	2.2	*0.4*	2.7	*0.4*	0.4	*0.1*	0.5	*0.09*
liver	10	*7.1*	6.7	*3.9*	5.3	*5.2*	4.9	*3.7*	0.9	*1.0*	0.9	*0.7*
lungs	3.8	*0.3*	2.8	*0.2*	2.1	*0.2*	2.7	*0.3*	0.4	*0.04*	0.4	*0.04*
lymph node	1.7	*0.03*	1.9	*0.03*	0.5	*0.01*	0.6	*0.01*	0.07	*nq*	0.07	*nq*
mamm. glands	2.4	*nq*	1.7	*nq*	0.9	*0.01*	1.0	*0.01*	nq	*nq*	0.07	*nq*
skel. muscle	0.6	*0.2*	0.5	*0.1*	0.3	*0.08*	0.3	*0.09*	0.09	*0.03*	0.08	*0.03*
ovaries			1.8	*0.02*			1.0	*0.01*			0.09	*nq*
pancreas	2.1	*0.2*	1.7	*0.1*	0.7	*0.05*	0.8	*0.08*	0.1	*0.01*	0.1	*0.01*
pituitary	1.2	*nq*	1.0	*nq*	0.8	*nq*	0.8	*0.0*	nq	*nq*	nq	*nq*
plasma	2.1	*0.08*	1.7	*0.07*	0.8	*0.03*	0.8	*0.03*	0.04	*nq*	0.06	*nq*
skin	1.7	*0.5*	1.2	*0.3*	0.5	*0.2*	0.6	*0.2*	0.2	*0.09*	0.1	*0.06*
spleen	1.0	*0.05*	0.8	*0.03*	0.5	*0.03*	0.5	*0.02*	0.09	*0.01*	0.1	*nq*
stomach	3.8	*0.4*	4.0	*0.4*	0.8	*0.09*	0.8	*0.08*	0.1	*0.01*	0.1	*0.01*
stomach cont.	2.2	*3.2*	1.1	*2.4*	0.3	*0.5*	0.7	*0.9*	nq	*nq*	nq	*nq*
testes	0.7	*0.1*			0.3	*0.08*			0.06	*0.02*		
thymus	0.9	*0.06*	0.7	*0.03*	0.5	*0.02*	0.5	*0.02*	0.07		0.071	*nq*
thyroid	1.6	*nq*	1.2	*nq*	0.9	*nq*	0.8	*nq*	nq	*nq*	nq	*nq*
uterus			0.9	*0.03*			0.6	*0.02*			0.1	*nq*
total		*58*		*52*		*22*		*22*		*4.6*		*3.2*

nq = below quantifiable limit (0.005 mg eq./kg).

Table 6.1.1.1-10 Distribution of radioactivity in tissues and organs [mg etridiazole equivalents/kg and % AR] at t_{max}, ½ t_{max} and 168 hours post-dose of 150 mg ^{14}C-etridiazole /kg bw.

	8 hours (t_{max})		24 hours (t_{max})		36 hours (1/2 t_{max})		60 hours (1/2 t_{max})		168 hours		168 hours	
	male		female		male		female		male		female	
tissue	µg/g	% AR	µg/g	% AR	µg/g	% AR	µg/g	% AR	µg/g	% AR	µg/g	% AR
adrenals	107	0.02	73	0.02	16	nq	18		4.9	nq	6.9	nq
urin. bladder	37	0.01	35	0.01	15	nq	11		5.0	nq	4.3	nq
blood	28	0.7	22	0.4	15	0.4	14	0.3	6.7	0.2	6.6	0.2
bone	18	0.04	11	0.02	10	0.02	6.0	0.01	5.6	0.01	3.0	0.01
bone marrow	21	nq	18	nq	16	nq	12		2.5	nq	2.2	nq
brain	15	0.07	9.8	0.06	4.8	0.02	3.9	0.02	1.7	0.01	1.5	0.01
carcass (res.)	63	29	39	19	11	5.0	7.9	3.9	4.8	2.9	3.9	2.1
eyes	8.4	0.01	5.9		3.9	nq	2.9	nq	1.7	nq	1.3	nq
fat	567	1.4	546	1.7	14	0.04	31	0.2	1.8	0.02	2.5	0.02
heart	21	0.05	15	0.03	9.3	0.03	8.6	0.02	3.8	0.01	3.5	0.01
intest. content	59	9.6	20	2.7	12	1.6	9.1	1.4	0.2	0.04	0.2	0.04
intest. tract	75	2.1	36	1.1	13	0.4	9.1	0.2	1.9	0.06	1.8	0.05
kidneys	101	0.6	65	0.4	35	0.2	35	0.2	12	0.09	13	0.07
liver	148	3.9	91	2.9	96	4.2	75	2.1	24	1.1	19	0.6
lungs	35	0.1	20	0.07	12	0.04	11	0.03	3.9	0.02	3.7	0.01
lymph node	94	0.02	66	0.03	8.8	nq	8.6	0.01	2.6	nq	2.4	nq
mamm. glands	78	0.01	345	0.2	7.6	nq	9.0	nq	4.8	nq	3.2	nq
skel. muscle	21	0.2	15	0.1	5.9	0.06	4.2	0.05	3.1	0.04	2.0	0.02
ovaries			131	0.06			13	0.01			1.3	nq
pancreas	62	0.1	52	0.1	16	0.04	12	0.04	4.0	0.02	4.1	0.01
pituitary	18	nq	19	nq	11	nq	11	nq	2.2	nq	3.0	nq
plasma	27	0.03	18	0.03	13	0.02	11	0.02	1.4	nq	1.6	nq
skin	62	0.6	35	0.5	12	0.1	8.9	0.08	8.5	0.2	3.8	0.07
spleen	19	0.03	13	0.02	10	0.02	8.7	0.01	3.3	0.01	3.5	0.01
stomach	246	0.8	43	0.2	13	0.05	11	0.04	3.9	0.02	3.5	0.01
stomach cont.	168	15	40	3.1	0.5	0.03	0.1	0.01	0.081	0.01	nq	nq
testes	15	0.12			6.2	0.05			2.1	0.02		
thymus	22	0.04	17	0.02	10	0.02	8.5	0.01	2.5	0.01	2.1	nq
thyroid	34	nq	28	nq	14	nq	7.9	nq	4.4	nq	4.1	nq
uterus			6.1	0.01			11	0.01			3.3	nq
total		65		33		12		8.6		4.7		3.2

nq = below quantifiable limit (0.005 mg eq./kg).

Conclusions

The absorption, distribution and excretion of ^{14}C-etridiazole was investigated after a single oral dose of 5 and 150 mg/kg bw. Peak ^{14}C-concentrations in blood were observed after 4 hours in both sexes after a dose of 5 mg/kg bw, but only after 8 and 21 hours in males and females, respectively, dosed with 150 mg/kg bw. The elimination half-life at 5 mg/kg bw (14 hours for both sexes) was much shorter than at 150 mg/kg bw (36 and 60 hours in males and females, respectively). After a single oral dose of 5 and 150 mg/kg bw, after 168 hours 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). Radioactivity in tissues (including the residual carcass) at 168 hours post-dose 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.

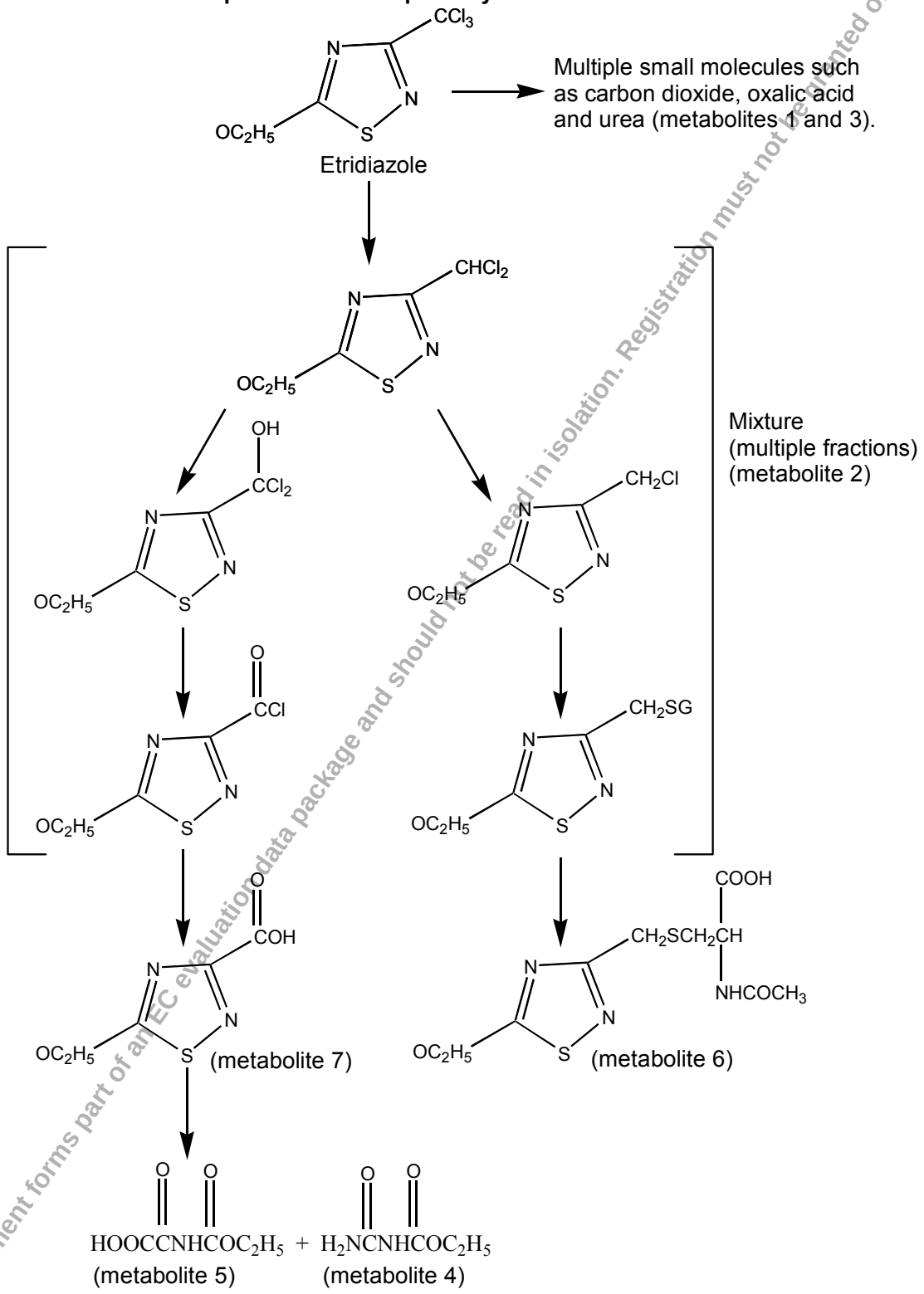
Acceptability

The study is **acceptable.**

B.6.1.2 Proposed metabolic pathway

The proposed metabolic pathway of etridiazole in rat is shown in Fig. 6.1.2.1.

Fig. 6.1.2.1 Proposed metabolic pathway of etridiazole in rat



B.6.1.3 List of identified compounds during *in vivo* metabolism studies with etridiazole

Table 6.1.3.1 presents an overview of the metabolite identification achieved during *in vivo* metabolism studies.

Table 6.1.3.1 Identified compounds during *in vivo* metabolism studies with etridiazole

Code	Compounds	Description	Matrix
Metabolite 1	multitude of minor metabolites	Parent	Rat, urine
Metabolite 2	mixture (≥5 fractions)	Metabolite	Rat, urine
Metabolite 3	oxalic acid	Metabolite	Rat,urine
Metabolite 4	ethyl (aminocarbamyl) carbamate	Metabolite	Rat, urine
Metabolite 5	N-carbethoxy oxamic acid	Metabolite	Rat, urine
Metabolite 6	N-acetyl cysteinyl conjugate of etridiazole	Metabolite	Rat, urine
Metabolite 7	etridiazole carboxylic acid	Metabolite	Rat, urine

[1] Free and glucose-conjugated

B.6.1.4 Summary and conclusions

Absorption

In one study, 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% 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, 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, 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, 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, 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, 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 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%).

B.6.2 ACUTE TOXICITY INCLUDING IRRITANCY AND SKIN SENSITISATION (ANNEX IIA 5.2)

B.6.2.1 Acute toxicity

STUDY 1

Characteristics

reference	:	Warshawsky, L.D., 1994	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	700, 850, 1000, 1400 mg/kg bw (both sexes)
year of execution	:	1993	vehicle	:	Corn oil
test substance	:	Terrazole Technical (Etridiazole), Lot no. 208S052, purity 99.4%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 401 (1987)
species	:	Rat, Crl:CD BR VAF/Plus	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	Male rats: 1141 mg/kg bw Female rats: 945 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987).

Results

Mortality: 2/5 males and 3/5 females given 1000 mg/kg bw were found dead 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.

Symptoms of toxicity: 39/40 animals showed 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: On day 8, weight loss or decreased weight gain was observed in the surviving animals dosed 850, 1000 and 1400 mg/kg bw. On day 15, these losses were regained with some additional weight gain. No remarkable changes were observed in the 700 mg/kg bw group.

Pathology: No significant observations were seen at necropsy for animals surviving to study termination from any of the dosage levels. In 9/16 animals that died during the study, no significant observations were observed either. In 4/16 animals (1000 mg/kg bw), foci of the glandular and/or nonglandular stomach mucosa, red or yellow foci of the liver and tan discoloration of the liver were observed. In 3/16 animals (1400 mg/kg bw) tan discoloured liver and red discoloured lung were observed.

Acceptability

No data on the temperature and humidity is present. Nevertheless, this study is considered acceptable, since the study was performed in accordance with OECD 401 (1987).

Conclusions

The acute oral LD_{50} of Terrazole Technical was found to be 1141 mg/kg bw in male rats and 945 mg/kg bw in females rats.

STUDY 2

Characteristics

reference	:	Warshawsky, L.D., 1994	exposure	:	24 hours on a skin area of 10% of the total body surface area (semi-occlusive exposure).
type of study	:	Acute dermal toxicity study	doses	:	5000 mg/kg bw (both sexes)
year of execution	:	1993	vehicle	:	None; undiluted test substance
test substance	:	Terrazole Technical (Etridiazole), Lot no. 208S052, purity 99.4%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 402 (1987)
species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 5000 mg/kg bw

Study design

The study was performed in accordance with OECD 402 (1987).

Results

Mortality: No mortality was observed.

Symptoms of toxicity: Inappetence was observed in all males and 4/5 females. Decreased defecation and/or no stool was observed in 3/5 males and 2/5 females. These effects disappeared within a week after treatment.

Body weight: 4/5 males and 4/5 females had lost weight during the first 8 days after treatment. These 8 animals regained this weight loss by day 15. An increased body weight on day 15 compared to day 1 was observed in 6/10 rabbits.

Pathology: No visible abnormalities were observed.

Acceptability

The study is considered acceptable. No data was present on temperature and humidity in the animal room.

Conclusions

The acute dermal LD_{50} of Terrazole Technical in rabbits was found to be greater than 5000 mg/kg bw in both males and females.

STUDY 3

Characteristics

reference	:	Hilaski, R.J., 1994	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.

Acceptability

Humidity is only 4-12%, but this is not unusual when testing aerosols. The study is considered acceptable.

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.

B.6.2.2 Irritation and sensitisation

STUDY 1

Characteristics

reference	:	Warshawsky, L.D., 1994	exposure	:	4 hours, semi-occlusive, application area 6.25 cm^2
type of study	:	Acute dermal irritation study	doses	:	0.5 ml
year of execution	:	1993	vehicle	:	None
test substance	:	Terrazole Technical (Etridiazole), Lot no. 208S052, purity 99.4%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 404 (1992)
species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	6 males	**Effect**	:	Not skin irritating

Study design

The study was performed in accordance with OECD 404 (1992). However, the amount of animals tested could have been lower (n=3).

Results

The results are summarised in tables 6.2.2.1 and 6.2.2.2.

Table 6.2.2.1 Individual irritation scores

Scores observed after	0.5 hours	24 hours	48 hours	72 hours
Erythema	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Oedema	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

Table 6.2.2.2 Mean value irritation scores

Animal	mean 24-72 hrs	
	erythema	oedema
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0

Acceptability

The study is considered acceptable.

Conclusions

Etridiazole does not have to be classified as irritating to skin.

STUDY 2

Characteristics

reference	:	Warshawsky, L.D., 1994	exposure	:	Single instillation
type of study	:	Acute eye irritation study	doses	:	0.1 ml
year of execution	:	1993	vehicle	:	None
test substance	:	Terrazole Technical (Etridiazole) Lot no. 208S052, purity 99.4%	GLP statement	:	Yes
route	:	Ocular	guideline	:	In accordance with OECD 405 (1987)
species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	6 males	**Effect**	:	Not eye irritating

Study design

The study was performed in accordance with OECD 405 (1987).

Results

The results are summarised in tables 6.2.2.3 and 6.2.2.4. At 1 hour after exposure, rabbits showed clear ocular discharge and blanching, which had disappeared within 24 hours.

Table 6.2.2.3

Scores observed after	1 hour	24 hours	48 hours	72 hours
Cornea/opacity	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Iris	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva redness	2, 2, 2, 3, 2, 2	2, 1, 1, 1, 1, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva chemosis	4, 3, 3, 3, 2, 2	1, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva discharge	3, 3, 3, 3, 3, 3	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

Table 6.2.2.4 Mean value irritation scores

Animal	mean 24-72 hrs			
	corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1	0	0	0.7	0.3
2	0	0	0.3	0
3	0	0	0.3	0
4	0	0	0.3	0
5	0	0	0.3	0
6	0	0	0.3	0

Acceptability

The study is acceptable, although less animals could have been used (n=3).

Conclusions

Etridiazole does not need classification for eye irritation.

STUDY 3

Characteristics

reference	:	Parcell, B.I., 1993	exposure	:	Intradermal and topical induction, topical challenge (occlusive, 24h).
type of study	:	Skin sensitisation study (GPMT)	doses	:	20% intradermal induction 100% topical induction 100 and 50% challenge 20, 10, 5 and 1% rechallenge
year of execution	:	1992	vehicle	:	Alembicol D
test substance	:	Etridiazole, Batch no. SI 3429, purity 99.3%	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 406
species	:	Guinea-pig, Dunkin/Hartley	acceptability	:	Acceptable
group size	:	20 controls (females) 20 test animals (females)	**Effect**	:	Skin sensitising

Study design

The study was performed in accordance with OECD 406 and conducted according to Magnusson and Kligman. Formalin was used as the positive control.

Dose levels were based on the results of a range-finding study using 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10, 20, 40, 50, 60, 70, 80, 90, and 100% for intradermal injections and 30, 50, 70, and 100% for topical applications. Well-defined erythema and oedema was observed after intradermal injections of up to 20%. Intradermal injections of 40% and above induced necrosis and well-defined oedema. No skin reaction was noted after any of the topical applications.

Intradermal induction was performed with a 20% test substance concentration in Alembicol D (product of coconut oil). Topical induction was initiated after intradermal induction with treatment of 10% concentration of sodium lauryl sulphate in petrolatum. One day later the undiluted test substance was dermally applied. Fourteen days later, challenge was performed with dermal application of undiluted and 50% test substance in Alembicol D. One week after the first challenge, a rechallenge was performed with 20, 10, 5 and 1% of Etridiazole in Alembicol D. The rechallenge was done with both the control and the test animals.

Results

After intradermal induction with 20% etridiazole, well-defined to moderate oedema and erythema was observed. Control animals showed slight erythema and oedema.

After topical induction with undiluted etridiazole, slight to moderate erythema was observed. In control animals mainly slight erythema was noted.

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.

Acceptability

The study is considered acceptable.

Conclusions

In this study, etridiazole has been found to be a skin sensitizer.

B.6.2.3 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in table 6.2.3.1, 6.2.3.2 and 6.2.3.3, respectively.

Table 6.2.3.1 Acute toxicity studies, LD_{50}/LC_{50} values

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle	Reference
Terrazole Technical	1141 mg/kg bw (males) 945 mg/kg bw (females)	Rat	Oral	Corn oil	Warshawsky, L.D., 1994
Terrazole Technical	> 5000 mg/kg bw (males and females)	Rabbit	Dermal	None	Warshawsky, L.D., 1994
Terrazole Technical	> 5.7 mg/l	Rat	Inhalation	None	Hilaski, R.J., 1994

Table 6.2.3.2 Skin and eye irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
Terrazole Technical	Not skin irritating	Rabbit	Dermal	None	Warshawsky, L.D., 1994
Terrazole Technical	Not eye irritating	Rabbit	Ocular	None	Warshawsky, L.D., 1994

Table 6.2.3.3 Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
Etridiazole	Skin sensitising	Guinea-pig	Dermal	Alembicol D	Parcell, B.I., 1993

Etridiazole needs to be classified as harmful if swallowed (Xn, R22) on the basis of its acute oral toxicity in rats according to Directive 2001/59/EC. Etridiazole does not need to be classified on the basis of its acute dermal and inhalation toxicity. Etridiazole is considered not irritating to the skin and eyes according to the criteria given in Annex VI of Directive 2001/59/EC. Etridiazole needs to be classified as a skin sensitizer (Xi, R43).

B.6.3 SHORT-TERM TOXICITY (ANNEX IIA 5.3)

B.6.3.1 Subacute oral studies

No data were submitted by the notifier.

A subacute dose range study in dogs was submitted (Larson, 1963), which was further discussed at the semichronic data on dogs.

