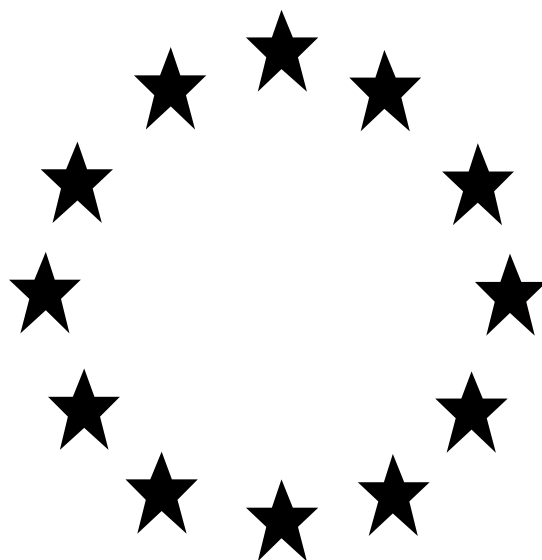




Draft Assessment Report (DAR)

- public version -

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

DICHLOBENIL

**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

January 2008

B.6 TOXICOLOGY AND METABOLISM

The toxicity studies have been carried out with the test substance dichlobenil (also called 2,6-dichlorobenzonitrile, H133, CASORON) and the preparation Casoron G (6.75% dichlobenil).

The metabolite tested is 2,6-dichlorobenzamide (BAM), soil metabolite of dichlobenil and becomes available as residue in plants.

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

B.6.1.1 Toxicokinetic studies

B.6.1.1.1 Excretion study

Characteristics of the study

Reference	: Koorn <i>et al.</i> , 1987	Route	: oral (gavage) and i.v.
Year of execution	1987	Group size	: 5 sex/dose (intact), 3/dose (bile duct cannulated, males only)
Guideline	: OECD 417	Exposure	: single
GLP	yes	Doses	: 6.7 mg/kg bw (oral), 5.6 mg/kg bw (i.v.)
Test substance	: [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87A29C; appearance not indicated, s.a. 145 MBq/g	Vehicle	: 1% tragacanth-gum (oral) PEG 400/benzylalcohol/isopropylidene glycol (15/2/60% (w/v)) in water (i.v.)
Purity	99.7 (radiochemical), 103.3% (chemical)	Acceptable	: limited
Species	rat, Sprague-Dawley		

Study design

Animals were exposed as described above and held in excretion cages. Urine was collected at 6, 24, 48, 72, 96 and 168 hours after administration, faeces was collected at 24, 48, 72, 96 and 168 hours. In bile cannulated animals bile was collected at 2, 5 and 24 hours after administration and urine at 5 and 24 hours. At 168 hours the intact animals were sacrificed, and blood and the liver were collected.

Radioactivity in cage washes, urine, faeces, plasma and liver were measured by (combustion) LSC.

Results

Table 6.1.1.1 Cumulative excretion in rats of total radioactivity, 24 and 168 h after oral or i.v. exposure to [phenyl-U-¹⁴C]-dichlobenil (Excretion expressed as percentage of the administered radiolabel)

Fraction