B.6.3.2 Subacute dermal studies

STUDY 1

Characteristics

Reference	:	Goldenthal, 2002	exposure	:	4 weeks, 6 hours/day, 10% body surface, dorsal area, semi-occlusive
type of study	:	subacute dermal toxicity	dose	:	0, 20, 400 and 1000 mg/kg bw/day
year of execution	:	2001	vehicle	:	none
test substance	:	Etridiazole techn., lot no 0065942, purity 98.1%	GLP statement	:	Yes
Route	:	Dermal	guideline	:	In accordance with OECD 410 (1981)
Species	:	Rats, CD (Crl:CD(SD)IGS BR	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL local**	:	1000 mg/kg bw/day
			NOAEL systemic		20 mg/kg bw/day

Study design

The study was performed in accordance with OECD 410 (1981).

Results

The results are summarised in table 6.3.2.1.

Table 6.3.2.1

Dose (mg/kg bw/d)	**0**		**20**		**400**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none								
Clinical signs	No treatment-related findings								
Body weight (gain)	No treatment-related findings								
Food consumption	No treatment-related findings								
Ophthalmoscopy	No treatment-related findings								
Neurobehavioural examination	No treatment-related findings								
Functional observations									
- mean hindlimb splay							dc		
Motor activity	No treatment-related findings								
Haematology									
- reticulocyte count							i	ic	
Clinical chemistry	No treatment-related findings								
Urinalysis									
- pH							dc	dc	

Dose (mg/kg bw/d)	0		20		400		1000		dr
	m	f	m	f	m	f	m	f	
Organ weights									
- liver					$ic^{a,r}$	$i^{a,r}$	$ic^{a,r}$	i^{a}, ic^{r}	m
Pathology									
Macroscopy	No treatment-related findings								
Microscopy									
- liver: centrilobular hypertrophy	0/10	0/10	0/10	0/10	7/10	0/10	10/10	8/10	m
- kidney: chronic progressive nephropathy	1/10	1/10					4/10	0/10	
- heart: cardiomyopathy	0/10	0/10					2/10	0/10	

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

Acceptability

The study is considered acceptable.

Conclusions

Dermal exposure to test substance concentrations of 0, 20, 400 and 1000 mg/kg bw/day did not result in local skin effects. No treatment-related effects on mortality, clinical observations, body weight, food consumption, ophthalmoscopy, neurobehavioural examination, motor activity and clinical biochemistry were noted. At the functional observations, a statistically significant decrease in mean hindlimb splay was noted in males at 1000 mg/kg food. This decrease might be due to a rather high control value, and is therefore considered to be of no toxicological relevance. Reticulocyte count was significantly increased at 1000 mg/kg bw in females (133% of control) and increased in males (125% of control; one male extremely high value). In the absence of haematological correlates this effect is considered to be not toxicologically relevant. The pH of urine in males and females at 1000 mg/kg bw was significantly decreased (0.5 pH unit relative to control). Absolute and relative liver weight were significantly increased at 400 and 1000 mg/kg bw in males (116-117% and 126-128% of control, resp.). In females absolute and relative liver weight was increased at 400 (111% of control) and 1000 mg/kg bw (113-114% of control; only increase in relative liver weight at 1000 mg/kg bw was statistically significant). Histopathology showed minimal centrilobular hypertrophy of the liver in males at 400 mg/kg bw and both sexes at 1000 mg/kg bw. Cardiomyopathy was observed in 2 males at the high dose level. Chronic progressive nephropathy was observed in one animal of both sexes in the control group and four males at 1000 mg/kg bw.

In the absence of local effects the NOAEL for local effects is set at 1000 mg/kg bw/day. Based on increased liver weights and concomitant centrilobular hypertrophy the NOAEL for systemic effects is set at 20 mg/kg bw/day.

B.6.3.3 Subacute inhalation studies

STUDY 1

Characteristics

reference	:	Hoffman, 2002	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**	:	<15 mg/m^3
			NOAEL systemic		15 mg/m^3

[1] **EQUIVALENT TO 0, 4.0, 20.2 AND 54.0 MG/KG BW/DAY FOR MALES AND FEMALES (USING 45 L/KG BW/H (RAT RESPIRATION RATE), SEE THE GUIDANCE DOCUMENT AOEL)**

Study design

The study was performed in accordance with OECD 412 (1981). Larynx, nasopharyngeal tissue, trachea, lungs, liver and gross lesions and masses were investigated for all 4 groups, other tissues were investigated in control group and at 200 mg/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								

Dose (mg/m^3)	0		15		75		200		dr
	m	f	m	f	m	f	m	f	
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

Acceptability

The study is considered acceptable.

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.

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).

B.6.3.4 Semichronic oral studies

STUDY 1

Characteristics

Reference	:	Richards, 1994	exposure	:	13 weeks, diet; 4 weeks recovery
Type of study	:	13-week oral toxicity study	dose	:	0, 50, 600 and 1250 mg/kg food (nominal)[1]
year of execution	:	1993	vehicle	:	none
test substance	:	Etridiazole, batch 2065048, purity 99.3%	GLP statement	:	yes
route	:	oral	guideline	:	predominantly in accordance with OECD 408 (1998)
species	:	rat, Crl:CD(SD)BR	acceptability	:	acceptable
group size	:	10/sex/dose (+5/sex/dose as recovery group)	**NOAEL**	:	2.7 mg/kg bw/day for males and 3.3 mg/kg bw/day for females

[1]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 (corrected for mean measured concentration of the test substance 93%, 85% and 90% of nominal concentration, respectively)

Study design

The study was generally in compliance with OECD 408 (1998). However, functional observations were not performed. Blood was collected in week 12 and week 17 (recovery group) and urine in week 13. At necropsy additional blood samples were collected for measurement of T_3, T_4 and TSH.

Results

The results are summarised in table 6.3.4.1a and 6.3.4.1b

Table 6.3.4.1a. Results after 13 weeks.

Dose (mg/kg food)	0		50		600		1250		dr
	m	f	m	f	m	f	m	f	
Mortality	none								
Clinical signs	no treatment-related findings[1]								
Body weight gain						d	dc	dc	
Food consumption							d	d	
Ophthalmoscopy	no treatment-related findings								
Haematology									
- reticulocytes							i	ic	
- platelet count							i	ic	
- prothrombin time					dc		dc		
- activated partial thromboplastin time					dc		dc		
Clin. Chemistry									
- ASAT							dc		
- ALAT							dc		
- sodium							dc		
- potassium							ic		
- chloride							dc		
- total cholesterol					ic		ic		
- glucose							d	d	
- bilirubin							ic		
- T_3								dc	
Urine analysis									
- chloride					ic	ic	ic	ic	m
- total chloride					ic		ic	ic	m

Dose (mg/kg food)	0		50		600		1250		dr
	m	f	m	f	m	f	m	f	
Organ weights									
- liver					i^a, ic^r	i^a, ic^r	i^a, ic^r	i^a, ic^r	m/f
Pathology									
macroscopy									
- liver, large	1/10	0/10	1/10	0/10	3/10	0/10	7/10	0/10	m
microscopy									
- liver: centrilobular hypertrophy	0/10	0/10	0/10	0/10	10/10	6/10	10/10	10/10	m/f
- kidney: hyaline droplets	6/10	0/10	5/10	0/10	9/10	0/10	10/10	0/10	

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
a/r absolute/relative
1 clinical signs were not reported, only in the result section it was stated that no treatment-related clinical signs were seen

Table 6.3.4.1b. Results after 4-week recovery period.

Dose (mg/kg food)	0		50		600		1250		dr
	m	f	m	f	m	f	m	f	
Mortality	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
Clinical signs	no treatment-related findings[1]								
Body weight gain						ic	ic	ic	
Food consumption	no treatment-related findings								
Ophthalmoscopy	no treatment-related findings								
Haematology									
- activated partial thromboplastin time					dc				
Clin. Chemistry	no treatment-related findings								
Organ weights									
- liver					ic^r	$i^{a,r}$	ic^r	$i^{a,r}$	
Pathology									
macroscopy	no treatment-related findings								
microscopy									
- kidney: hyaline droplets	5/5	0/5	4/5	0/5	5/5	0/5	5/5	0/5	

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
1 clinical signs were not reported, only in the result section it was stated that no treatment-related clinical signs were seen

Acceptability

The study is considered acceptable.

Conclusions

Rats were administered 0, 50, 600 or 1250 mg/kg food of etridiazole for 13 weeks. One male in the control group was killed in extremis in week 17 due to a damaged eye after blood sampling. Body weight gain was decreased at 600 mg/kg food in females (90% of control) and 1250 mg/kg food in both sexes (78 and 64% of control for males and females, respectively). During the recovery phase higher body weight gains were observed in females previously treated at 600 mg/kg food (>400% of control) and males and females previously treated at 1250 mg/kg food (156% and >300% of control) than in controls. Food consumption was significantly decreased at 1250 mg/kg food in males (93% of control) and females (82% of control). Food consumption was restored in the recovery group. No treatment-related clinical signs were observed.

At the end of the exposure period reticulocytes and platelet count was increased in males and females at 1250 mg/kg food (females statistically significant; 146 and 126% of control, resp.). Prothrombin time and activated partial thromboplastin time (APTT) were significantly decreased in males at 600 and 1250 mg/kg food (92 and 84-86% of control, resp.). At the end of the recovery period APTT was still decreased at 600 mg/kg food only (82% of control). In the absence of a dose-relationship the latter deviation is considered to be not toxicologically relevant.

Aspartate aminotransferase and alanine aminotransferase were significantly decreased in males at 1250 mg/kg food (82 and 84% of control); a decrease in both parameters is not considered to be toxicologically relevant. Sodium and chloride were slightly reduced (99 and 98% of control, resp.) and potassium was significantly increased in males at 1250 mg/kg food (110% of control) at the end of the treatment period. Glucose was decreased at 1250 mg/kg food in males and females (87 and 82% of control, resp.). Cholesterol was significantly increased in males at 600 and 1250 mg/kg food at the end of the exposure period (136 and 143% of control, resp.). Bilirubin was significantly increased in males at 1250 mg/kg food (122% of control). Both parameters had returned to control levels at the end of the recovery period. At necropsy T_3 levels were significantly decreased in females at 1250 mg/kg food (61% of control). T_3 values were similar to control values at the end of the recovery period. Changes in sodium, chloride and potassium in males correlate with the histopathological observations in the kidneys. Changes in cholesterol indicate a disturbed liver function.

Urinalysis showed that chloride was significantly increased in males (158-176% of control) and females (131-137% of control) at 600 and 1250 mg/kg food. Total chloride was also significantly increased in males at 600 and 1250 mg/kg food (202-216% of control) and females at 1250 mg/kg food (167% of control).

Relative liver weights were significantly increased at 600 and 1250 mg/kg food at the end of the exposure period in males (117-139% of control) and in females (114-129% of control). Absolute liver weights were also increased at 600 and 1250 mg/kg food in males (122-133% of control) and females (107-114% of control) (not examined statistically). After recovery relative liver weights were still increased in males at 600 and 1250 mg/kg food (112-115% of control) and increased in females (111-112% of control) and absolute liver weights were only increased in females (105-113% of control).

At necropsy the incidence of liver enlargement was increased in males at 600 and 1250 mg/kg food. Histopathology showed a concomitant centrilobular hypertrophy in the liver all males at 600 and 1250 mg/kg food. Centrilobular hypertrophy was also found in 6/10 and all females at 600 and 1250 mg/kg food. Effects were not observed in animals sacrificed after 4 weeks recovery. An increased incidence

of hyaline droplets in the kidneys was noted at 600 and 1250 mg/kg food in males. It should be noted that a high incidence of hyaline droplets was also noted in control and low dose animals (5-6/10). After recovery, hyaline droplets were observed in the kidneys of males in all groups. In the absence of historical data it is not clear whether the slightly increased incidence of hyaline droplets at 600 and 1250 mg/kg food at the end of treatment is an effect of the test substance or not. The possible effect would be considered to be related to α_{2u}-globulin, which is specific for male rats and therefore, the effect would not be considered to be relevant for human risk assessment and derivation of the NOAEL (see also B6.8.2).

The study results indicate that the liver and kidney are potential targets of etridiazole in rats. The NOAEL is set at 50 mg/kg food (equal to 2.7 mg/kg bw for males and 3.3 mg/kg bw for females) based on effects on the liver and kidneys.

STUDY 2

Characteristics

Reference	:	Ambrose, 1964	exposure	:	3 months, diet
Type of study	:	3-months oral toxicity study	dose	:	0, 100, 400 and 1600 mg/kg food
year of execution	:	unknown	vehicle	:	None
test substance	:	Om 2424 (etridiazole), purity unknown	GLP statement	:	No
route	:	Oral	guideline	:	Not in accordance with OECD 408 (1998)
species	:	Dog, beagle	acceptability	:	Not acceptable
group size	:	2/sex/dose	**NOAEL**	:	-

Study design

The study is not in compliance with OECD 409 (1998). The dose levels were based on a 28-day study (Larson, 1963) with 1 mongrel dog/dose at doses of 312, 1250, 5000 and 20000 mg/kg food Olin 2424 (no control group). Only body weight, food consumption, limited haematological parameters and histopathology (not specified) were investigated. The dog at 5000 mg/kg food died in week 4. Decreased body weight and food consumption were observed at 1250-20000 mg/kg food. Focal periportal fatty changes were observed at 20000 mg/kg food.

In the main study (Ambrose, 1964), etridiazole was administered at 0, 100, 400 and 1600 mg/kg food for 3 months. The following parameters were investigated: clinical signs, body weight, haematology (haematocrit, haemoglobin, total and differential white cell count), urinalysis, liver function (EXP, ALAT, ASAT), organ weights (heart, spleen, kidney, liver and testes) and histopathology (heart, lung, liver, kidney, spleen, gastro enteric, urinary bladder, skin, skeletal muscle, mesenteric lymph nodes, bone marrow, brain, pituitary, thyroid, pancreas, adrenal and gonad).

Results

In the 3-month study Beagle dogs (2/sex/dose) were administered 0, 100, 400 or 1600 mg/kg food of OM-2424 (etridiazole, unknown batch and purity). No mortality was observed. Body weight was reduced relative to the control group at 400 mg/kg bw (1 female) and at 1600 mg/kg food in both sexes. Food consumption was reduced at 1600 mg/kg food in both sexes. No treatment-related findings were observed for haematological parameters (haematocrit, haemoglobin, white blood cell count and differential white cell count). Limited urinalysis was stated to show no effect. Alkaline

phosphatase was increased at 1600 mg/kg food in males (177% of control). No effect was observed on bromsulphalein and ASAT. For organs weights results are only available combined for males and females (mean). Absolute spleen weight was significantly decreased at 1600 mg/kg food and relative spleen weight was significantly decreased at all three exposure levels (58-63% of control). Absolute and relative liver weight was increased at 1600 mg/kg food (153-199%). The only effect observed with histopathology was marked congestion of the spleen in one male at 400 mg/kg food.

Acceptability

The study is not considered acceptable for the overall evaluation of etridiazole, because:

- limited number of animals used
- unknown purity of the test substance
- study was not performed under GLP
- number of parameters investigated is limited

STUDY 3

Characteristics

Reference	:	Goldenthal, 2002	exposure	:	1-year, diet
Type of study	:	1-year oral toxicity study	dose	:	0, 160, 500 and 1000 mg/kg food/ (nominal)[1]
year of execution	:	2001/2002	vehicle	:	none
test substance	:	Terrazole techn. (etridiazole), Lot no. 065942, purity 99%	GLP statement	:	yes
route	:	oral	guideline	:	in accordance with OECD 452 (1981)
species	:	dog, Beagle	acceptability	:	acceptable
group size	:	4/sex/dose	**NOAEL**	:	3.1 and 4.3 mg/kg bw/day for males and females, resp.

[1]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 (corrected for mean measured concentration of the test substance 90%, 91% and 90% of nominal concentration, respectively and for instability (86, 74 and 74% at 160, 500 and 1000 mg/kg food)

Study design

The study was generally in compliance with OECD 452 (1981). Haematological parameters and urinalysis were not determined after 3 months of exposure. Dosage levels were based on a 2-year dog study (Larson, P.S., 1968; MRID #0001697) at dose levels of 10, 100 and 1000 mg/kg food. After 1 year body weight gain was decreased, alkaline phosphatase levels were increased and liver weight was increased at 1000 mg/kg bw. The NOEL was established at 100 mg/kg food.

Results

The results are summarised in table 6.3.4.2.

Table 6.3.4.2. Results.

Dose (mg/kg food)	0		160		500		1000		dr
	m	f	m	f	m	f	m	f	
Mortality	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	
Clinical signs	No treatment-related findings								
Body weight gain							dc	d	
Food consumption	No treatment-related findings								

Dose (mg/kg food)	0		160		500		1000		dr
	m	f	m	f	m	f	m	f	
Ophthalmoscopy	No treatment-related findings								
Haematology									
- platelet count							ic^1		
- APTT							ic^2	ic^2	
Clin. Chemistry									
- ALP					ic^1	i^1	ic^1	ic^1	
- urea nitrogen							dc^2	d^2	
- creatinine							dc^1	dc^1	
- total protein							dc^3,d^2	dc^2	
- albumin							d^3,dc^2	dc^1	
- A/G ratio							dc^2	dc^1	
- cholesterol							i^1	i^3	
Urinalysis	No treatment-related findings								
Organ weights									
- liver			$i^{a,r}$		$i^{a,r}$	$i^{a,r}$	i^a,ic^r	i^a,ic^r	
- kidney							d	ic^r	
- thyroid							i^a,ic^r	i^a,ic^r	
Pathology									
macroscopy	No treatment-related findings								
microscopy	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
[1] at 6 and 12 months
[2] at 12 months
[3] at 6 months

Acceptability

The study is acceptable. Diets are prepared once a week. However, stability measurements of Terrazole technical in diet (p. 20) showed that after 7 days only 86% and 74% of Terrazole technical were found in the diet for 160 and 1000 mg/kg food, respectively. Periodic analyses of the test substance concentrations in the diet were 144, 455 and 899 mg/ kg food, i.e. (90, 91 and 90% of nominal). The report suggests that these reduced concentrations are due to binding of the test article to food proteins with the decrease being directly related to how long the dietary samples were frozen prior to analysis. However, no proof was given. Since it is not clear whether corrections for measured concentrations and instability were included in the compound consumption given (p. 22 of report), the reviewer calculated the corrected values from body weight at the end of the study and average food consumption.

Conclusions

Dogs were administered 0, 160, 500 or 1000 mg/kg food of Terrazole technical for 12 months. No treatment-related mortality or clinical signs were observed.

Decreased body weight was noted at 1000 mg/kg food in males and females at the end of the study (88 and 82% of control for males and females, respectively). No treatment-related findings on food consumption and ophthalmoscopy were observed.

Platelet count was significantly increased at 1000 mg/kg food in males after 6 and 12 months of exposure (132-133% of control; 124-135% of pre-test), while values in other males decreased during

exposure. APTT was significantly increased relative to control value after 12 months of exposure in males and females at 1000 mg/kg food (114-115% of control); 1000 mg/kg food was the only level at which APTT increased relative to pre-test value.

Alkaline phosphatase was increased during the whole exposure period at 500 mg/kg food in males (401-517% of control) and females (217-283% of control), and at 1000 mg/kg food in males (416-455% of control) and females (495-539% of control). Blood urea nitrogen was significantly decreased in males (71% of control) and females (74% of control) at 1000 mg/kg food at the end of the exposure period. Creatinine was significantly decreased during the whole exposure period in males (74-75% of control) and females (63-66% of control) at 1000 mg/kg food. Total protein was decreased at 1000 mg/kg food in males (90-92% of control) during the whole exposure period and after 12 months in females (94% of control). Albumin and albumin/globulin ratio were significantly decreased at 1000 mg/kg food in males (83-90% and 84% of control, respectively) and females (82-85% and 71-74% of control, respectively). Cholesterol was increased at 1000 mg/kg food during the whole exposure period in males (127-145% of control) and increased in females at 6 months (147% of control). Other deviations were not considered to be toxicologically relevant. The decreased total protein, albumin and albumin/globulin ratio may be related to decreased food consumption and/or liver dysfunction.

Urinalysis showed no treatment-related effects. The deviations in ALP, cholesterol and proteins at 500 and/or 1000 mg/kg food point to perturbations in liver function.

Absolute and relative liver weights were increased in males at all levels (absolute: 114-120% of control; relative: 113-139% of control), and in females at 500 and 1000 mg/kg food (absolute: 119-128% of control; not dose-related); relative: 120-143% of control. The increased liver weight in males at 160 mg/kg bw was only slight (114% for absolute weight and 113% for relative weight) and not accompanied by changes in clinical parameters and is therefore, not considered to be toxicologically relevant. Absolute kidney weight was decreased in males at 1000 mg/kg food (82% of control). Relative kidney weight was significantly increased in females at 1000 mg/kg food (131% of control). Absolute thyroid (including parathyroid) weight was increased and relative thyroid weight was significantly increased (133-138% of control) at 1000 mg/kg food in both genders (114-116% of control).

Macroscopy and microscopy revealed no treatment-related findings.

The NOAEL is set at 160 mg/kg food (= 3.1 mg/kg bw/d for males and 4.3 mg/kg bw/d for females) based on increased alkaline phosphatase and increased liver weight at 500 mg/kg food.

B.6.3.5 Summary

The results of the subacute and semichronic toxicity studies are summarised in tables 6.3.5.1 and 6.3.5.2, respectively.

Table 6.3.5.1 Subacute studies with etridiazole

Duration	Species	Route	NOAEL mg a.i./kg bw/day	LOAEL mg a.i./kg bw/day	Critical effects	Reference
4 weeks	rat	dermal	local: 1000 systemic: 20	local: - systemic: 400	- no local effects observed - increased liver weight and centrilobular hypertrophy	Goldenthal, 2002
4 weeks	rat	inhala-tion	local: <15 mg/m^3 systemic: 15 mg/m^3 (M: 1.5; F: 2.34)	local: 15 mg/m^3 systemic: 75 mg/m^3	- squamous metaplasia in larynx mucosa - reduced body weight gain, increased potassium	Hoffman, 2002

Table 6.3.5.2 Semichronic studies with etridiazole

Duration	Species	Route	NOAEL mg a.i./kg bw/day	LOAEL mg a.i./kg bw/day	Critical effects	Reference
13 weeks	rat	oral	2.7 (M) 3.3 (F)	29.5 (M) 35.2 (F)	increased cholesterol, increased liver weights, and centrilobular hypertrophy.	Richards, 1994
3 months	dog	oral	-[1]	-	Decreased body weight, reduced spleen weight, congestion of the spleen	Ambrose, 1964
12 months	dog	oral	3.1 (M) 4.3 (F)	8.1 (M) 9.3 (F)	increased alkaline phosphatase, increased liver weight.	Goldenthal, 2002

[1]NOAEL could not be derived due to the limited number of animals, unknown purity of etridiazole, limited number of parameters investigated and no GLP-protocol.

Four weeks of dermal exposure of rats to 0, 20, 400 and 1000 mg/kg bw/day resulted in increased liver weights and concomitant centrilobular hypertrophy at 400 mg/kg bw/day and above. The NOAEL for systemic effects was set at 20 mg/kg bw/day. As no local effects were observed the NOAEL for local effects was established at 1000 mg/kg bw/day.

After subacute inhalation exposure of rats to 0, 15, 75 and 200 mg/m^3, a reduced body weight gain and an increased potassium concentration, of which the toxicological relevance was unclear, were observed at 75 mg/m^3 and above. Based on these effects a NOAEL of 15 mg/m^3 was established for systemic effects. For local effects no NOAEL could be derived, because squamous metaplasia of the larynx mucosa was observed at all dose levels.

Dietary exposure of rats to 0, 50, 600 and 1250 mg/kg food/day of etridiazole (equal to 0, 2.7, 29.5 and 64.7 mg/kg bw/day for males and 0, 3.3, 35.2 and 73.6 mg/kg bw/day for females) for 13 weeks caused decreased body weight at 600 mg/kg food in females and 1250 mg/kg food in both sexes. 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. 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. T_3 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 correlate with hyaline droplets observed in the kidneys of male animals. The incidence was slightly increased at 600 and 1250 mg/kg food, which is considered to be due to α_{2u}globulin (see B.6.8.2). 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.

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. An increased platelet count (males only) and APTT was 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. Increased alkaline phosphatase and cholesterol, and decreased protein parameters indicate a disturbed functioning of the liver. Concomitantly, liver weights were increased at 500 and 1000 mg/kg food. No macroscopic or microscopic findings were noted. Therefore, the NOAEL is 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).

Considering the identity of the observed effects at the LOAELs after oral, dermal and inhalation repeated exposure, classification with R48 is not considered necessary.

B.6.4 GENOTOXICITY (ANNEX IIA 5.4)

B.6.4.1 *In vitro*

STUDY 1

Study design and results

Type of study: Ames test, plate incorporation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: *S. typh.* TA 98 TA 100	 point mut. point mut.	 - -	 - -	rat liver	Arochlor 1254	Mutation test: 0.02, 0.06, 0.2, 0.6, 2.0 µl/plate Solvent: DMSO Positive and negative control: Included	Loveday, K.S., Seixas, G.S.A. 1981
Test substance: Terrazole (etridiazole), batch nr. 79-02-B, liquid, red/orange, 99.0% Toxicity observed at dose level: ≥ 0.6 µl/plate GLP statement: yes According to OECD 471: not mentioned							

Acceptability

The study does not fulfil the requirements of the recent OECD 471 guideline of 1997. Study is incomplete, only two strains tested, no independent repeat assay performed and the experiment is only performed in duplicate. The results can be used for the overall evaluation.

Conclusions

Etridiazole did not induce point mutations in *S. Typhimurium* strains TA98 and TA100.

STUDY 2

Study design and results

Type of study: Ames test, plate incorporation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
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	- - - - - - - - - - -	- - - - - 	rat liver	Aroclor 1254	*S. typh.* TA100 (- and + S9)*:* 23 concentrations ranging from 1 to 5000 µg/plate [1)] *S. typh.* TA1535, TA1537, TA1538, TA98 (- and + S9)*:* 1, 5, 10, 25, 50, 100, 250, 500, 750 and 1000 µg/plate [2)] *E.coli:* (-S9) : 250, 500, 750, 1000 and 2000 µg/disc Vehicle: DMSO Negative and positive controls included	Ercegovich, C.D., Rashid, K.A., 1977

[1)] Dose levels were based on a dose-range finding study with levels of 0.08-5000 µg/plate. No toxicity was observed.

Test substance: Terrazole (technical grade)(etridiazole), batch nr. 7.506, drum #4, purity 93.7%.
[2)] Toxicity was observed at 800 µg/plate (-S9) and 3000 µg/plate (+S9). No toxicity was observed in tester strain TA1535 and TA1538 in the presence of S9-mix
No precipitation was observed.
GLP statement: no
According to OECD 471: no, see acceptability

Acceptability

The study does not fulfil the requirements of the recent OECD 471 guideline of 1997. No independent repeat assay has been performed and the experiment is only performed on one plate.

However, considering the broad range of tested concentrations, the inclusion of a positive and negative control, and combining the results with the results of Study 1, the present study is considered suitable for the overall evaluation.

Conclusions

Etridiazole did not induce point mutations in *S. Typhimurium* and *E.coli*.

STUDY 3

Study design and results

Type of study: mammalian cells *in vitro*, gene mutation test, with independent repeat assay

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
CHO chinese hamster cells	gene mutations (HPRT)	-	-	rat liver	Arochlor 1254	Dose range finding (- and + S9): 0.00001, 0.0001, 0.001, 0.01 and 0.05 % [1)] Main experiment: (-S9) 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007 and 0.008 % and (+S9) 0.001, 0.0015, 0.002, 0.003, 0.004 and 0.005 % Solvent: DMSO Negative and positive controls included	Loveday, K.S., Gorodecki, J., 1981
Test substance: Terrazole (etridiazole), Batch 79-02-B, liquid, red-orange, 99.0% 1) Cytotoxicity observed at dose level: 0.01% and above No precipitation was observed GLP statement: yes According to OECD 476: not indicated							

Acceptability

The study was performed in accordance with OECD 476 of 1984. The study does not fulfil the requirements of the more recent OECD 476 guideline of 1997, such as cytotoxicity criteria and number of dose levels tested. However, the study is considered acceptable.

Conclusions

Etridiazole did not induce gene mutations in mammalian cells.

STUDY 4

Study design and results

Type of study: mammalian cells *in vitro*, cytogenetic assay, with independent repeat assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster ovary (CHO) cells	Sister chromatid exchange	-/+	-	rat liver	Arochlor 1254	Range finding (-S9): 10 concentrations ranging from 0.00001 to 0.5 % [1)] (+S9): 7 concentrations ranging from 0.001 to 0.007 % Main test: (- and +S9): 0.001, 0.002, 0.003, 0.004 and 0.005% Vehicle: DMSO Negative and positive controls included	Loveday, K.S., Donahue, B.A., 1982

Test substance: Terrazole (etridiazole), Batch 10626-1, liquid, purity 99.0% (based on reply on completeness check).
[1)] Cytotoxicity observed at dose level: 0.005% and above (-S9)
Not miscible in the cell medium at dose level: 0.01% and above (-S9) and precipitate was observed at dose levels of 0.006 and 0.007% (+S9)
GLP statement: yes
According to OECD 479: no

Results

dose	*-S9*		*+S9*	
	30-h treatment		*4-h treatment*	
%	*Frequency SCEs/cell ± SD*	*Mean Frequency SCEs/cell*	*Frequency SCEs/cell ± SD*	*Mean Frequency SCEs/cell*
0	11.6 ± 0.9	14.1	14.0 ± 1.1	14.8
0	16.5 ± 1.3		15.5 ± 0.9	
0.001	13.3 ± 0.9		15.2 ± 1.2	
0.002	19.8 ± 1.2		17.1 ± 1.0	
0.003	14.2 ± 0.9		17.0 ± 1.1	
0.004	17.8 ± 1.1		15.6 ± 1.1	
0.005	22.7 ± 1.3		14.7 ± 0.8	
pos. c.	54.4 ± 6.2		120.5 ± 13.0	

Acceptability

According the report the test substance induced statistically significant (one-way ANOVA) and concentration dependent sister chromatid exchanges in the absence of S9-mix. Although, if the results are judged for biological relevancy these increases are considered not relevant, since no more than two-fold increase has been observed. No independent repeat assay performed. However, the study is considered acceptable.

Conclusions

According to the author of the report, etridiazole induced statistically significant and concentration dependent sister chromatid exchanges in the absence of S9-mix. This might be caused by the longer treatment period in the non-activated assay. However since no biological significant increase was observed and no historical control data of the solvent control were added, this increase is considered equivocal.

STUDY 5

Study design and results

Type of study: mammalian cells *in vitro*, cytogenetic assay, with independent repeat assay

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster ovary (CHO) cells	-Sister chromatid exchange -Chromosome aberration	+ +	 +	rat liver	Arochlor 1254	SCE [1]: Main test (-S9): 0.001, 0.002, 0.003, 0.004 and 0.005% CA test [1]: Main test 1: (- and + S9): 0.003, 0.004, 0.005 and 0.006% Main test 2: (-S9): 0.004, 0.005 and 0.006% Vehicle: DMSO Negative and positive controls included	Loveday, K.S., 1982

Test substance: Terrazole (etridiazole), Batch 79-02-B, liquid, 99.0%
[1] Dose levels were selected on the basis of toxicity and solubility limits observed in previous SCE tests.
No information was added about precipitation to this study
GLP statement: yes
According to OECD 473 and 479: no

Results CA Between brackets number of flask evaluated

Main 1

	-S9		*+S9*	
dose	*6 h treatment/ harvest*		*2 hours treatment/6-h harvest*	
%	*mitotic index %*	*Mean break frequency.*	*mitotic index %*	*Mean break frequency.*
0	3.4	0.035 (2)	4.9	0.065 (3)
0.003	2.5	0.066 (2)	1.5	0.152 (2)
0.004	3.6	0.082 (2)	0.8	0.064 (2)
0.005	2.2	0.096 (2)	1.7 *	0.169 (1)
0.006	1.1	0.290 (1)	0.5 *	0.108 (1)
pos. c.	ND	0.350 (1)	ND	0.151 (1)

* one slide no metaphases

Main 2

	-S9	
dose	*6 h treatment/ harvest*	
%	*mitotic index %*	*Mean break frequency.*
0	13.1	0.037 (4)
0.004	ND	0.036 (3)
0.005	3.0	0.103 (2)
0.006	3.4	0.103 (2)
pos. c.	ND	0.084 (2)

ND Not determined

Results SCE

	-S9
dose	*30-h treatment*
%	*Frequency SCEs/cell*
0	9.2 (3)
0.003	16.3 (2)
0.004	19.9 (2)
0.005	17.6 (2)
pos. c.	66.5 (1)

Acceptability

The chromosome aberration study does not fulfil the requirements of the recent OECD 471 guideline of 1997. Although an independent repeat assay has been performed, in this repeat experiment only very toxic dose levels are tested and the positive control substance showed no clastogenic response. Furthermore only the mean value of the historical background has been given and not the laboratorial low and high results are reported. For the SCE study no historical background data are given, no dose dependent relationship is observed and no toxicity data are reported. Although etridiazole induced statistically significant increases in both the chromosome aberration study and the SCE study, the results of this study should not be considered for the overall evaluation.

Conclusions

Etridiazole induced statistically significant increases in both the chromosome aberration study and the SCE study. The clastogenicity of the chromosome aberration study was confined only to very toxic concentrations and since the results of both the chromosome aberration study and the SCE study could not be compared to the historical control data the results of this study are equivocal.

STUDY 6

Study design and results

Type of study: microbial test, *mitotic recombination assay*, with independent repeat assay

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Saccharomyces cerevisiae, D6	Mitotic aneuploidy	-	-	rat liver	Phenobarbital/ ß-naphthoflavone	Dose range finding (- and + S9): 6.4, 20.5, 64, 205 and 640 µg/ml[1)] Both Main experiment: (- and + S9): 40, 80, 160, 320 and 640 µg/ml Solvent Ethanol Negative and positive controls included	Edwards, C.N., McSheehy, T.W., 1987
Test substance: Etridiazole technical, Batch SI 1281, clear liquid, 98.1% 1) No cytotoxicity was observed up to and including the dose level of 640 µg/ml No precipitation was observed GLP statement: yes According to OECD 481: yes							

Acceptability

The study was performed in accordance with OECD 481 of 1986 and the study is considered acceptable.

Conclusions

Etridiazole technical did not induce mitotic aneupoloidy in *Saccharomyces cerevisiae*, D6.

B.6.4.2 *In vivo*

STUDY 1

Study design and results

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
mouse, NMRI, KFM 18/sex (male and female/dose (vehicle control, low and mid dose), 20/sex/dose (high dose)	micronuclei (bone marrow)	-	Pilot study: 700, 1000 and 2500 mg/kg bw [1)] Main test: 1000 mg/kg bw Sampling times: 24, 48 and 72 hours Solvent control 2% CMC Negative and positive controls included	Banduhn, N., 1985
Test substance: Terrazole technical (etridiazole), Batch CM-8561 S045017, liquid, purity min. 95% 1) The LD50 dose was estimated to be 1467 mg/kg bw GLP statement: yes According to OECD 474: yes				

Dose/effect	0	1000 mg/kg bw 24 hours sampling time	1000 mg/kg bw 48 hours sampling time	1000 mg/kg bw 72 hours sampling time
Mortality	0/10	0/10	0/10	0/10
Clinical signs[A]	After treatment to terminal sacrifice: slight to severe sedation and ataxia 1 to 6 hours after treatment: tremor was observed in males and females 24 hours and 32 hours after treatment: 3 male animals had ptoses of the eyes 72 hours after treatment: one female had a ruffled fur			
% PCE	Male: - Female: -	Male: - Female: -	Male: - Female: -	Male:+ Female: +
MPCE [% of PCE]	No treatment related effects			

- no reduction in the ratio of PCE/NCE
+ reduction in the ratio of PCE/NCE

Acceptability

The study was performed in accordance with OECD 474 of 1984. The study fulfils the requirements of the more recent OECD 474 guideline of 1997.

Conclusions

Etridiazole is not mutagenic in this *in vivo* mouse micronucleus assay.

STUDY 2

Study design and results

Type of study: mammalian cells *in vivo*, cytogenetic assay

Indicator cells	Endpoint	Result	Dose range	Reference
rat, Sprague-Dawley 15/sex (male and female)/ vehicle and positive control 21/sex (male and female)/, low and mid dose 28/sex (male and female)/ high dose	Chromosome aberration (bone marrow)	-	Pilot study: 357, 549.3, 845, 1300 and 2000 mg/kg bw [1)] Main test: 175, 350 and 700 mg/kg bw Sampling times: 6, 24 and 48 hours Solvent control 0.05% CMC Negative and positive controls included	McEnaney, S. 1995
Test substance: Etridiazole (Technical), Batch 206S048 (SI 3429), brown liquid, 99.3% 1) The LD50 dose was estimated to be 976 mg/kg bw GLP statement: yes According to OECD 475: yes				

Results

treatment	*6-h sampling*				*24-h sampling*				*48-h sampling*			
	male		*female*		*male*		*female*		*male*		*female*	
[mg/kg]	*MI %*	*Cells with Aberr.*	*MI %*	*Cells with Aberr.*	*MI %*	*Cells with Aberr.*	*MI %*	*Cells with Aberr.*	*MI %*	*Cells with Aberr.*	*MI %*	*Cells with Aberr.*
0	4.6	0	2.4	0	2.9	1	3.6	0	2.3	0	3.7	2
175	-	-	-	-	2.6	2	2.3	0	-	-	-	-
350	-	-	-	-	3.6	3	4.6	0	-	-	-	-
700	2.3	1	3.2	1	1.9	1	3.0	4	2.6	0	1.3	0
pos. c.	-	-	-	-	-	55	-	63	-	-	-	-

- = not evaluated

Acceptability

The study was performed in accordance with OECD 475 of 1997.

Conclusions

Etridiazole is not clastogenic in this *in vivo* Chromosome aberration study.

STUDY 3

Study design and results

Type of study: *in vivo-in vitro* rat hepatocyte UDS test

Indicator cells	Endpoint	Result	Dose range	Reference
rat, male Sprague-Dawley 5/dose (vehicle control, low and mid dose), 7/dose (high dose)	Unscheduled DNA synthesis (liver hepatocytes)	-	DRF: 100, 500, 1000 and 1500 mg/kg bw [1)] Main study: 250, 500 and 1000 mg/kg bw [2)] Vehicle: corn oil Negative and positive controls included	San, R. H. C., Sly, J. E., 1995

Test substance: Terrazole (Etridiazole), batch nr. 306S052, reddish-brown liquid, purity 98.7%.
1) Toxicity observed at dose level: 500 mg/kg bw and above
2) Toxicity observed at dose level: 500 and 1000 mg/kg bw only for the 12-16 h harvest group
GLP statement: yes
According to OECD 486: yes

Results

dose	*1-3 hours after dosing*	*12-16 hours after dosing*
mg/kg	*Mean net grains per nucleus ± SD .*	*Mean net grains per nucleus ± SD*
0	-2.6 ± 0.2	-2.3 ± 0.1
250	-2.5 ± 0.2	-2.2 ± 0.1
500	-2.5 ± 0.3	-2.4 ± 0.2
1000	-2.5 ± 0.2	-2.6 ± 0.2
PC	8.7 ± 1.7	9.4 ± 0.8

Acceptability

The study fulfils the requirements of the recent OECD 486 guideline of 1997.

Conclusions

Etridiazole is not genotoxic in the DNA-repair assay

STUDY 4

Study design and results

Type of study: *in vivo-in vitro* rat replicative DNA synthesis (RDS)

Indicator cells	Endpoint	Result	Dose range	Reference
rat, male F344/DuCrJ 4/dose (vehicle control, ½ MTD and MTD dose),	replicative DNA synthesis (liver hepatocytes)	+	DRF: 500, 1000 and 2000 mg/kg bw [1)] Main study: 250 and 500 mg/kg bw [2)] Sampling times: 24, 39 and 48 hours Vehicle: corn oil Negative controls included	Miyagawa, M., 1995
Test substance: Etridiazole, batch nr. 306S052, purity 98.7%. 1) The MTD which produced no mortality was set at 500 mg/kg bw 2) No toxicity was observed in 2 or 3 days after dosing GLP statement: no According to OECD 486: no				

Results

dose		
mg/kg	*Time after dosing*	*RDS incidence ± SD (%).*
0	0	0.65 ± 0.19
250	24	5.38 ± 2.95
250	39	3.35 ± 2.25
250	48	1.25 ± 0.59
500	24	0.90 ± 0.55
500	39	2.49 ± 0.88
500	48	0.60 ± 0.38

Acceptability

There is no OECD 486 guideline of this study. However Etridiazole showed positive responses in the number of cells with labelled hepatocytes.

Conclusions

Etridiazole is positive in the *in vivo-in vitro* replicative DNA synthesis test

B.6.4.3 Summary

The results from the *in vitro* and *in vivo* genotoxicity studies are summarised in table 6.4.3.1 and 6.4.3.2, respectively.

Table 6.4.3.1 *In vitro* genotoxicity studies

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
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-B, liquid, red-orange, 99.0%	Chinese hamster ovary cell line CHO	gene mutations (HPRT)	-	-	Loveday, K.S., Gorodecki, J., 1981
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.

Table 6.4.3.2 *In vivo* genotoxicity studies

Test substance	Type of study		Result	Reference
	Species	Endpoint		
Terrazole technical, Batch CM-8561 S045017, liquid, min. 95%	mouse, NMRI, KFM	Micronuclei (bone marrow)	-	Banduhn, N., 1985
Etridiazole (Technical), Batch 206S048 (SI 3429), brown liquid, 99.3%	rat, Sprague-Dawley	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 and TA1537, both with and without metabolic activation. Etridiazole was positive in a chromosome aberration test with Chinese hamster ovary cell line CHO. However these results were obtained at very toxic dose levels and the results could not be compared to the historical control data, therefore this study was not acceptable. Etridiazole was positive in two SCE studies (one study was considered not acceptable). In both studies statistically significant increases were observed, however if the results are judged for biological relevancy these increases are considered not relevant, since no more than a two-fold increase has been observed. Etridiazole was negative in a gene mutation test using CHO hamster cells. In addition, etridiazole was negative in an *in vivo/in vitro* DNA repair study using male rat hepatocytes, etridiazole was also negative in an *in vivo* mouse micronucleus test and an *in vivo* rat chromosome aberration test. Etridiazole was positive an *in vivo-in vitro* replicative DNA synthesis test.

Based on the *in vivo-in vitro* replicative DNA synthesis test in combination with the results of the studies summarized at the mechanistic data (B.6.8.2) it can be concluded that etridiazole possesses promotor activity, with a threshold level.
Based on the three well performed *in vivo* studies, in which etridiazole showed negative responses, etridiazole is considered to be non-genotoxic *in vivo*.

B.6.5 LONG-TERM TOXICITY AND CARCINOGENICITY STUDIES (ANNEX IIA 5.5)

B.6.5.1 Chronic toxicity and carcinogenicity

STUDY 1

Characteristics

reference	:	Trutter, 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):									

Dose (mg/kg food)	**0**		**100**		**640**		**1280**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
-hepatocellular adenoma	1 (2%)	2 (4%)	0	1 (2%)	0	2 (4%)	**8 (16%)**	**12 (24%)**	
-hepatocellular carcinoma	1 (2%)	0	1 (2%)	0	2 (4%)	0	1 (2%)	**12 (24%)**	
-cholangiocarcinoma	0	0	**1 (2%)**	0	0	0	**1 (2%)**	**11 (22%)**	
Thyroid:									
-follicular cell adenoma	6/50 (12%)	0/50	6/48 (12.5%)	1/31 (3%)	7/49 (14%)	0/25	**14/49 (29%)**	**4/50 (8%)**	
-follicular cell carcinoma	0/50	1/50 (2%)	1/48 (2%)	1/31 (3%)	**6/49 (12%)**	0/25	**4/49 (8%)**	1/50 (2%)	
-follicular cell adenoma/carcinoma	6/50 (12%)	1/50 (2%)	7/48 (15%)	2/31 (6%)	**13/49 (27%)**	0/25	**18/49 (37%)**	**5/50 (10%)**	m
Testis (n=50):									
-interstitial cell tumors	0	-	4 (8%)	-	4 (8%)	-	**10 (20%)**	-	
Kidney (n=50):									
-Tubule cell adenoma	0	0	**1 (2%)**	0	0	0	**1 (2%)**	0	
-Tubule cell carcinoma	0	0	0	0	**4 (8%)**	0	**1 (2%)**	0	
Mammary gland (n=50) fibroadenoma		9 (18%)		18 (36%)		17 (34%)		23 (46%)	
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

Acceptability

The study was predominantly performed in compliance with OECD guideline 453, however, no clinical biochemistry and urinalysis were performed.

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 weight increases 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:

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).

See also B.6.8.2.2 for a further discussion about the oncogenic effects observed in this study and the possible underlying mechanisms.

STUDY 2

Characteristics

reference	:	Goldenthal, 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.

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									

Dose (mg/kg food)	0		50		900/1300		1800/2000/1600		dr
	m	f	m	f	m	f	m	f	
macroscopy (n=50)									
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
microscopy (n=50) *neoplastic lesions*									
Liver:									
-hepatocellular adenoma	4	0	3	0	**12**	**5**	**11**	**11**	f
-hepatocellular carcinoma	0	0	0	0	**4**	0	0	**3**	
microscopy *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
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

Acceptability

The study is considered acceptable. However, 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.

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 (Borzelleca, 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 (Hayashi, 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 (Erker, 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.

B.6.5.2 Summary

The results of the chronic toxicity and carcinogenicity studies are summarised in tables 6.5.2.1.

Table 6.5.2.1 Chronic toxicity and carcinogenicity studies with etridiazole

Duration	Species	Route	NOAEL mg/kg bw/day	LOAEL mg/kg bw/day	Critical effects	Reference
104-	rat	Oral	-	5	Increased incidence of tubular cell	Trutter, 1988

weeks					karyomegaly in kidney.	
79-weeks	mouse	Oral	7.5	184.7	Reduced survival rate, hepatocellular adenoma/carcinoma, increased liver weight and reduced kidney weight, histopathological changes in liver, kidney, spleen, and heart.	Goldenthal, 2004

In a 2-year carcinogenicity study in rats, administration of etridiazole (dietary administration of 0, 100, 640 and 1280 mg/kg food) resulted in reduced body weight and food consumption at 640 and 1280 mg/kg food. Treatment at 640 and 1280 mg/kg food resulted, in females, in a mild increase in leukocyte count, with increased neutrophils and decreased lymphocytes, and in a mild decrease in erythrocyte count and haematocrit. At post mortem necropsy, abnormalities were noted in liver and liver weights were increased at 640 and 1280 mg/kg food. Neoplastic lesions were observed in liver (hepatocellular adenoma and/or carcinoma at 1280 mg/kg food and cholangiosarcoma at 1280 mg/kg food), thyroid (follicular adenoma and/or carcinoma at 640 and 1280 mg/kg food, testis (interstitial cell tumours at 1280 mg/kg food) and kidney (tubular cell tumours at 640 ppm). A variety of non-neoplastic lesions were noted in liver (at 640 and 1280 mg/kg food) and kidney (at all dose levels).
Based on the non-neoplastic lesions 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, kidney and testis.

In a 79-week carcinogenicity study in mice, etridiazole was administered via the diet at 0, 50, 900/1300 and 1800/2000/1600 mg/kg food. 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. Treatment at 900/1300 and 1800/2000/1600 mg/kg food resulted in reduced survival. 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. High dose males had lower body weights than controls from week 3 of treatment onwards. In addition, mid and high dose males, and high dose females showed lower food consumption than controls. Liver weights were increased, kidney and uterus weights reduced in mid and high dose groups. At post-mortem necropsy, abnormalities were noted in liver, kidney and spleen of mid and high dose groups.
Histopathological findings were recorded in mid and high dose groups and comprised findings in liver, kidneys and spleen. Neoplastic lesions (hepatocellular adenoma and carcinoma) were noted in liver of mid and high dose groups. A variety of non-neoplastic lesions were also noted in mid and high dose animals. 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, at dose levels clearly exceeding the MTD.

See also B.6.8.2.2 for a further discussion about the oncogenic effects observed in the carcinogenicity studies and the possible underlying mechanisms.

B.6.6 REPRODUCTIVE TOXICITY (ANNEX IIA 5.6)

B.6.6.1 Reproductive toxicity

STUDY 1

Characteristics

reference	:	Turck, 2003	exposure	:	Diet starting 10 weeks before mating (F0) or at weaning (F1), throughout mating, gestation and lactation periods until scheduled necropsy
type of study	:	2-generation reproduction study	doses	:	0, 80, 320 and 800 mg/kg food for males and 0, 80, 320 and 640 mg/kg food for females[1]
year of execution	:	2002	vehicle	:	None
test substance	:	Terrazole® tech., Lot no. IRC012320, purity 97.9%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 416
species	:	Rats, Sprague-Dawley [Crl:CD®(SD)IGS BR	acceptability	:	Acceptable
group size	:	30 males and 40 females/dose	**NOAEL**par	:	5.3 mg/kg bw/day
			NOAELdev	:	5.3 mg/kg bw/day
			reproductive effects	:	≥ 53.3 mg/kg bw/day for males and ≥ 42.7 mg/kg bw/day for females

[1] equivalent to 0, 5.3, 21.3 and 53.3 mg/kg bw/day in males and 0, 5.3, 21.3 and 42.7 mg/kg bw/day in females, using a default food conversion factor of 15; during mating males in the high dose group received 640 mg/kg food

Study design

The study was performed in accordance with OECD guideline 416 (2001). In addition, blood samples were taken from 20 pregnant females on day 20 of gestation, 20-day old foetuses from 10 litters/group, all 4-day old culled F1 pups, and one F1 weanling/sex/litter not selected for the next parental generation. Blood samples were analysed for T_3, T_4 and TSH.

Results

The results of the study are summarized in table 6.6.1.1.

Table 6.6.1.1.

Dose (mg/kg food)	0		80		320		800/640		dr
	m	f	m	f	m	f	m	f	
F_0 animals									
Mortality	no treatment-related findings								
Clinical signs	no treatment-related findings								
Body weight gain							dc	dc	
Food consumption							dc	dc	
Mating/fertility/gestation	no treatment-related findings								
Oestrus cycle	no treatment-related findings								
Sperm evaluation	no treatment-related findings								
Organ weight									
- liver							i^a,ic^r	ic^r	
- kidneys						ic^r	ic^r	$ic^{a,r}$	
- thyroid							ic^r		
- testes					ic^r		ic^r		
- adrenal								dc^a	
- pituitary					dc^a		dc^a		

Dose (mg/kg food)	0		80		320		800/640		dr
	m	f	m	f	m	f	m	f	
Pathology									
Clinical									
day 20 gestation (females (n=20) and foetuses (10 litters/group))	no treatment-related findings								
terminal:									
- T_3					ic	dc	ic	dc	
- T_4								dc	
- TSH								d	
Macroscopy	no treatment-related findings								
Microscopy									
Kidney:									
- chronic progress. nephropathy	0/3	0/0	0/0	0/0	0/0	0/0	7/8	0/1	
F_1 pups									
Litter size	no treatment-related findings								
Post implantation loss (%)	no treatment-related findings								
Live birth index	no treatment-related findings								
Viability index	no treatment-related findings								
Lactation index	no treatment-related findings								
Sex ratio	no treatment-related findings								
Clinical signs	no treatment-related findings								
Body weight									
day 4 post partum							dc	dc	
day 7 post partum					dc	dc	dc	dc	
days 14 post partum					dc	dc	dc	dc	
day 21 post partum					dc	dc	dc	dc	
Sexual maturation									
- vaginal opening	32.0		32.5		32.5		33.3*		
- preputial separation (days)	no treatment-related findings								
Organ weights									
- brain							ic^r	dc^a, ic^r	
- spleen							dc^a	$dc^{a,r}$	
- thymus								dc^a	
- thyroid					ic^r	ic^a	ic^r	ic^a	
Pathology									
Clinical									
- F_1 foetuses day 20 of gestation	no treatment-related findings								
- T_3 on lactation day 4							dc	d	
- T_3 on lactation day 21						dc	dc	dc	
Macroscopy	no treatment-related findings								
F_1 animals									
Mortality	no treatment-related findings								
Clinical signs (n = 30)	no treatment-related findings								
Body weight gain									
- premating period					dc	dc	dc	dc	m/f
Food consumption									
- premating period					d	d	dc	dc	m/f
- gestation period								dc	
- lactation period								dc	
Mating/fertility/gestation									
- copulatory interval		2.6		3.1		3.9*		2.6	

Dose (mg/kg food)	0		80		320		800/640		dr
	m	f	m	f	m	f	m	f	
Oestrus cycle	no treatment-related findings								
Sperm evaluation	no treatment-related findings								
Organ weight									
- liver				ic^{r}		ic^{r}	ic^{a,r}	ic^{a,r}	f
- pituitary							dc^{a}		
- brain				dc^{a}		dc^{a}	ic^{r}	dc^{a}, ic^{r}	
- adrenals							ic^{r}		
- epididymes							ic^{r}		
- kidney					ic^{r}	dc^{a}	ic^{r}	dc^{a}	
- seminal vesicles					ic^{a,r}		ic^{r}		
- ovaries								ic^{r}	
Pathology									
Macroscopy	no treatment-related findings								
Microscopy	no treatment-related findings								
F_2 pups									
Litter size	no treatment-related findings								
Post implantation loss	no treatment-related findings								
Live birth index	no treatment-related findings								
Viability index	no treatment-related findings								
Lactation index	no treatment-related findings								
Sex ratio	no treatment-related findings								
Body weight							dc	dc	
Organ weights									
- brain							dc^{a}, ic^{r}	dc^{a}, ic^{r}	
- spleen							dc^{a}	dc^{a}	
- thymus							dc^{a,r}	dc^{a}	
- thyroid							ic^{r}	dc^{a}	
- pituitary									
Pathology									
Macroscopy	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
* statistically significant when compared to controls (p =< 0.05)

Acceptability

The study is considered acceptable. In the absence of histopathology of the kidneys in F_0 animals at 80 and 320 mg/kg food kidney weight changes at 320 mg/kg food in F_0 animals are considered toxicologically relevant.

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.

B.6.6.2 Teratogenicity studies

STUDY 1

Characteristics

reference	:	Wahlberg, 1982	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		
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

Acceptability

The study was considered acceptable. 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.

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.

STUDY 2

Characteristics

reference	:	Knickerbocker, 1979	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. 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*	

Dose (mg/kg bw/day)	0	Positive control	1.7	5	15	45	dr
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

Acceptability

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). The study is considered acceptable, as in the mid dose group no developmental effects were noted, and based on the results of the teratology and reproduction study in rats no external changes are to be expected.

Potential critical effects of the test substance (liver, kidneys, thyroid) were not studied. Consequently, the observed NOAEL for maternal effects might not be accurate.

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 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. 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.

B.6.6.3 Summary

The results of reproduction toxicity and teratogenicity studies are summarised in table 6.6.3.1.

Table 6.6.3.1 Summary of reproduction toxicity and teratogenicity studies with etridiazole

Species		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical effects	Reference
2-Generation reproduction toxicity					
rat	parental	5.3	21.3	Decreased body weight and food consumption (F_1 –generation), changes in T_3 and changes in weight of seminal vesicles, pituitary, kidneys and liver.	Turck, 2003
	developmental	5.3	21.3	Decreased body weight, changes in thyroid weight and decreased T_3 concentration (F_0 - offspring).	
	reproduction	M: ≥ 53.3; F: ≥ 42.7	-	No effects.	
Teratogenicity					
rat	maternal	30	75	Mortality, clinical signs, decreased body weight.	Wahlberg, 1982
	developmental	30	75	Decreased foetal weight, anasarca, retarded ossification of various bones.	
	teratogenicity	≥ 75	-	No effects.	
rabbit	maternal	15	45	Mortality, decreased body weight.	Knicker-bocker, 1979
	developmental	15	45	Decreased foetal weight, reduction of live foetuses per dam, increase of dams with resorptions	
	teratogenicity	15	45	Missing sternebrae, tail defects, underdeveloped hind limbs, crossed hind legs, and open eyes.	

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.

B.6.7 NEUROTOXICITY / DELAYED NEUROTOXICITY (ANNEX IIA 5.7)

B.6.7.1 Acute oral neurotoxicity and delayed neurotoxicity

No studies were submitted and are not considered necessary.

B.6.7.2 Semichronic oral 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. Therefore, studies are not considered necessary.

B.6.8 FURTHER TOXICOLOGICAL STUDIES (ANNEX IIA 5.8)

B.6.8.1 Toxicological data on metabolites

B.6.8.1.1 Toxicity studies on metabolites

Studies with the metabolite 5-ethoxy-1,2,4-thiadiazol-3-carboxylic acid (also called etridiazole acid) were submitted. This metabolite is found in soil and water.

STUDY 1

Characteristics

reference	:	Cerven, 2002	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	2000 mg/kg bw
year of execution	:	2001/02	vehicle	:	distilled water
test substance	:	5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (metabolite of etridiazole) Lot no. AGD1785-129, purity 98.8%	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 401
species	:	Rat, Wistar	acceptability	:	Acceptable
group size	:	5/sex	**LD_{50}**	:	> 2000 mg /kg bw (both sexes)

Study design

The study was performed in accordance with OECD 401 (1987).

Results

Mortality: No mortality occurred.

Symptoms of toxicity: all animals appeared normal during the observation period

Body weight: No treatment related findings were noted in the animals.

Pathology: One male had slightly mottled kidneys. Other animals were all without abnormalities.

Acceptability

The study is considered acceptable.

Conclusions

The oral LD_{50} of 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid in rats was found to be greater than 2000 mg/kg bw in males and females.

STUDY 2

Characteristics

Reference	:	Goldenthal, 2004	exposure	:	13 weeks, diet; 4 weeks recovery
Type of study	:	13-week oral toxicity study	dose	:	0, 50, 600 and 1250 mg/kg food (nominal)[1]
year of execution	:	2003	vehicle	:	none
test substance	:	5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid (metabolite of Terrazole) Lot no. AC-1834-CMP-118 and AC-1834-CMP-149, purity 99.6%	GLP statement	:	yes
route	:	oral	guideline	:	predominantly in accordance with OECD 408 (1998)
species	:	rat, Crl:CD®(SD)IGS BR	acceptability	:	acceptable
group size	:	10/sex/dose (+5/sex/dose as recovery group)	**NOAEL**	:	38.9 mg/kg bw/day for males and 47.5 mg/kg bw/day for females

[1]equal to 0, 3.2, 38.9 and 79.2 mg/kg bw/d for males and 0, 3.8, 47.5 and 94.5 mg/kg bw/d for females

Study design

The study was generally in compliance with OECD 408 (1998). However, functional observations were not performed. Blood was collected at necropsy. Histopathology was not performed after recovery due to the absence of effects at the end of the exposure period.

Results

The results are summarised in table 6.8.1.1a and 6.81.1b

Table 6.8.1.1a. Results after 13 weeks.

Dose (mg/kg food)	0		50		600		1250		dr
	m	f	m	f	m	f	m	f	
Mortality	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	
Clinical signs	no treatment-related findings								
Body weight gain							d	dc	
Food consumption							d	d	
Ophthalmoscopy	no treatment-related findings								
Haematology									
- leukocyte count:								dc	
- lymphocytes								dc	
- eosinophils								dc	
- large unstained cells								dc	
Clin. Chemistry	no treatment-related findings								
Organ weights									
- prostate							dc[a,r]		
Pathology									
macroscopy	no treatment-related findings								
microscopy									
- prostate:minimal subacute inflammation	5/10						8/9		

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

Table 6.8.1.1b. Results after 4-week recovery period.

Dose (mg/kg food)	0		50		600		1250		dr
	m	f	m	f	m	f	m	f	
Mortality	no treatment-related findings								
Clinical signs	no treatment-related findings								
Body weight gain								d	
Food consumption	no treatment-related findings								
Ophthalmoscopy	no treatment-related findings								
Haematology - MCH								dc	
Clin. Chemistry	no treatment-related findings								
Organ weights - liver								i^a,ic^r	
Pathology									
macroscopy	no treatment-related findings								
microscopy	not performed								

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

Acceptability

It should be noted that no histopathology was performed in the 50 and 600 mg/kg food groups. As no further changes were noted at both dose levels, and the findings in the prostate were not considered to be adverse, it is concluded that this omission does not affect the acceptability of the study. The study is considered acceptable.

Conclusions

Rats were administered 0, 50, 600 or 1250 mg/kg food of 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid for 13 weeks. One male at 1250 mg/kg food was found dead on day 44. The animal was in a generally bad condition and red material around the eye, lacrimation, swelling of the nose/muzzle, and black discoloration of the fur in the anogenital region were noted. Death was established to be caused by inflammation/septicemia of unknown origin and was not considered to be test substance related. No treatment-related clinical signs were observed in the other animals during the 90-day exposure period or recovery period. Body weight gain was decreased at 1250 mg/kg food in males (92% of control) and females (84% of control) and recovered during the recovery phase for males and partly for females (90% of control). Food consumption was decreased (sporadically significant) at 1250 mg/kg food in males (93% of control) and females (95% of control). Food consumption was restored in the recovery group.

Leukocyte count was decreased at 1250 mg/kg food in females after treatment (67% of control) and was restored during the recovery phase. The decreased leukocyte count consisted of a decrease of lymphocytes, eosinophils and large unstained cells. MCH was slightly, but significantly decreased at

1250 mg/kg food in females at the end of the recovery period only (95% of control). Since MCH was not decreased at the end of the treatment period, the decrease at the end of the recovery period is not considered to be toxicologically relevant.

Clinical chemistry values were all within normal values at the end of the exposure period and after recovery.

Absolute and relative prostate weight was significantly decreased in males at 1250 mg/kg food (67-72% of control) at the end of the exposure period. After recovery, absolute and relative prostate weight was increased (111-119% of control). Relative liver weights were slightly, but significantly increased at 1250 mg/kg food in females at the end of the recovery period only (112% of control). This change was not considered to be toxicologically relevant, since no change was observed at the end of the treatment-period.

No macroscopic abnormalities were observed. Histopathological examination showed a slight increased incidence of minimal subacute inflammation of the prostate in males at 1250 mg/kg food compared to the control group.

The NOAEL is set at 600 mg/kg food (equal to 38.9 mg/kg bw for males and 47.5 mg/kg bw for females) based on reduced body weights and changes in prostate weight.

STUDY 3

Study design and results

Type of study: Ames test, plate incorporation method,

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: *S. typh.* TA 98 TA 100 TA 1535 TA 1537 B. *E.coli* WP2uvrA	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	Arochlor 1254	DRF test: 6.7, 10, 33, 100, 333, 667, 1000, 3333, 5000 µg/plate Mutation test: 100, 333, 1000, 3333 and 5000 µg/plate Solvent: DMSO Positive and negative control: Included	Wagner, V.O., Klug, M.L. 2002
Test substance: 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid, batch nr. AGD1785-129, pale yellow powder, 98.8% No toxicity was observed No precipitation was observed GLP statement: yes According to OECD 471: yes							

Acceptability

The study fulfils the requirements of the recent OECD 471 guideline of 1997.

Conclusions

5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid did not induce point mutations in *S. Typhimurium* and *E.coli*.

STUDY 4

Study design and results

Type of study: mammalian cells *in vitro*, cytogenetic assay,

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster ovary (CHO) cells	Chromosome aberration	+	-	rat liver	Arochlor 1254	DRF test: nine concentration ranging from 0.1742 to 1742 µg/ml Main test 1: (- and +S9): 435.5, 871 and 1742 µg/ml Main test 2: (-S9): 0.004, 0.005 and 0.006% Vehicle: DMSO Negative and positive controls included	Gudi, R., Brown, C. 2002
Test substance: 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid, batch nr. AGD1785-129, pale yellow powder, 98.8% No toxicity was observed up to and including 1742 µg/ml (10 mM) No precipitation was observed precipitation to this study GLP statement: yes According to OECD 473: yes							

Results CA

See below. Values printed bold represented a statistically significant difference from the control.

dose µg/ml	*-S9*		*-S9*		*+S9*	
	4 h treatment/ 16 hr recovery		*20 h continuous treatment*		*4 h treatment/ 16 hr recovery*	
	Mean mitotic index %	*Aberrations per cell (mean)*	*mitotic index %*	*Average aberrations per cell.*	*mitotic index %*	*Average aberrations per cell.*
0	8.7	0.005	6.8	0.000	8.0	0.030
435.5.	7.8	0.010	7.0	0.005	7.3	0.040
871	6.9	0.005	6.4	**0.035** (1)	7.3	0.025
1742	6.6	0.005	6.8	**0.110**	7.2	0.005
pos. c.	6.8	**0.205**	5.2	**0.195**	8.0	**1.400**

(1) This number of aberrant cells of the treatment group 871 µg/ml was within the historical solvent control range

Acceptability

The chromosome aberration study fulfils the requirements of the recent OECD 473 guideline of 1997.

Conclusions

5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid induced statistically significant increases in the non-activated 20 hours exposure group. 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid was found to be negative for the induction of structural chromosome aberrations in CHO cells in the non-activated and S9 activated 4 hour exposure groups.

STUDY 5

Study design and results

Type of study: mouse micronucleus test

Indicator cells	Endpoint	Result	Dose range	Reference
mouse, ICR Pilot study 2/male/low doses 5/sex)/ highest dose Toxicity assay 5/sex/dose Main study 5/sex/ low and mid dose 15/sex/ low and mid dose	micronuclei (bone marrow)	-	Pilot study: 1, 10, 100, 1000 and 2000 mg/kg bw [1)] Toxicity assay: 200, 400, 600 and 800 mg/kg bw [2)] Main test: 37.5, 75 and 150 mg/kg bw [3)] Sampling times: 24 and 48 hours Solvent control Corn oil Negative and positive controls included Dose route: ip	Gudi, R., Krsmanovic, L. 2002
Test substance: 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid, batch nr. AGD1785-129, pale yellow powder, 98.8% Toxicity 1) In all dose levels clinical signs were observed and in the two highest dose levels all animals died 2) At the dose level of 200 mg/kg 1 out of 5 animals died (male and female), at the dose level of 400 mg/kg 4 out of 5 animals died (male) and 2 out of 5 animals died (female), at the dose level of 600 and 800 mg/kg all animals died (male and female) 3) In all dose levels clinical signs were observed (piloerection and lethargy). No animal died. GLP statement: yes According to OECD 474: yes				

Acceptability

The study fulfils the requirements of the more recent OECD 474 guideline of 1997.

Conclusions

5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid is not mutagenic in this *in vivo* mouse micronucleus assay.

B.6.8.1.2 Summary

An acute oral toxicity study, a semichronic oral toxicity study and genotoxicity studies were performed with one water and soil metabolite: 5-ethoxy-1,2,4-thiadiazol-3-carboxylic acid.

The acute oral LD50 of 5-ethoxy-1,2,4-thiadiazol-3-carboxylic acid is found to be greater than 2000 mg/kg bw.

In a 13-week oral toxicity study with rats (0, 50, 600 and 1250 mg/kg food), a NOAEL of 600 mg/kg food (equal to 38.9 mg/kg bw for males and 47.5 mg/kg bw for females) is established, based on reduced body weights and changes in prostate weight. Histopathological examination showed a slight increased incidence of minimal subacute inflammation of the prostate in males at 1250 mg/kg food compared to the control group.

5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid did not induce point mutations in S. typhimurium tester strains TA98, TA100, TA1535 and TA1537, both with and without metabolic activation. 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid was positive in a chromosome aberration test with Chinese hamster ovary cell line CHO in the non-activated 20 hours exposure group. However, 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid was negative in an *in vivo* mouse micronucleus test.

In conclusion, 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid is considered to be non genotoxic.

Conclusion

Metabolite 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid showed to be less toxic than etridiazole for acute and semichronic toxicity. Both etridiazole and 5-ethoxy-1,2,4-thiadiazole-3-carboxylic acid are considered non-genotoxic.

B.6.8.1.3 Further toxicity data on metabolites

In Volume 3, Annex B, B.8.1.1 and B.8.4.3 it is concluded that also dichloro-etridiazole may volatilise from soil and water, although at lower quantities compared to etridiazole. Therefore, exposure of the worker to dichloro-etridiazole during re-entry activities cannot be excluded. No toxicological data are available for this metabolite. However, in the proposed metabolic pathway (see Fig. 6.1.2.1) it is assumed that the first step in the rat metabolism is a conversion of etridiazole to dichloro-etridiazole.

The notifier submitted modeling data for the metabolites dichloro-etridiazole, 3-hydroxymethyl etridiazole and 5-hydroxyethoxy etridiazole acid, using Derek for Windows version 9.0.0, a program to perform (Q)SAR analyses (Freeman, 2006c). Additional Derek modeling on etridiazole and etridiazole acid was conducted for comparison. Additional modeling on 5-hydroxyethoxy etridiazole acid was conducted using Multicase MC Web from Multicase Incorporated Version 1.8. Multicase predicts various toxicological endpoints on the basis of structural fragments, thus providing a 'chemical' explanation to the observed property. It assumes that the presence of molecular fragments previously found in a number of active compounds is indicative of potential activity and a reasonable basis to assess the activity of new molecules. The program has a high predictive value and shows 95-100% concordance with experimental results for compounds that were part of the database used to train the program and 70-85% when they were not.

Results

Derek for Windows modeled results in both bacterium and mammalian species. The superendpoints, or toxicological effects, examined included carcinogenicity, mutagenicity, irritation, respiratory sensitization, skin sensitization, thyroid toxicity and miscellaneous endpoints. Miscellaneous endpoints consisted of alpha-2mu-globulin nephropathy, anaphylaxis, antichlolinesterase activity, bladder urothelial hyperplasia, cerebral oedema, chloracne, cumulative effect on white blood cell count and immunology, cyanide-type effects, developmental toxicity, hepatotoxicity, oestrogenicity, peroxisome proliferation, phospholipidosis, phototoxicity, pulmonary toxicity, teratogenicity, testicular toxicity and uncoupler of oxidative phosphorylation.

For the dichloro-etridiazole metabolite, negative results (no structural alerts for toxicity) were found for the following toxicological endpoints: carcinogenicity, irritation, respiratory sensitization, thyroid toxicity and miscellaneous endpoints. Positive results for mutagenicity are presented below. For comparison, also the results from the Derek analysis of etridiazole and etridiazole acid are presented.

For 5-hydroxy-ethoxyetridiazole acid, negative results (no structural alerts for toxicity) were found for the following toxicological endpoints: carcinogenicity, irritation, respiratory sensitization, skin

sensitization, thyroid toxicity and miscellaneous endpoints. Positive results for mutagenicity are presented below.

For 3-hydroxymethyl etridiazole, negative results were found for all toxicological endpoints examined: carcinogenicity, genotoxicity, irritation, respiratory sensitization, skin sensitization, thyroid toxicity and miscellaneous endpoints.

Positive Derek results

Compound	Derek result
dichloro-etridiazole	plausible for *in vitro* bacterial mutagenicity
	open for mammalian mutagenicity
5-hydroxy-ethoxyetridiazole acid	plausible for *in vitro* bacterial mutagenicity
	plausible for *in vitro* mammalian mutagenicity
3-hydroxymethyl etridiazole	negative, no structural alerts
etridiazole	plausible for bacterial mutagenicity
	open for mammalian mutagenicity
etridiazole acid	negative, no structural alerts

Plausible: The weight of evidence supports the proposition
Open: There is no evidence that supports or opposes the proposition.

Discussion and interpretation

In examining all the data available, it is unlikely that the metabolites dichloro-etridiazole, 5-hydroxy-ethoxyetridiazole acid and 3-hydroxymethyl etridiazole pose a hazard to human health. Examining the results as a total toxicological experimental data package would be examined, each metabolite would not be considered to be a human hazard. The positive mutagenicity results would be analyzed in conjuction with other negative mutagenicity results, carcinogenicity results and developmental and teratogenic test results; the result of the data analysis being that the metabolites are not hazardous to human health.

In examining the mutagenicity results, it is unlikely that the metabolites of etridiazole are mutagenic. Etridiazole has undergone both *in vitro* and *in vivo* mutagenicity testing with a determination of no genotoxic risk *in vivo*. Therefore, the positive result in the Derek model for etridiazole is negated by testing results. The dichloro-etridiazole metabolite of etridiazole is the first step in the dechlorination metabolic pathway. The mechanism of mutagenicity of both etridiazole and dichloro-etridiazole is based on the presence of the halide atoms. However, etridiazole is not mutagenic experimentally and hence its dechlorinated metabolite dichloro-etridiazole is not expected to be mutagenic either. This is supported by the further dechlorination of the dichloro derivative to the corresponding acid metabolite, etridiazole acid, which is non-mutagenic experimentally and considered to be non-mutagenic by the Derek estimations. Negative results with etridiazole acid (also called 5-ethoxy-1,2,4-thiadiazol-3-carboxylic acid) include Ames assay and *in vivo* mouse micronucleus test (see B.6.8.1.1).

The second step in the metabolic process involves the metabolism of the dichloro-etridiazole metabolite to etridiazole acid and 3-hydroxymethyl etridiazole. Both of these metabolites are considered non-mutagenic by Derek modeling.

The 5-hydroxy-ethoxyetridiazole acid is formed from a detoxicification reaction of etridiazole acid; rendering etridiazole acid more water soluble, readily excretable, and capable of undergoing additional detoxification conjugation reactions. The detoxification steps provide a pathway for the compound to be excreted possibly prior to any potential aldehyde formation. This is a typical detoxification pathway, and although the SAR structure is potentially mutagenic, the likelihood of mutagenic effects is small.

Additional modeling in Multicase for 5-hydroxyethoxy etridiazole acid was conducted for comparison. 5-hydroxyethoxy etridiazole acid gave a 21% chance of mutagenicity with this compound in bacteria (Ames test) and 88% chance for mammalian mutagenicity in the mouse lymphoma L5178Y cell line. Multicase did not identify the same biopheres or SAR alerts as Derek. No biophores or SAR alerts were found in common between the Multicase and Derek systems and in addition, Multicase considered the biophore 'CS-NH' deactivating in the Ames assay model. The biophore or SAR alert for mammalian mutagenicity differed between Multicase and Derek. Considering all this data, the weight of evidence suggests that 5-hydroxyethoxy etridiazole acid is not a genotoxic hazard.

In the Derek predictions, mutagenicity was the only toxicological endpoint affected. Furthermore, a positive result in mammalian mutagenicity alone is not a sufficient evidence of a toxicologically relevant effect or predictor of real life effects. No carcinogenicity, reproductive or developmental toxicity was predicted based on the metabolite structures.

Conclusion

The overall interpretation of the Derek modeling of the metabolites dichloro-etridiazole, 5-hydroxy-ethoxyetridiazole acid and 3-hydroxymethyl etridiazole, is negative for human health hazard.

B.6.8.2 Mechanistic data

B.6.8.2.1 Mechanistic studies

STUDY 1

Characteristics

reference	:	Tanaka, 1995	exposure	:	7, 14 or 28 days, diet
type of study	:	Promotor activity detection test	doses	:	0, 0, 640, 1280 mg/kg food[1] (see also study design)
year of execution	:	1994-1995	Vehicle	:	Not applicable
test substance	:	Terrazole (etridiazole), lot No. 401S002, purity 97.5%	GLP statement	:	No
route	:	oral	Guideline	:	No guideline available
species	:	male rats, Crj:CD	Acceptability	:	Acceptable
group size	:	12/group			

1 equal to 0, 0, 62 and 120 mg/kg body weight/day.

Study design

The study was designed to investigate whether etridiazole had promotor activity in liver-, thyroid- and testis tumour development (these types of tumours developed in a 2-year carcinogenicity study in rats with etridiazole at 640 or 1280 mg/kg food, Trutter 1988). This mechanistic study consisted of 4 groups, each containing 12 male, individually housed rats: a negative control group (diet only), a positive control group (known liver tumour promotor Phenobarbital, 1000 mg/kg food equal to 96 mg/kg bw/day), and two etridiazole treated groups (640 and 1280 mg/kg food). Four rats per group were treated for 7, 14 and 28 days.

Clinical signs, body weight and food intake were recorded during treatment. After 7, 14 and 28 days blood samples were taken and haematological and clinical biochemistry parameters (as included in routine repeated dose toxicity testing), thyroid function (TSH, T3, T4) and testis function (prolactin, LH, testosterone) were investigated. At these timepoints 4 rats/group were killed, macroscopic findings recorded and liver, testes, thyroid and pituitary gland were examined (organ weights, biochemical parameters, immunohistochemistry and histopathology (light microscopy and on liver also electron microscopy after 28 days of treatment). Biochemical parameters examined were metabolizing enzymes and connexin 32 protein in liver. Immunohistochemistry was performed to detect TSH, FSH, and LH in pituitary; T4 and thyroglobulin in thyroid; and connexin 32 in liver (latter staining only after 28 days of treatment).

Results

The results are summarized in Table 6.8.2.1.

Table 6.8.2.1

Etridiazole dose (mg/kg food)	**0**	**0**	**640**	**1280**	
Phenobarbital dose (mg/kg food)	**0**	**1000**	**0**	**0**	**dr**
Mortality	0	0	0	0	
Clinical signs		No treatment-related findings			
Body weight (gain)				dc	
Food consumption				dc	
Haematology, clinical biochemistry		No treatment-related findings			
Organ weights (after 28 days)					
-Liver		ic[ar]	ic[ar]	ic[ar]	m
-Thyroid				dc[ar]	
-Pituitary				dc[ar]	
Pathology (after 28 days)					
macroscopy		No treatment-related findings			
light microscopy					
Liver:					
-centrilobular hypertrophy	0/4	4/4	4/4	4/4	m
-centrilobular vacuolation	0/4	0/4	0/4	2/4	
Thyroid:					
-enlarged follicular cells	0/4	4/4	3/4	4/4	m
-decrease in follicular size	0/4	0/4	3/4	4/4	m
electron microscopy of liver					
-proliferation of SER		i	i	i	m
Immunohistochemistry					
Thyroid:					
-positive for T4	0/4	4/4	0/4	3/4	m
-positive for thyroglobulin	0/4	4/4	0/4	2/4	m
Liver:			Not		
-centrilobular conexxin 32		d	examined	d	
Drug metabolyzing enzymes in liver					
-P450, MCD, ECD, PCD		ic			
-P450, MCD, ECD				dc	
-UDP-GT		ic	ic	ic	
-GST (dcnb and/or cdnb)		ic	ic	ic	
-CYP2B1		ic		ic	
-CYP2C13		dc		dc	
-connexin 32 protein		dc		dc	

dr dose related (etridiazole)
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

Acceptability

The study was considered acceptable. However, it is noted that the test diets were not analysed for accuracy and homogeneity.

Conclusions

Treatment with the test substance at 1280 mg/kg food resulted in reduced body weights and food consumption. After 28 days, liver weights were increased in the low dose (relative weight was 113% of control), high dose (relative weight was 132% of control) and positive control group (145% of control). Furthermore, absolute thyroid and pituitary weight were reduced in the high dose group, thyroid weight was 67% of negative controls and pituitary weight was 78% of negative controls. No

relevant findings were noted in testes in this study. Microscopic examination of the thyroid showed a decreased size of follicles after etridiazole treatment for 28-days, correlating with the reduced thyroid weight; and enlarged follicular epithelial cells after both etridiazole as well as phenobarbital treatment. Immunohistochemistry showed that T_4 en thyroglobulin levels were higher in the positive control group than in the etridiazole treated groups, indicating stimulation of the thyroid after both types of treatment. This is probably secondary to liver changes: treatment with both xenobiotics induces liver enzymes that are also involved in breakdown of thyroid hormones. No other relevant findings were noted in thyroid. No significant changes were observed in any hormone levels, i.e., TSH, T_3, T_4, prolactin, LH or testosterone, in etridiazole treated animals.

Centrilobular hepatocyte hypertrophy was noted in both etridiazole groups and the positive control group after 28-days of treatment, which represented increased proliferation of the smooth endoplasmatic reticulum. This was considered to reflect increased induction of metabolizing enzymes. A discrepancy between the positive control group and etridiazole treated group(s) was recorded with regard to phase I metabolizing enzymes. Etridiazole treatment resulted in a decrease in CYP450, MCD and ECD, whereas phenobarbital resulted in an increase in these enzymes. CYP450 isoenzyme CYP2B1 was markedly increased after phenobarbital treatment and only slightly increased after etridiazole treatment. CYP2C13 showed a decrease in positive controls and was slightly increased after etridiazole treatment. These differences indicate etridiazole has a different biochemical profile than the known tumour promotor Phenobarbital. Furthermore, connexin 32 levels were studied in liver. Connexin 32 is a protein of the gap junction, involved in intercellular communication. A reduction in gap junctions lead to reduced intercellular communication, and is known to be related to (the promotion stage) of carcinogenesis. Etridiazole (as well as phenobarbital) treatment resulted in reduced connexin 32 in liver, as was demonstrated in this study, both biochemically and by immunohistochemistry. Etridiazole treatment reduced connexin 32 protein levels more (approximately 80% decrease) than phenobarbital treatment (approximately 40% decrease). This all suggests that etridiazole may act as a tumour promotor, comparable to phenobarbital, and may play an important role in the liver tumour development in the 2-year carcinogenicity study in rats. 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.Concerning the development of thyroid and testis tumors, this study did not provide informative results.

STUDY 2

Characteristics

reference	:	Tanaka, 1995	exposure	:	14 or 28 days, diet
type of study	:	Promotor activity detection test, additional low dose study	doses	:	0, 100, 200, 400 mg/kg food[1]
year of execution	:	1995	Vehicle	:	Not applicable
test substance	:	Terrazole (etridiazole), lot No. 504S039, purity 97.3%	GLP statement	:	No
route	:	oral	Guideline	:	No guideline available
species	:	male rats, Crj:CD	Acceptability	:	Acceptable
group size	:	8/group			

1 equal to 0, 9, 18 and 37 mg/kg body weight/day.

Study design

The study was designed to investigate whether etridiazole had promotor activity in liver-, thyroid- and testis tumour development (these types of tumours developed in a 2-year carcinogenicity study in rats with etridiazole at 640 or 1280 mg/kg food, Trutter 1988). In a previous mechanistic study (Tanaka, 1995) dose levels of etridiazole of 640 and 1280 mg/kg food were used and this study suggested etridiazole had promotor activity. The present mechanistic study was designed to find a threshold level for this promotor activity. The study consisted of 4 groups, each containing 8 male, individually housed rats: a negative control group (diet only), and three Etridiazole treated groups (100, 200 and 400 mg/kg food). Four rats per group were treated for 14 and 28 days.

Clinical signs, body weight and food intake were recorded during treatment. After 14 and 28 days blood samples were taken and haematological and clinical biochemistry parameters (as included in routine repeated dose toxicity testing) were evaluated. At both timepoints 4 rats/group were killed, macroscopic findings recorded and liver, testes, thyroid and pituitary gland were weighed and examined by light microscopy (H&E staining). Furthermore, drug metabolizing enzymes (e.g. CYP450, GST, and UDP-GT) and connexin 32 levels were determined in liver tissue.

Results

The results are summarized in Table 6.8.2.2.

Table 6.8.2.2

Dose (mg/kg food)	**0**	**100**	**200**	**400**
Mortality, clinical signs, food consumption, haematology, clinical biochemistry, organ weights, macroscopy	No treatment-related findings			
Body weight (gain)				d
After 28 days: **microscopy**				
Liver:				
-centrilobular hypertrophy	0/4	0/4	0/4	2/4
Thyroid:				
-enlarged follicular cells	0/4	0/4	1/4	2/4
-decrease in follicular size	0/4	0/4	1/4	1/4
Drug metabolyzing enzymes in liver				
-UDP-GT				I
-GST (dcnb)				I
Connexin 32 in liver				d

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

Acceptability

The study was considered acceptable. However, it is noted that the test diets were not analysed for accuracy and homogeneity.

Conclusions

Treatment with the test substance at 400 mg/kg food resulted in reduced body weights. Microscopic examination of the liver showed mild centrilobular hypertrophy in 2/4 males after 28 days of treatment. This finding correlated with increased induction of phase II metabolizing enzymes after 28 days.

Furthermore, connexin 32 levels were studied in liver. A reduction in this protein of the gap junction, involved in intercellular communication, is known to be related to (the promotion stage of) carcinogenesis. Etridiazole treatment at 400 mg/kg food for 28 days resulted in reduced connexin 32 in liver (approximately 80% of controls). In the thyroid enlarged follicular cells and a decrease in follicular size was observed in 1 or 2 males after treatment at 200 and 400 mg/kg food. These thyroid effects may be related to the enzyme induction in the liver, although enzyme induction is only seen at 400 mg/kg food.

In conclusion, etridiazole at 400 mg/kg food was considered to act as a promotor based on induction of phase II liver enzymes and reduction of connexin 32 protein in liver. These effects were not noted at 100 or 200 mg/kg food, indicating that etridiazole has a threshold level for promotor activity.

STUDY 3

Characteristics

reference	:	Hagiwara, 1995	exposure	:	6 weeks, diet
type of study	:	Liver medium-term Bioassay	doses	:	See study design
year of execution	:	1994-1995	Vehicle	:	not applicable
test substance	:	Terrazole technical (etridiazole), lot No. 306S052, purity 98.7%	GLP statement	:	Yes
Route	:	Oral	Guideline	:	No guideline available
species	:	male rats	Acceptability	:	Acceptable
Group size	:	15 or 20/group; see study design			

Study design

The study was designed to investigate whether etridiazole had promotor activity in liver tumour development (these types of tumours developed in a 2-year carcinogenicity study in rats with Etridiazole at 640 or 1280 mg/kg food, Trutter 1988). This type of study was performed for over two hundred chemicals to predict hepatocarcinogenicity and promoting or inhibitory activity. The study design was as follows:

Group	Pretreatment	Etridiazole treatment, mg/kg food (mg/kg bw/day)	No. of animals (5/cage)
1	DEN	0 (0)	20
2	DEN	100 (7)	20
3	DEN	640 (42)	20
4	DEN	1280 (88)	20
5	DEN	0 (positive control, phenobarbital)	20
6	Saline	0 (0)	15
7	Saline	640 (40)	15
8	Saline	1280 (82)	15

Animals were treated once, two weeks prior to start of etridiazole treatment, intraperitoneally, with N-nitrosodiethylamine (DEN) as an initiator (or saline as control) at a dose level of 200 mg/kg bw. Two weeks later test diets were provided, group 5 received diet containing 500 mg/kg food phenobarbital (known tumour promoter) as a positive control. This dose was equal to 35 mg/kg bw/day. Clinical signs, body weight and food intake were recorded during the study. After 1 week of treatment partial hepatectomy (two-thirds) was performed and after 6 weeks of treatment all surviving animals were killed, examined macroscopically and livers were collected and weighed. Sections of three liver lobes per animal were stained immunohistochemically for GST-P and analysed quantitatively. Preneoplastic GST-P positive liver foci served as an endpoint marker for hepatocarcinogenicity. For statistical analysis, groups 2 to 5 were compared to group 1 and group 7 and 8 were compared to group 6.

Results

The results are summarized in Table 6.8.2.3.

Table 6.8.2.3

Pretreatment	DEN					Saline			
Etridiazole dose (mg/kg food)	**0**	**100**	**640**	**1280**	**0**	**0**	**640**	**1280**	
Phenobarbital dose (mg/kg food)	**0**	**0**	**0**	**0**	**500**	**0**	**0**	**0**	**dr**
Mortality	1/20	0/20	0/20	1/20	1/20	0/15	0/15	0/15	
Clinical signs	No treatment-related findings								
Body weight (gain)			dc	dc			dc	dc	yes
Food consumption (%)		103	121	146	137		121	142	
Organ weights									
-Liver		ic^{r}	ic^{ar}	ic^{ar}	ic^{ar}		ic^{ar}	ic^{ar}	Yes
Pathology									
macroscopy									
Liver:									
- tan area	0/20	0/20	1/20	1/20	1/20	0/20	0/20	0/20	Yes
- white spots (one or more)	0/20	0/20	0/20	1/20	1/20	0/20	0/20	0/20	Yes
GST-P positive foci liver									
-number and size			ic	ic	ic				

dr dose related (Etridiazole)
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

Acceptability

The study was considered acceptable.

Conclusions

Observed mortality and clinical signs recorded were related to the surgical procedure of partial hepatectomy and unrelated to treatment. Reduced body weights were noted as a result of treatment with etridiazole at 640 or 1280 mg/kg food. At necropsy only minor findings (white spots or tan area) were noted in liver in individual animals treated with DEN and 640 or 1280 mg/kg food etridiazole or 500 mg/kg food phenobarbital. Absolute and/or relative liver weights were increased in a dose dependent way after treatment with etridiazole at 100, 640 or 1280 mg/kg food (increases varied from 103 to 146% of controls). Etridiazole treatment at 640 and 1280 mg/kg food markedly increased both the number and size of GST-P positive liver foci (increases varied from 231 to 796% of controls). Treatment with the positive control resulted in comparable effects, with the magnitude of changes between those seen at 640 or 1280 mg/kg food etridiazole.

In conclusion, this study showed that etridiazole possesses promoter activity at 640 and 1280 mg/kg food (42 and 88 mg/kg bw/day), but not at 100 mg/kg food (7 mg/kg bw/day) for hepatocarcinogenicity. In addition, no initiation potential was noted with doses up to 1280 mg/kg food (88 mg/kg bw/day) etridiazole.

B.6.8.2.2 Overall evaluation of the oncogenic properties of etridiazole

In B.6.8.2.2, several subjects will be presented:

- an overall evaluation of the mechanistic studies
- an evaluation of etridiazole carcinogenicity studies by Cardona, submitted by the notifier
- a summary of a position paper with regard to the kidney tumours, submitted by the notifer
- comments with regard to the oncogenic properties of etridiazole by the RMS
- conclusion

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 by Tanaka (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 lead 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).

Evaluation of etridiazole carcinogenicity studies by Cardona, 1997

Summary of evaluation by Cardona

Four carcinogenicity studies were evaluated by Cardona. These comprised one study in mice (Erker, 1981) and three studies in rats (Borzelleca, 1968; Hayashi, 1983 and Trutter, 1988), which were also evaluated by the RMS in this DAR, see B.6.5.1, study 5, 3, 4 and 1, respectively. Based on these studies it was concluded by Cardona, 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 the study by Trutter (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.

Additional position paper with regard to kidney tumours

The notifier submitted an additional position paper (Freeman, E, 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. 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.

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.

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.

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.

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).

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.

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 these observations, the RMS agrees with the notifier that although the mode of action has not actually been proven (by determination of the presence of the alpha-2-microglobulin in the hyaline droplets and binding of etridiazole to alpha-2-microglobulin) it is reasonable to assume that the kidney tumours in the rat are alpha-2-microglobulin associated, and therefore not relevant for human risk assessment.

Conclusion

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 the argumentation in B.6.8.2.2, it is concluded that classification of etridiazole as Carcinogen Category 3 should be considered (Xn, R40), in line with the current ECB classification.

B.6.9 MEDICAL DATA AND INFORMATION (ANNEX IIA 5.9)

B.6.9.1 Medical surveillance on manufacturing

STUDY 1

Characteristics

Reference	:	Kehrig, 2004	Examinations	:	Once a year
Type of study	:	Medical surveillance plant workers			
year of execution	:	2003-2004	GLP statement	:	no
test substance	:	Etridiazole	guideline	:	Not available
species	:	Workers	acceptability	:	Acceptable
group size	:	60 employees			

Study design

In an etridiazole plant, medical surveillances of all workers involved in handling etridiazole were gained over the production periods January - June 2003 and January – April 2004. The examinations included medical examination (history, physical examination, vision and hearing testing), laboratory examinations (full blood count, AST, ALR, γ-GT, creatinine, cholesterol, urine) and a technical examination (lung function, ECG, ergometry, chest X-ray, sonography).

Results

Ten workers showed skin irritation during 1 or 2 weeks and 5 workers showed long lasting exzemas (weeks to months), clinically due to a type IV sensitization. No further changes were noted.

Conclusion

In a production plant of etridiazole, skin irritation and skin sensitization were noted among workers.

B.6.9.2 Clinical cases of poisoning incidents

In Document M-II, section 5.9.2, reference is made to clinical cases and incidences. As only a summary of data is available, no evaluation of data was made.
The following data were included in Document M-II:

Data reported in the U.S.A.

A pesticide incident occurred in 1997 (incident # 5351-1), when a woman experienced dizziness, shortness of breath, and malaise. She was participating in a study involving four workers who hand-potted soil for nearly three days in a greenhouse. All applications were made at appropriate intervals but one post-treatment was done at four hours instead of twelve hours required by label. No other workers experienced any adverse effects, and the symptoms of the affected woman eventually dissipated with no sequelae.

Reference

Letter of Uniroyal Chemical Co., Inc of June 9, 1997 to U.S. EPA containing a case incident report of possible toxic effects in compliance with EPA FIFIRA Section 6(a)(2) § 159.184 – Toxic or adverse effect incident report.

Poison Control Centre data 1993 through 1996

A total of 30 unintentional exposures were reported to the Toxic Exposure Surveillance System from 1993 through 1996. All thirty cases involved adults and older children aged six to nineteen, nine of which had a minor outcome, two with moderate outcome, and none that were considered as life threatening. Eight cases were seen in a healthcare facility, none were hospitalised, and none were admitted for critical care. There were too few cases with outcome determined to do a meaningful comparison on the number of symptomatic cases. The percent of cases seen in a healthcare facility was only slightly above the average for all pesticides. These comparisons are shown in the table below (Table 5.9.2).

California data – 1992 through 1995

Detailed descriptions of 10 cases submitted to the Californian Pesticide Illness Surveillance Program (1982 – 1995) were reviewed. In one case, etridiazole was judged to be responsible for the health effects. In this one case, a worker handled moist soil that was treated with etridiazole and experienced eye and skin illness for two years. The case did not require hospitalisation and was not known to take time off work due to the exposure. These findings do not seem to be relevant for the EU, since agricultural workers do not come into direct contact with the treated soil.

On the list of top 200 chemicals for which the National Pesticide telecommunications Network (NPTN) received calls from 1984-1991 inclusively, etridiazole was not reported to be involved in human incidents. Relatively few incidents of illness have been reported due to etridiazole.

Table 5.9.2 Comparison between etridiazole and all pesticides for percent clinical cases for adults and children (six years & older) reported to Poison Control Centres, 1993-1996

Pesticide	SYM*	MOD*	LIFE-TH*	HCF*	HOSP*	ICU*
Etridiazole	85%	15%	0%	27%	0%	0%
All pesticides	72%	12%	0.37%	21%	7.6%	3.3%

** Symptomatic cases based on those cases with a minor, major, or fatal medic21%al outcome. Denominator for SYM. MOD, and LIFE-TH is total case where medical outcome was determined. Denominator for HCF is all exposures. Denominator for HOSP and ICU is in all cases seen in a health care facility.*
SYM = symptomatic outcome, MOD = moderate or more severe outcome, LIFE-TH = life-threatening or fatal outcome, HCF = seen in health care facility, HOSP = hospitalised and ICU = or seen in an intensive care unit.
Extracted from the U.S.EPA Preliminary Risk Assessment report on etridiazole of January 03, 2000.

Conclusion (by the notifier): Few incidents of illness have been reported in the U.S.A. due to exposure to etridiazole, and the vast majority of those have been of a minor nature.
Since etridiazole is only used on a relatively small scale in E.U. countries, the product never gave any reason to be included in health surveillance programs for pesticides. To the best of our knowledge, no cases of poisoning have ever been reported to us.

B.6.9.3 Observations on exposure of the general population

In Document M-II, section 5.9.3 the notifier stated: not applicable/no data reported.

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

In Document M-II, section 5.9.4, reference is made to the toxic/adverse effects described in a number of clinical cases (see B.6.9.2). From medical surveillance data of the manufacturing plant (see also B.6.9.1), it is evident that allergic skin reactions may be possible if inappropriate personal protection measures are taken.

B.6.9.5 First aid measures and therapeutic regimes

In any cases of exposure, the notifier recommends to seek medical advice immediately and show the label or the Safety Data Sheet, if possible.

Inhalation:	Move to fresh air. Obtain medical attention immediately.
Skin contact:	Remove contaminated clothing. Rinse affected skin with copious amounts of water, using a mild cleaning agent. Obtain medical attention if contact has been widespread and prolonged, or if irritation persists.
Eye contact:	Immediately flush eyes with water and continue washing for several minutes. Obtain medical attention if discomfort persists.
Ingestion:	Do not induce vomiting. Rinse mouth with water. Give two glasses of milk or water at once. Obtain medical attention in all cases.

For therapeutic regimes, the notifier referred to the MSDS. However, no advise for treatment is included in the MSDS of either AATERRA ME or etridiazole technical.

However, as there are no specific effects anticipated, there is no specific treatment. Treat symptomatically.

B.6.9.6 Expected effects of poisoning

Effects of etridiazole poisoning in man are not known.

B.6.10 SUMMARY OF MAMMALIAN TOXICOLOGY AND PROPOSED ADI, AOEL ARFD AND DRINKING WATER LIMIT (ANNEX IIA 5.10)

This toxicological dossier contains studies with the test substance etridiazole, which is also known under the name Terrazole.

B.6.10.1 Toxicokinetics

Absorption

In one study, 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% AR, 14-16% AR and 4.2-7.4% AR, respectively, was excreted with urine, faces 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, 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, 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, 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, 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, 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 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%).

B.6.10.2 Toxicodynamics

Acute toxicity

Etridiazole needs to be classified as harmful if swallowed (Xn, R22) on the basis of its acute oral toxicity in rats. Etridiazole does not need to be classified on the basis of its acute dermal and inhalation toxicity and is considered not irritating to the skin and eyes. Etridiazole needs to be classified as a skin sensitizer (Xi, R43).

Short-term and semichronic toxicity

Four weeks of dermal exposure of rats to 0, 20, 400 and 1000 mg/kg bw/day resulted in increased liver weights and concomitant centrilobular hypertrophy at 400 mg/kg bw/day and above. The NOAEL for systemic effects was set at 20 mg/kg bw/day. As no local effects were observed the NOAEL for local effects was established at 1000 mg/kg bw/day.

After subacute inhalation exposure of rats to 0, 15, 75 and 200 mg/m^3, a reduced body weight gain and an increased potassium concentration, of which the toxicological relevance was unclear, were observed at 75 mg/m^3 and above. Based on these effects a NOAEL of 15 mg/m^3 was established for systemic effects. For local effects no NOAEL could be derived, because squamous metaplasia of the larynx mucosa was observed at all dose levels.

Dietary exposure of rats to 0, 50, 600 and 1250 mg/kg food/day of etridiazole (equal to 0, 2.7, 29.5 and 64.7 mg/kg bw/day for males and 0, 3.3, 35.2 and 73.6 mg/kg bw/day for females) for 13 weeks caused decreased body weight at 600 mg/kg food in females and 1250 mg/kg food in both sexes. 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. 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. T_3 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 correlate with hyaline droplets observed in the kidneys of male animals. The incidence was slightly increased at 600 and 1250 mg/kg food, which is considered to be due to α_{2u}globulin (see mechanistic data). 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.

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. An increased platelet count (males only) and APTT was 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. Increased alkaline phosphatase and cholesterol, and decreased protein parameters indicate a

disturbed functioning of the liver. Concomitantly, liver weights were increased at 500 and 1000 mg/kg food. No macroscopic or microscopic findings were noted. Therefore, the NOAEL is 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).

Genotoxicity

Etridiazole did not induce point mutations in *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537, both with and without metabolic activation. Etridiazole was positive in a chromosome aberration test with Chinese hamster ovary cell line CHO. However these results were obtained at very toxic dose levels and the results could not be compared to the historical control data, therefore this study was not acceptable. Etridiazole was positive in two SCE studies (one was not considered acceptable). In both studies statistically significant increases were observed, however if the results are judged for biological relevancy these increases are considered not relevant, since no more than a two-fold increase has been observed. Etridiazole was negative in a gene mutation test using CHO hamster cells. In addition, etridiazole was negative in an *in vivo/in vitro* DNA repair study using male rat hepatocytes, etridiazole was also negative in an *in vivo* mouse micronucleus test and in an *in vivo* rat chromosome aberration test. Etridiazole was positive an *in vivo-in vitro* replicative DNA synthesis test.

Based on the *in vivo-in vitro* replicative DNA synthesis test in combination with the results of the studies summarized at the mechanistic data it can be concluded that etridiazole possesses promotor activity, with a threshold level.
Based on the three well performed *in vivo* studies, in which etridiazole showed negative responses, etridiazole is considered to be non-genotoxic.

Long-term toxicity

In a 2-year chronic toxicity and carcinogenicity study in rats, administration of etridiazole (dietary administration of 0, 100, 640 and 1280 mg/kg food) resulted in reduced body weight and food consumption at 640 and 1280 mg/kg food. Treatment at 640 and 1280 mg/kg food resulted, in females, in a mild increase in leucocyte count, with increased neutrophils and decreased lymphocytes, and in a mild decrease in erythrocyte count and haematocrit. At post mortem necropsy, abnormalities were noted in liver and liver weights were increased at 640 and 1280 mg/kg food. Neoplastic lesions were observed in liver (hepatocellular adenoma and/or carcinoma at 1280 mg/kg food and cholangiosarcoma at 1280 mg/kg food), thyroid (follicular adenoma and/or carcinoma at 640 and 1280 mg/kg food, testis (interstitial cell tumours at 1280 mg/kg food) and kidney (tubular cell tumours at 640 ppm). A variety of non-neoplastic lesions were noted in liver (at 640 and 1280 mg/kg food) and kidney (at all dose levels).
Based on the non-neoplastic lesions 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, kidney and testis.

In a 79-week carcinogenicity study in mice, etridiazole was administered via the diet at 0, 50, 900/1300 and 1800/2000/1600 mg/kg food. 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. Treatment at 900/1300 and 1800/2000/1600 mg/kg food resulted in reduced survival. 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. High dose males had lower body weights than controls from week 3 of treatment onwards. In addition, mid and high dose males, and high dose females showed lower food consumption than controls. Liver weights were increased, kidney and uterus weights reduced in mid and high dose groups. At post-mortem necropsy, abnormalities were noted in liver, kidney and spleen of mid and high dose groups.

Histopathological findings were recorded in mid and high dose groups and comprised findings in liver, kidneys and spleen. Neoplastic lesions (hepatocellular adenoma and carcinoma) were noted in liver of mid and high dose groups. A variety of non-neoplastic lesions were also noted in mid and high dose animals. 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 at dose levels clearly exceeding the MTD.

Mechanistic data

Based on chronic toxicity and carcinogenicity data in rats, it was concluded that etridiazole has an oncogenic effect on rat liver, thyroid, kidney and testis. Furthermore, based on the carcinogenicity data in mice, it was concluded that etridiazole has an oncogenic effect on mouse liver.

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 by Tanaka (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 lead 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).

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.

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.

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.

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).

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.

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 these observations, the RMS agrees with the notifier that although the mode of action has not actually been proven (by determination of the presence of the alpha-2-microglobulin in the hyaline droplets and binding of etridiazole to alpha-2-microglobulin) it is reasonable to assume that the kidney tumours in the rat are alpha-2-microglobulin associated, and therefore not relevant for human risk assessment.

Conclusion

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 the argumentation in B.6.8.2.2, it is concluded that classification of etridiazole as Carcinogen Category 3 should be considered (Xn, R40), in line with the current ECB classification.

Reproduction and developmental toxicity

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.

Neurotoxicity

No acute or semichronic oral neurotoxicity studies with etridiazole 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.

Toxicological data with metabolites

Metabolite 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid showed to be less toxic than etridiazole for acute and semichronic toxicity. Both etridiazole and 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid are considered non-genotoxic.

Derek modeling of the metabolites dichloro-etridiazole, 5-hydroxy-ethoxyetridiazole acid and 3-hydroxymethyl etridiazole, is negative for human health hazard.

B.6.10.3 ADI

The ADI has to be derived from the results of toxicity studies with experimental animals. The calculation of the ADI is based on the highest dose at which no adverse effect is observed in the most appropriate study in the most sensitive species. Etridiazole was tested in several subacute, semi-chronic and chronic studies in dogs, rats and mice, in a reproduction study in rats, and in teratogenicity studies in rats and rabbits. Furthermore, it was established in several *in vitro* and *in vivo* genotoxicity tests that etridiazole does not have genotoxic potential.

The critical effects of etridiazole were considered to be effects on liver and kidneys. Several repeated dose studies, including the 2-generation reproduction toxicity study and teratogenicity studies were considered for the establishment of the ADI (table 6.10.3.1).

Table 6.10.3.1 Studies considered for the establishment of the ADI

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Semichronic toxicity studies			
13-week, oral, rat (diet)	2.7	29.5	Richards, 1994
1-year, oral, dog (diet)	3.1	8.1	Goldenthal, 2002
Chronic toxicity studies			
2-year, oral, rat	< 5.0	5.0	Trutter, 1988
18-month, oral, mouse	7.5	185	Goldenthal, 2004
Reproduction and teratogenicity studies			
2-generation, oral, rat	Parental and developmental: 5.3	21.3	Turck, 2003
Teratogenicity, oral, rat	Maternal and developmental: 30	75	Wahlberg, 1982
Teratogenicity, oral, rabbit	Maternal and developmental: 15	45	Knicker-bocker, 1979

Unfortunately, no clear NOAEL was established in the chronic study in rats, in which non-neoplastic lesions (tubular cell karyomegaly in kidney) were noted at the lowest dose level of 5 mg/kg bw/day.

Based on the LOAEL of 5 mg/kg bw/day in the chronic study in rats, an ADI might be derived based on application of an additional safety factor 10 for extrapolation of a LOAEL to a NOAEL. Based on a overall safety factor of 1000 an ADI of 0.005 mg/kg bw/day can be derived. This provides a Margin of Safety of 6000 for tumour development, observed at 30 mg/kg bw/day (640 ppm) in the 2-year rat study.

B.6.10.4 ARfD (acute reference dose)

An ARfD was not allocated by the notifier.

According to the Guidance for the setting of an Acute Reference Dose (Document 7199/VI/99 rev. 5) an ARfD should not be allocated if:

- the pesticide has shown a very low acute oral toxicity (e.g. no adverse clinical sings and deaths have been observed at the limit dose for acute LD50 testing), and
- the toxicological profile was based only on e.g. mild effects, which are not relevant for acute intake (e.g. adaptive liver enlargement, chronic body weight reduction, reduced food intake), and
- the toxicological profile has not other alerts for acute toxicity.

Furthermore, residue data should be taken into account to conclude on the necessity of an ARfD.

Based on the submitted data on etridiazole, the following considerations were made:

- The acute oral LD_{50} of etridiazole was found to be 1141 mg/kg bw in male rats and 945 mg/kg bw in females rats.
- In the acute, semichronic or chronic toxicity studies, no specific effects were observed relevant for acute intake.
- For etridiazole, there were no alerts observed for acute toxicity, e.g. acute neurotoxicity.
- In the teratogenicity study in rats and rabbits skeletal malformations/variations were noted in foetuses at maternal toxic levels.
- Because of insufficient residue data (see B.7), no conclusion on the necessity of an ARfD can be based on submitted residue data.

Based on the above data, it can be concluded that the criteria for not allocating an ARfD are not met, since the LD50 value <2000 mg/kg bw. Although there are no triggers from repeated dose toxicity studies indicating acute toxic effects for etridiazole, based on the above considerations on the acute toxicity and residue data of etridiazole, it was concluded that allocation of an ARfD is considered justified.

The calculation of the acute reference dose is based on the highest dose at which no adverse effect is observed in the most appropriate acute toxicological endpoint of relevance to humans, derived from the most appropriate study in the most appropriate species.

The following studies were considered relevant for the establishment of the ARfD:

- The acute oral toxicity study in rats: a LOAEL of 700 mg/kg bw could be established. As clinical signs (decreased activity and decreased defecation) were noted at all dose levels, no NOAEL could be established.
- The subacute oral toxicity in rats (mechanistic study, 28 days): a NOAEL of 200 mg/kg food (18 mg/kg bw/d) was established (with a limited number of parameters).
- The 2-generation study in rats: a NOAEL of 5.3 mg/kg bw/day for parental and developmental effects.

- The teratogenicity study in rabbits: a NOAEL of 15 mg/kg bw/day was established for maternal and developmental effects.
- The teratogenicity study in rats: a NOAEL of 30 mg/kg bw/day was established for maternal and developmental effects.

The 2-generation reproduction study and the subacute oral toxicity study are considered less suitable for the establishment of an ARfD, due to the length of exposure, the limited number of investigated parameters, and since observed effects are more likely to be cause due to repeated exposure or due to indirect effects on the parental animals.

The latter can also not be excluded for the teratogenicity studies. However, considering the shorter exposure time of the teratogenicity studies, and the absence of a clear NOAEL for acute toxicity of etridiazole, the NOAEL of 15 mg/kg bw/day from the teratogenicity study in rabbits is considered to be most suitable NOAEL for the derivation of an ARfD.

Application of a safety factor of 100 for inter- and intraspecies differences results in an ARfD of 0.15 mg/kg bw/day.

B.6.10.5 AOEL

AATERRA ME is used in glasshouses, on substrate grown cucumber, tomato and pepper, and on non-soil-bound ornamentals.

Application of AATERRA ME on cucumber, tomato and pepper takes place once or twice during growth stage BBCH 81. Application of AATERRA ME on ornamentals takes place before planting. Although multiple cultures might be produced within one year in glasshouses, the assumption of semi-chronic use of AATERRA ME is justified (only one or two applications per culture, and one culture takes at least 4 months).

Exposure of bystanders can be excluded, since AATERRA ME is used in glasshouses.

For the use of AATERRA ME, re-entry activities should be considered for inspection, binding and harvesting of cucumber, tomato, pepper and ornamentals. The exposure period will not exceed three months.

Etridiazole was tested in several subacute, semi-chronic and chronic oral studies in dogs, rats, and mice, in a reproduction study in rats and in teratogenicity studies in rats and rabbits (see table 6.10.5.1).

Table 6.10.5.1 Studies considered for the establishment of the AOEL

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
Dermal and inhalation toxicity studies			
28-day, inhalation, rat	4 (15 mg/m^3)	20 (75 mg/m^3)	Hoffman, 2002
28-day, dermal, rat	20	400	Goldenthal, 2002
Semichronic toxicity studies			
13-week, oral, rat (diet)	2.7	29.5	Richards, 1994
1-year, oral, dog (diet)	3.1	8.1	Goldenthal, 2002
Chronic toxicity studies			
2-year, oral, rat	< 5.0	5.0	Trutter, 1988
18-month, oral, mouse	7.5	185	Goldenthal, 2004
Reproduction and teratogenicity studies			
2-generation, oral, rat	Parental and developmental: 5.3	21.3	Turck, 2003
Teratogenicity, oral, rat	Maternal and developmental: 30	75	Wahlberg, 1982
Teratogenicity, oral, rabbit	Maternal and developmental: 15	45	Knicker-bocker, 1979

As etridiazole is extensively metabolised, route-specific toxicity cannot be excluded. Therefore, if available, toxicity studies for the route concerned should be considered to calculate route-specific AOELs. In case of etridiazole subacute dermal and inhalation studies were available.

In a 28-day inhalation study, a NOAEL of 15 mg/m^3 (equivalent to 4 mg/kg bw/day) was established for systemic effects. The observed effects at the LOAEL of 75 mg/m^3 (20 mg/kg bw/day) were equivalent to the effects observed after oral exposure, mainly liver effects. The effect levels for both oral and respiratory route are in the same order of magnitude. Therefore, it is concluded that derivation of a specific AOEL for respiratory exposure is not considered necessary.

In a 28-day dermal toxicity study, a NOAEL of 20 mg/kg bw/day was derived, based on increased liver weights and concomitant centrilobular hypertrophy. At the NOAEL the estimated area concentration is 0.1 mg/cm^2 (assuming a body weight of 0.2 kg and a corresponding 400 cm^2 body surface, and using the reported 10% exposed body surface). For an area dose of 10 µg/cm^2, a dermal absorption value of 18% was derived (see B.6.12).This dermal absorption value can be considered a worst-case value for an area dose of 0.1 mg/cm^2. This means that a dermal dose of 20 mg/kg bw/day is comparable to a systemic dose of about 3.6 mg/kg bw/d. The observed effects at the LOAEL were equivalent to the effects observed after oral exposure, mainly liver effects. The NOAELs for both oral and dermal route are in the same order of magnitude. Therefore, it is concluded that derivation of a specific AOEL for dermal exposure is not considered necessary.

The semi-chronic AOEL for systemic exposure is set on the basis of the most relevant NOAEL from semi-chronic oral toxicity studies. The most relevant NOAELs (2.7 and 3.1 mg/kg bw/day) were obtained from a 13-week rat study and a 1-year dog study, respectively.

The most relevant NOAEL of 3.1 mg/kg bw/day in the 1-year dog study is used as a starting point for the establishment of the AOEL. Application of a safety factor of 100 for inter- and intraspecies differences, results in an AOEL of 0.03 mg/kg bw/day. This provides a Margin of Safety of 1000 for tumour development, observed at 30 mg/kg bw/day (640 ppm) in the 2-year rat study.

Occupational risk assessments will be based on the AOEL of 0.03 mg/kg bw/day.

B.6.10.6 Drinking water limit

According to Council Directive 97/57/EC, exposure to etridiazole through the drinking water should account for not more than 10% of the ADI. If it is assumed that the average daily consumption of water amounts to 2 litre per person of 60 kilogram, a drinking water limit of (60x0.005)/10)/2 mg/l, i.e. 0.015 mg/l can be established.

According to Document 8064/VI/79 of the European Commission, the EU drinking water limit for pesticides of 0.1 µg/l is applicable for etridiazole.

B.6.11 ACUTE TOXICITY INCLUDING IRRITANCY AND SKIN SENSITISATION OF THE PREPARATION

B.6.11.1 Acute toxicity

STUDY 1

Characteristics

reference	:	Sanders, A., 1998	exposure	:	Once by gavage
type of study	:	Acute oral toxicity study	doses	:	2000, 3162 and 5000 mg/kg bw
year of execution	:	1997	vehicle	:	None
test substance	:	AATERRA ME, batch no. AEF 017408 00 1H55 A101	GLP statement	:	Yes
route	:	Oral	guideline	:	In accordance with OECD 401 (1987)
species	:	Rat, Sprague-Dawley CD (Crl: CD BR)	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	Male rats: 3628 mg/kg bw Female rats: 2515 mg/kg bw

Study design

The study was performed in accordance with OECD 401 (1987). Dose levels were based on a range finding study in which one male and one female were treated with 1000, 2000 and 5000 mg/kg bw. Animals treated with 5000 mg/kg bw were found dead within 2-3 days and showed overt clinical signs (among others ataxia, emaciation, hunched posture and lethargy). The clinical signs were also noted in animals given 2000 mg/kg.

Results

Mortality: 1/5 females given 2000 mg/kg were killed in extremis on day 7. One male and one female given 3162 mg/kg were found dead on day 2. Three females given 3162 mg/kg were killed in extremis between days 2 and 14. In the 5000 mg/kg dose group, within 3 days three males were found dead and two males were killed in extremis. In the same dose group, three females were found dead within 2 days, and two females were killed in extremis on day 7.

Symptoms of toxicity: Hunched posture, ataxia, lethargy, decreased respiratory rate, laboured respiration, emaciation, pilo-erection, ptosis, splayed or tiptoe gait and red/brown staining around the snout was noted in all dose groups. These clinical signs disappeared before the end of the study. Only in the highest dose group chromodacryorrhoea in 2 females and (occasional) body tremors in all males and two females was observed.

Body weight: Surviving animals mostly showed a loss in bodyweight during the first week, and a gain in the second week.

Pathology: Surviving animals showed no pathological changes. Common findings in killed or dead animals included haemorrhagic lungs, discoloured liver, discoloured kidneys, haemorrhagic gastric mucosa. Other findings included ulcerated or sloughing non-glandular stomach epithelium and haemorrhagic intestines.

Acceptability

This study is considered acceptable.

Conclusions

The acute oral LD_{50} of AATERRA ME was found to be 3628 mg/kg bw in male rats and 2515 mg/kg bw in female rats.

STUDY 2

Characteristics

Reference	:	Sanders, A., 1998	exposure	:	24 hours on a skin area of 10% of the total body surface area (semi-occlusive exposure).
type of study	:	Acute dermal toxicity study	doses	:	5000 mg/kg bw (both sexes)
year of execution	:	1997	vehicle	:	None
test substance	:	AATERRA ME, batch no. AEF 017408 00 1H55 A101, 710 g/L etridiazole	GLP statement	:	Yes
Route	:	Dermal	guideline	:	In accordance with OECD 402 (1987)
Species	:	Rat, Sprague-Dawley CD (Crl: CD BR)	acceptability	:	Acceptable
group size	:	5/sex/dose	**LD_{50}**	:	> 5000 mg/kg bw

Study design

The study was performed in accordance with OECD 402 (1987).

Results

Mortality: No mortality occurred.

Symptoms of toxicity: No treatment related findings.

Body weight: No treatment related findings.

Pathology: No treatment related findings.

Acceptability

This study is considered acceptable.

Conclusions

The acute dermal LD_{50} of AATERRA ME in rats was found to be greater than 5000 mg/kg bw in males and females.

B.6.11.2 Irritation and sensitization

STUDY 1

Characteristics

reference	:	Sanders, A., 1998	exposure	:	4 hours, semi-occlusive, application area 6.25 cm^2
type of study	:	Skin irritation study	doses	:	0.5 ml
year of execution	:	1997	vehicle	:	None
test substance	:	AATERRA ME, batch no. AEF 017408 00 1H55 A101, 710 g/L etridiazole	GLP statement	:	Yes
route	:	Dermal	guideline	:	In accordance with OECD 404 (1992)
species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	6 males	**Effect**	:	Skin irritating

Study design

The study was performed in accordance with OECD 404 (1992).

Results

The results are summarised in tables 6.11.2.1 and 6.11.2.2.

Table 6.11.2.1 Individual irritation scores

Scores observed after	1 hours	24 hours	48 hours	72 hours	7 days	14 days
Erythema	1,2,2,2,2,1	2,2,2,2,2,2	2,2,2,2,2,2	2,2,2,2,2,2	0,0,0,0,?,?	0,0,0,0,0,0
Oedema	1,1,2,2,2,1	1,1,2,2,2,2	1,1,1,1,2,1	1,1,1,1,2,1	0,0,0,0,0,0	0,0,0,0,0,0

?: adverse skin reaction prevented accurate evaluation of erythema.

Table 6.11.2.2 Mean value irritation scores

Animal	mean 24-72 hrs	
	erythema	Oedema
1	2	1
2	2	1
3	2	1.3
4	2	1.3
5	2	2
6	2	1.3

Crust formation was observed at all rabbits on day 7, and in 4 rabbits on day 14. Therefore erythema could not be accurately evaluated on day 7 for 2 animals. Two rabbits appeared normal on day 14.

Acceptability

The study is considered acceptable. The amount of animals tested could have been lower (n=3).

Conclusions

AATERRA ME should be classified as a skin irritant (Xi R38).

STUDY 2

Characteristics

Reference	:	Sanders, A., 1998	exposure	:	Single instillation
type of study	:	Acute eye irritation study	doses	:	0.1 ml
year of execution	:	1997	vehicle	:	None
test substance	:	AATERRA ME, batch no. AEF 017408 00 1H55 A101, 710 g/L etridiazole	GLP statement	:	Yes
Route	:	Ocular	guideline	:	In accordance with OECD 405 (1987)
Species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	4 males and 2 females	**Effect**	:	Eye irritating

Study design

The study was performed in accordance with OECD 405 (1987). A local anaesthetic (0.5% proxymetacaine hydrochloride) was instilled into the eyes before treatment.

Results

The results are summarised in tables 6.11.2.3 and 6.11.2.4.

Table 6.11.2.3

Scores observed after	1 hour	24 hours	48 hours	72 hours	7 days	14 days
Cornea/opacity	d,1,1,d,0,d	1,1,1,1,1,1	1,1,2,1,2,2	1,1,2,1,2,2	1,0,0,0,0,1	0,0,0,-,0,0
Iris	1,1,1,1,0,1	1,1,1,1,1,1	1,1,1,1,1,1	0,1,1,1,1,1	0,0,0,0,0,0	0,0,0,-,0,0
Conjunctiva redness	2,2,2,2,2,2	2,2,2,2,2,2	2,2,2,2,2,3	2,2,2,2,2,2	1,1,1,0,1,1	0,0,0,-,0,0
Conjunctiva chemosis	2,2,2,2,2,2	2,2,2,2,2,2	2,2,2,2,2,2	1,2,2,2,2,2	0,0,1,0,1,0	0,0,0,-,0,0
Conjunctiva discharge	3,3,2,3,3,3	1,2,2,3,2,3	1,1,2,2,1,3	1,1,1,1,1,2	1,0,0,0,0,0	0,0,0,-,0,0

d = dulling of the normal lustre of the cornea
- = animal sacrificed, since no ocular changes were noted after 7 days.

Table 6.11.2.4 Mean value irritation scores

Animal	mean 24-72 hrs			
	corneal opacity	Iris	Conjunctiva redness	Conjunctiva chemosis
1 (m)	1	0,7	2	1,7
2 (f)	1	1	2	2
3 (m)	1,7	1	2	2
4 (f)	1	1	2	2
5 (m)	1,7	1	2	2
6 (m)	1,7	1	2,3	2

Acceptability

The study is considered acceptable. The amount of animals tested could have been lower (n=3).

Conclusions

AATERRA ME should be classified as an eye irritant (Xi R36).

STUDY 3

Characteristics

Reference	:	Sanders, A., 1998	exposure	:	Intradermal and topical induction, topical challenge (occlusive, 24h).
type of study	:	Skin sensitisation study (GPMT)	doses	:	0.1% intradermal induction 100% topical induction 50% and 25% challenge
year of execution	:	1997	vehicle	:	Distilled water
test substance	:	AATERRA ME, batch no. AEF 017408 00 1H55 A101	GLP statement	:	Yes
Route	:	Dermal	guideline	:	In accordance with OECD 406 (1992)
Species	:	Guinea-pig, Dunkin/Hartley	acceptability	:	Acceptable
group size	:	10 controls (females) 20 test animals (females)	**Effect**	:	Sensitising

Study design

The study was performed in accordance with OECD 406 (1992) and conducted according to Magnusson and Kligman. 2-Mercaptobenzothiazole was used as the positive control.

Dose levels were based on the results of a range-finding study using 0.1, 0.5, 1.0 and 5.0% for intradermal injections and 25, 50, 75 and 100% for topical applications. Severe erythema and necrosis of the skin was observed after intradermal injections of 0.5% and above. Topical application of 50% test substance revealed the maximum non-irritant concentration.

Intradermal induction was performed with 0.1% test substance in distilled water. Topical induction with undiluted test substance was initiated one week after intradermal induction. Fourteen days later, challenge was performed with dermal application of 50% and 25% test substance in distilled water. Observations of the challenge sites were made 24 or 48 hours after removal of the dressings.

Results

24 and 48 hours after intradermal induction of 0.1% AATERRA ME, very slight to well-defined erythema was observed. Control animals showed no or very slight erythema.

One hour after topical induction with undiluted AATERRA ME, very slight to moderate erythema and very slight oedema was observed in all animals. After 24 hours, erythema could often not be evaluated due to adverse reactions (crust formation, superficial cracking of the epidermis and desquamation). Very slight to slight oedema was noted in all animals after 24 hours. In control animals no erythema and oedema was noted.

After topical challenge with 25% test substance, 11/20 animals were sensitised. After 24 hours, very slight to well-defined erythema was observed in 11/20 animals and very slight oedema was observed in 9/20 animals. Five animals showed very slight erythema 48 hours after challenge. After topical challenge with 50% test substance, all animals were sensitised. After 24 hours, very slight to well-defined erythema in all animals and very slight to well-defined oedema in 12/20 animals was observed. After 48 hours, 12/20 animals showed very slight erythema and 7/20 animals showed very slight oedema. Some animals showed desquamation both after 24 and 48 hours. In control animals no erythema and oedema was noted.

Acceptability

The study is considered acceptable. Erythema and oedema are not scored according to the Magnusson and Kligman grading scale, but evaluated according to the Draize scales.

Conclusions

In this study, AATERRA ME has been found to be a skin sensitizer.

B.6.11.3 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in table 6.11.3.1, 6.11.3.2, and 6.3.11.3, respectively.

Table 6.11.3.1 Acute toxicity studies, LD_{50}/LC_{50} values AATERRA ME

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle	Reference
AATERRA ME	3628 mg/kg bw (males) 2515 mg/kg bw (females)	Rat	Oral	None	Sanders, A., 1998
AATERRA ME	> 5000 mg/kg bw (males and females)	Rat	Dermal	None	Sanders, A., 1998

Table 6.11.3.2 Skin and eye irritation studies AATERRA ME

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
AATERRA ME	skin irritating	Rabbit	Dermal	None	Sanders, A., 1998
AATERRA ME	eye irritating	Rabbit	Ocular	None	Sanders, A., 1998

Table 6.11.3.3 Sensitisation studies AATERRA ME

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
AATERRA ME	Skin sensitising	Guinea-pig	Dermal	Distilled water	Sanders, A., 1998

According to the Directive 2001/59/EC, AATERRA ME does not need to be classified on the basis of its acute oral and dermal toxicity in rats. AATERRA ME needs to be classified as a skin irritant (Xi R38) and an eye irritant (Xi R36) according to the criteria given in Annex VI of Directive 2001/59/EC. AATERRA ME also needs to be classified as a skin sensitizer (Xi R43).

An acute inhalation study with AATERRA ME was not submitted by the notifier. Considering the volatility of the active substance (vapour pressure 1.43 Pa) and the use of AATERRA ME in glasshouses, performance of an acute inhalation study with AATERRA ME should be considered necessary. However, since AATERRA ME contains 700 g/L etridiazole, an acute inhalation study with etridiazole is available, and data on the formulants do not indicate a risk for inhalation, performance of an acute inhalation study is not considered necessary.

B.6.12 DERMAL ABSORPTION

STUDY 1

Characteristics

reference	:	Chadwick, 1985	exposure	:	0.5, 1, 2, 4 and 10 h non-occlusive
type of study	:	*in vivo* dermal absorption	doses	:	5 mg/cm^2 and 0.5 mg/cm^2. High dose represents worker exposure to undiluted formation, low dose to formulation under conditions of use.
year of execution	:	1985	vehicle	:	High dose: 25% (w/v) solution in xylene. Low dose± 2.5% (w/v) solution in aqueous xylene solution.
test substance	:	[^{14}C]-Terrazole, Lot no AC-840-33, Specific activity 12.3 mCi/mmole, Radiochemical purity 97%.	GLP statement	:	No, but QA unit report included.
route	:	Dermal	Guideline	:	No
species	:	Rat, Sprague-Dawley, male	acceptability	:	Not acceptable
group size	:	3/dose/sacrifice time	**Result**	:	-

Study design

The percutaneous absorption and distribution of [14C]-Terrazole were studied in two groups of 15 male Sprague-Dawley rats. The groups received a single dermal application at 0.5 and 5 mg/cm^2 skin by application of 200 µL of the formulation test substance to a skin area of 20 cm^2. The application area was subsequently dried with a hair dryer, set at cool. Then, the exposed area was covered with a non-occlusive protective device.

Animals were exposed for 0.5, 1, 2, 4 or 10 hours. During this time, urine and faeces were collected. Urine and faeces samples consisted of the total amount of urine in addition to the contents of the urine bladder or faeces excreted during the exposure period, respectively. Cage wash was performed at the end of the collection period. At the end of the exposure period, animals were anesthetized with pentobarbital and a blood sample was taken by cardiac puncture. The protective device was removed. The exposed skin was washed with three gauze pads dipped in xylene and one dry gauze pad. Then, the treated area of skin was removed (free of underlying muscle). Skin stripping was not performed. The carcass was frozen on dry ice and skinned prior to homogenisation. The unshaved skin was also kept for analysis. All samples were stored at -20°C.

The levels of radioactivity were determined by (solubilisation or combustion) LSC in all samples of blood, urine, faeces, carcasses and unshaved skin, cage wash, treated skin area and skin wash. The protective device used to cover the treated skin area was extracted using acetonitrile; the extract was subsequently analysed by LSC.

Acceptability

This study was not considered acceptable for risk assessment purposes of ATTERRA ME and was not fully evaluated since:

- Recoveries were too low in both treatment groups varying from 30 to 59% of the applied radioactivity. A probable explanation is that the compound evaporated during application.
- 30 to 50% of the administered radioactivity in both treatment groups appeared to be associated with the adhesive foam and bandages of the protective device. This amount may not have been available for absorption.

- Etridiazole was diluted in xylene. The formulation AATERRA ME does not contain xylene, it is therefore unknown what the relevance is of the administered formulation for the dermal absorption of AATERRA ME.
- The study was not performed under GLP conditions.

STUDY 2
Characteristics

reference	:	Dow *et al.* 2003	exposure	:	4 and 10 hours, occlusive
type of study	:	*in vivo* dermal absorption	doses	:	0.1, 1 and 10 µg/cm^2. High dose represents operator exposure.
year of execution	:	2002	vehicle	:	Terrazole®-25 EC formulation diluted with water to produce 1 mg/mL suspension to obtain high dose. Subsequent 10-times dilution steps with water produced medium and low dose.
test substance	:	[^{14}C]-etridiazole, formulated as Terrazole®-25 EC*, Lot no: CSL-99-866-29-17, Specific activity: 30.3 mCi/mmole, Radiochemical purity ≥99%.	GLP statement	:	Yes
route	:	Dermal	guideline	:	Draft OECD guideline 428
species	:	Rat, Sprague-Dawley [Crl:CD®(SD)IGS BR®], males	acceptability	:	Yes
group size	:	4/dose/sacrifice time	**Result**	:	Dermal absorption 30% from low dose, 19% from medium dose and 18% from high dose.

* In the study report, this formulation was described as Terrazole®-30 EC, but it is in fact Terrazole®-25 EC

Study design

The percutaneous absorption and distribution of [^{14}C]-etridiazole were studied in three groups of 8 male Sprague-Dawley rats. The groups received a single dermal application at three nominal dose levels: 0.1, 1 and 10 µg/cm^2 skin by application of 100 µL of the formulation test substance to a skin area of 10 cm^2. In view of the high volatility of the test substance and to prevent other expectable losses, the treated skin area was covered with a protective device consisting of a set of rings topped with a Tenax™-membrane, shown to be effective in trapping volatilized etridiazole in two pilot studies (Cheng and Skillinger, 2001, MRID # 45503601 and Gay, 2002, MRID # 45675301, both not available for evaluation). Directly after application of the test substance, the device was glued to the skin using cyanomethacrylate. Application of the test substance and installation of the protective device were performed under O_2/CO_2 anaesthesia. In another study (McManus, 1995, MRID NO. 4365801, not available for evaluation) it was demonstrated that 4-7% of the administered dose was eliminated through the lungs, therefore, expired air was not collected.

Animals were exposed for 4 or 10 hours. During this time, urine and faeces were collected. Urine and faeces samples consisted of the total amount of urine or faeces excreted during the exposure period, in addition the contents of the urine bladder or intestines, respectively. Cage wash was performed at the end of the collection period. Immediately after the exposure periods of 4 or 10 hours, animals were anesthetized with O_2/CO_2, and the protective device was removed and placed in ethyl ether. The exposed skin was washed five times using the following procedure: 1. with natural sponge moistened with a 5% (v/v) Liquid Ivory®/HPLC grade water solution, 2. with a sponge moistened with water and 3. with a dry sponge. The resulting 15 sponges per animal were combined in one container to allow analysis of skin washes. Immediately following the skin wash cycles, the treated area of skin was

removed. Then the animals were exsanguinated. Skin stripping was not performed. Terminal blood and carcasses were retained for analysis.

The levels of radioactivity were determined by (solubilisation) LSC in all samples of blood, urine, faeces, carcasses, cage wash, treated skin and skin wash. The protective device used to cover the treated skin area was extracted using ethyl acetate sonication; the extract was subsequently analysed by LSC.

Results

Total recovery (group means) was 96-103% AR. Results are given in Table 6.12.1.1.

Table 6.12.1.1 Excretion and retention in rats of total radioactivity after single dermal exposure

Values are expressed as percentage of the administered radiolabel.

Dose Level	Low dose 0.099 µg a.s./cm²		Medium dose 0.975 µg a.s./cm²		High dose 10.25 µg a.s./cm²	
Sacrifice time	**(4 h)**	**(10 h)**	**(4 h)**	**(10 h)**	**(4 h)**	**(10 h)**
Urine	3.2	4.6	4.6	7.7	1.4	8.8
Faeces	0.09	1.5	0.1	0.2	0.09	0.06
Cage Wash	ND[1]	ND[1]	1.6	2.1	5.0	1.9
Total Excretion	3.3	6.2	6.3	10	6.5	11
Residues (blood + carcass)	26	16	8.3	6.8	9.9	5.9
Systemic Absorption	29	22	15	17	16	17
Skin treated area	1.0	0.8	2.2	2.4	0.4	0.5
Skin Wash	2.5	ND[1]	2.7	1.5	1.8	1.4
Cover and O-Ring	71	79	80	75	78	80
Dislodged Dose	74	79	83	77	80	81
Total Recovery	104	102	100	96	96	99

[1]ND – not detected

Considering the high volatility of the test substance, causing the rapid evaporation of the test substance from the application site, exposure duration of either 4 or 10 hours is considered to be of no great influence on dermal absorption. The amount of radioactivity present in blood and carcass after 4h was for all doses higher than after 10h. However, the amount excreted (urine, faeces and cash wash) was higher after 10 h exposure, than after 4 h. Most of the applied radioactivity, about 73-83%, evaporated from the surface of the skin and was trapped by the application site protective device. The majority of the applied dose therefore was classified as not absorbed and was comparable for all dose groups and exposure times.

Due to the volatility of the test substance, it is not clear whether the animals were exposure for the full 4 or 10 hours exposure period. Therefore, worst-case values on dermal absorption (either of the 4 or 10 hour exposure period) will be taken for risk assessment purposes.

Up to 2.4% of applied radioactivity remained in/on the treated skin after washing and could be absorbable. It was not investigated to what extent this amount becomes systemically available after cessation of the exposure. Therefore, the potentially absorbed dose is taken as a measure for the dermal absorption of [^{14}C]-etridiazole, as shown in Table 6.12.1.2.

Table 6.12.1.2 Mean systemically available dose and potentially available dose at different time points

Absorption is expressed as percentage of the administered radiolabel.

Dose Level	Low dose		Medium dose		High dose	
	0.099 µg a.s./cm²		0.975 µg a.s./cm²		10.25 µg a.s./cm²	
Time	systemically available[1]	potentially available[2]	systemically available[1]	potentially available[2]	systemically available[1]	potentially available[2]
4 h	29	30	15	17	16	16
10 h	22	23	17	19	17	18

[1] systemically available dose: urine, faeces, blood, cage wash, carcass
[2] potentially absorbed dose = systemically available dose + treated skin site

Acceptability

According to the OECD guidance document for the conduct of skin absorption studies, post-exposure groups should be included in the study to follow the fate of the skin residue post-exposure. Because the high volatility of the test substance was foreseen, the exposure was performed under occluded conditions, allowing a high recovery of AR, thereby reflecting a worst case scenario.

Due to the evaporation of the test substance, it is not clear whether the animals were exposed for the full 4 or 10 hours exposure period. Therefore, worst-case values on dermal absorption (either of the 4 or 10 hour exposure period) will be taken for risk assessment purposes.

The potentially available dose is taken as a measure for the dermal absorption of etridiazole, for risk assessment purposes.

The test substance was applied using a 25 EC formulation. Since this is an EC formulation with a high content of organic solvent, the results of this study can be regarded as worst-case estimates of dermal absorption of etridiazole formulated as AATERRA ME.

Conclusion

The dermal absorption of etridiazole in rats *in vivo* from a 25 EC formulation was 30% after a single application of test material at low dose (0.1 µg/cm^2), 19% at medium dose (1 µg/cm^2) and 18% at high dose (10 µg/cm^2). These dermal absorption values can be regarded as worst-case estimates for the formulation AATERRA ME.

B.6.13 TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES

MSDS's for the formulants of AATERRA ME were submitted by the notifier.

Considering human toxicological endpoints: One of the formulants is labelled with R41 and R38. However, these endpoints (skin and eye irritation) are covered in B.6.11.

B.6.14 EXPOSURE DATA

AATERRA ME 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 contains 700 g/L etridiazole. AATERRA ME is applied in glasshouses by drip-irrigation. No exposure studies were submitted by the notifier. Exposure data were derived using models.

B.6.14.1 Exposure calculations

Operators

According to the notifier, exposure of the operator only occurs during mixing and loading since it is assumed that for application of AATERRA ME by drip-irrigation operators are not exposed. However, considering the volatility of etridiazole (as indicated by the vapour pressure of 1.43 Pa and Henry's law constant of 3.02 $Pa.m^3/mol$), and the atmospheric DT_{50} of 1.556 days, occurrence of etridiazole in air cannot be excluded *a priori.* Significant losses of etridiazole through volatilisation were also observed in soil and water/sediment studies (see Volume 3, Annex B, B.8.1.1 and B.8.4.3). Respiratory exposure of the operator to etridiazole during application (inspection during drip irrigation) cannot be excluded therefore.

Internal operator exposure values during mixing and loading, with and without personal protective equipment (PPE) were calculated using the UK and the German model. For risk assessment purposes, the 75th percentile of the UK-model was used (UK-75th) and the geometric mean of the German model (DE-GM). These calculations are presented in appendix 3. Since etridiazole is volatile, it is possible that the respiratory exposure during mixing/loading is underestimated by the German model (within the UK model, it is assumed that there is no inhalation exposure during mixing and loading). The notifier submitted estimations of concentrations of etridiazole in air due to volatilisation (Klein, 2006). See appendix paragraph 4.1 for further information. Based on these estimates also, the respiratory exposure of the operator during mixing/loading and possible inspection activities during the drip-irrigation, was calculated, and compared with the exposure calculations based on the German model.

From the laboratory studies (see Volume 3, Annex B, B.8.1.1 and B.8.4.3) it can be concluded that also the soil/water metabolite dichloro-etridiazole may volatilise from soil and water, although at lower quantities compared to etridiazole. Therefore, respiratory exposure of the operator to dichloro-etridiazole during application (inspection during drip irrigation) cannot be excluded, and was calculated based on the submitted estimations of concentrations of dichloro-etridiazole in air due to volatilisation. See appendix 3, paragraph 4.1 for further information.

Also etridiazole acid is a major metabolite in soil and water. The notifier submitted an EPI Suite (version 3.12) estimation of the volatility of the etridiazole acid metabolite. Based on the estimated vapour pressure of 0.01 Pa and Henry's law constant of 7×10^{-4} $Pa.m^3/mol$ it can be assumed that exposure to the etridiazole acid metabolite via the inhalatory route will not significantly contribute to the total exposure..

Bystanders

The presence of bystanders should be kept to a minimum. This can easily be achieved in a greenhouse, where no person should be allowed in who is not involved in the application process. Therefore, no estimation of bystander exposure for application of AATERRA ME in greenhouses is performed.

Workers

According to the notifier, AAATERRA ME is applied to substrates (as a root and substrate fungicide) and therefore no dislodgeable residues are anticipated and no exposure of workers when handling treated plants. This is acceptable and it is indeed assumed that dermal exposre of the workers is negligible. However, during and after treatment via the irrigation system, workers can be present in the glasshouses for re-entry activities.

Given the volatility of etridiazole and the soil/water metabolite dichloro-etridiazole, occurrence of etridiazole and dichloro-etridiazole in air cannot be excluded *a priori*. Based on the estimated air concentrations of etridiazole and dichloro-etridiazole (submitted by the notifier), the respiratory exposure of the workers during re-entry activities was calculated. Based on the molecular structure and the Derek analysis (see B.6.8.1.3) it is assumed that the toxicity of dichloro-etridiazole is comparable to the toxicity of etridiazole. Therefore, the AOEL derived for etridiazole is also used for the risk assessment of dichloro-etridiazole (see appendix 3, 4.2 for more information).

The calculations of operator and worker exposure are presented in appendix 3.

Dermal absorption of etridiazole in humans when exposed to the undiluted formulation was estimated to be 18%, and 30% for the diluted formulation, based on the *in vivo* rat data described in section B.6.12. For mixing and loading activities, the dermal absorption for the undiluted formulation was used. For inhalation exposure a default value of 100% was used.

The basic assumptions, input data and calculations used in the risk assessment for the use of ATTERRA ME are further specified in Appendix 3.

B.6.14.2 Internal exposure and risk assessment

Internal exposures and risk- assessments, using the standard UK and DE models, are specified in Table 6.14.1.1.

Table 6.14.2.1 Operator internal exposure and risk assessment

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Mixing/loading AATTERRA ME for application on cucumber via drip irrigation						
UK- 75th	Respiratory	-	-	1.8	-	-
	Dermal	2.52	0.13	1.8	140	7

Model	Route	Estimated internal exposure (mg a.s./day)		AOEL Systemic * (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
	Total	**2.52**	**0.13**	**1.8**	**140**	**7**
DE- GM	Respiratory	0.0005**	0.0005**	2.1	0.02	0.02
	Dermal	0.3629	0.0036	2.1	17	0.2
	Total	**0.36**	**0.004**	**2.1**	**17**	**0.2**
Mixing/loading AATTERRA ME for application on tomato and pepper via drip irrigation						
UK- 75th	Respiratory	-	-	1.8	-	-
	Dermal	3.78	0.19	1.8	210	11
	Total	**3.78**	**0.19**	**1.8**	**210**	**11**
DE- GM	Respiratory	0.001**	0.001**	2.1	0.05	0.05
	Dermal	0.7258	0.007	2.1	35	0.3
	Total	**0.73**	**0.008**	**2.1**	**35**	**0.4**
Mixing/loading AATTERRA ME for application on ornamentals via drip irrigation						
UK- 75th	Respiratory	-	-	1.8	-	-
	Dermal	25.2	1.26	1.8	1400	70
	Total	**25.2**	**1.26**	**1.8**	**1400**	**70**
DE- GM	Respiratory	0.0084**	0.0084**	2.1	0.4	0.4
	Dermal	6.048	0.0605	2.1	288	3
	Total	**6.06**	**0.07**	**2.1**	**288**	**3**

* Assuming a body weight of 60 kg for UK-POEM and 70 kg for German model
\- No data available
** exposure might be an underestimation, because exposure to vapours is not considered in the German model, see also below for further calculations

Calculated data for respiratory exposure of the operators during mixing and loading might be underestimations, because exposure to vapours is not considered in the German model.
No data were available on the exposure of operators during application.

The notifier submitted estimations of concentrations of etridiazole and dichloro-etridiazole in air due to volatilisation (Klein, 2006). See appendix paragraph 4.1 for further information. Based on these estimates, the respiratory exposure of the operator was calculated.

Table 6.14.2.2 Estimated respiratory exposure of the operator

	Estimated respiratory exposure of the operator to etridiazole (mg/day)	Estimated respiratory exposure of the operator to dichloro-etridiazole (mg/day)
Vegetable crops	0.013 (= 0.6% of the AOEL)	0.0015 (= 0.07% of the AOEL)
Ornamentals	0.168 (= 8% of the AOEL)	0.019 (= 0.9% of the AOEL)

The calculations in Table 6.14.2.2 illustrate that the respiratory exposure of the operator was indeed underestimated by the German model. However, the additional calculations of the respiratory exposure based on estimated air concentrations, do not change the conclusions drawn based on calculations with UK-POEM and the German model.

Table 6.14.2.3 Worker internal exposure and risk assessment for etridiazole

Model *	Route	Estimated internal exposure (mg a.s./day)		AOEL systemic ** (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Re-entry exposure during and after application of AATERRA ME via drip irrigation in vegetables						
	Respiratory	0.067	n.a.	2.1	3	n.a.
	Dermal	-	-	-	-	-
	Total	**0.067**	**n.a.**	**2.1**	**3**	**n.a.**
Re-entry exposure during and after application of AATERRA ME via drip irrigation in ornamentals						
	Respiratory	0.840	n.a.	2.1	40	n.a.
	Dermal	-	-	-	-	-
	Total	**0.840**	**n.a.**	**2.1**	**40**	**n.a.**

* No model available. Respiratory exposure of the worker was calculated based on estimated air concentrations.
* * Assuming a body weight of 70 kg
n.a. Not applicable
- The dermal exposure is not quantifiable.

Table 6.14.2.3 Worker internal exposure and risk assessment for dichloro-etridiazole

Model *	Route	Estimated internal exposure (mg a.s./day)		AOEL systemic ** (mg a.s/day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Re-entry exposure during and after application of AATERRA ME via drip irrigation in vegetables						
	Respiratory	0.0076	n.a.	2.1	0.4	n.a.
	Dermal	-	-	-	-	-
	Total	**0.0076**	**n.a.**	**2.1**	**0.4**	**n.a.**
Re-entry exposure during and after application of AATERRA ME via drip irrigation in ornamentals						
	Respiratory	0.094	n.a.	2.1	4.5	n.a.
	Dermal	-	-	-	-	-
	Total	**0.094**	**n.a.**	**2.1**	**4.5**	**n.a.**

* No model available. Respiratory exposure of the worker was calculated based on estimated air concentrations.
* * Assuming a body weight of 70 kg
n.a. Not applicable
- The dermal exposure is not quantifiable.

It is possible that after volatilisation of etridiazole and dichloro-etridiazole, some deposition on the plants will subsequently occur. This might result in dermal exposure of the worker. However, the amount of deposition cannot be quantified. Furthermore, it should be taken into account that the dermal absorption is 30% (for a low area dose, see list of endpoints). Given the relatively low respiratory exposure (see % AOEL), it is not expected that the potential dermal absorption will contribute significantly to the total exposure.

Conclusions

Based on the available data from the exposure models and the additional calculations of the respiratory exposure, the following conclusions can be drawn:

- Safe uses for operators without PPE were identified for substrate grown cucumber, tomato and pepper, using the German model. Safe uses for operators with PPE were identified for substrate grown cucumber, tomato and pepper, and non-soil bound ornamentals using the German model and UK-POEM.
- No bystanders should be allowed in greenhouses during the application of AATERRA ME.
- Safe uses for workers without PPE were identified for substrate grown cucumber, tomato and pepper, and non-soil bound ornamentals, based on estimated air concentrations of etridiazole and dichloro-etridiazole.

B.6.15 REFERENCES RELIED ON

Section B.6 Toxicology and metabolism (Annex IIA, point 5, Annex IIIA, point 7)

References for the active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 5.1/01	Markham, P.	1994	Disposition and metabolism of terrazole in rats. Report [redacted], [redacted] No. 9314 Ter – 89, part 2 GLP, Unpublished.	Y	CHEM
IIA, 5.1/02	McManus, J.P., Markham, P.	1995	Metabolism of etridiazole (Terrazole) in the rat - metabolite identification and quantification Report [redacted] [redacted]. No.9314 TER – 89, part 1 GLP, Unpublished	Y	CHEM
IIA, 5.1/03	Thalacker, F.W.	1997	Pharmacokinetics and tissue distribution of 14C-etridiazole in rats [redacted], Project No. CHW 611-157, 4 April 1997 TER - 91 GLP, Unpublished	Y	CHEM
IIA, 5.2.1/01	Warshawsky, L.D.	1994	Acute oral toxicity study in rats Report [redacted] No.399-148 TER - 92 GLP, Unpublished	Y	CHEM
IIA, 5.2.2/01	Warshawsky, L.D.	1994	Acute dermal toxicity study in rabbits. Report [redacted] No.399-149 TER - 93 GLP, Unpublished	Y	CHEM
IIA, 5.2.3/01	Hilaski, R.J.	1994	EPA (FIFRA) acute inhalation toxicity evaluation on Terrazole technical in rats. Report [redacted] No.399-147 TER - 94 GLP, Unpublished	Y	CHEM
IIA, 5.2.4/01	Warshawsky, L.D.	1994	Primary dermal irritation test in rabbits following a 4 hour exposure period - Terrazole technical Report [redacted]. No.399-150 TER - 95 GLP, Unpublished	Y	CHEM
IIA, 5.2.5/01	Warshawsky, L.D.	1994	Primary eye irritation study in rabbits - Terrazole technical Report [redacted]. No.399-151 TER - 96 GLP, Unpublished	Y	CHEM
IIA, 5.2.6/01	Parcell, B.I.	1993	Etridiazole: skin sensitisation in the guinea-pig Report [redacted] No.921004D/URL 93/SS TER - 97 GLP, Unpublished	Y	CHEM
IIA, 5.3.2/01	Richards, J.F.	1994	Etridiazole: 13 week oral (dietary administration) sub-chronic toxicity study in the rat with a 4 week treatment-free period Report [redacted] No.217/14-1050 TER - 99	Y	CHEM

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			GLP, Unpublished		
IIA, 5.3.2/03	Goldenthal, E.I.	2002	One year dietary toxicity study in dogs Report [redacted] No.399-208 TER - 101 GLP, Unpublished	Y	CHEM
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IIA, 5.4.1/06	San, R.H.C., Sly, J.E.	1995	*In vivo - in vitro* rat hepatocyte unscheduled DNA synthesis assay Report [redacted] No.G94BN72.381 TER - 107 GLP, Unpublished	Y	CHEM
IIA, 5.4.1/07	Miyagawa, M.	1995	*In vivo-in vitro* replicative DNA synthesis (RDS) test using rat livers on etridiazole Report [redacted] No.4B547 TER - 108 GLP, Unpublished	Y	CHEM
IIA, 5.4.1/08	Edwards, G.N., Forster, M.A.	1987	Induction of mitotic aneuploidy in S. Cerevisiae D6 - test substance: etridiazole technical Report Life Science Research, Roma Toxicology Centre S.P.A., Italy No.131003-M-	Y	CHEM

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
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IIA, 5.4.2/01	Banduhn, N.	1985	Mouse micronucleus assay with Terrazole technical Report [redacted] No.053651 TER - 110 GLP, Unpublished	Y	CHEM
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IIA, 5.5/03	Trutter, J.A.	1988	Oncogenicity study in rats with Terrazole technical. Report [redacted] No.798-210 TER - 117 GLP, Unpublished	Y	CHEM
IIA, 5.5/02	Goldenthal, E.J.	2004	18-months dietary carcinogenicity study in mice. Report [redacted] No. 399-312 GLP, Unpublished	Y	CHEM
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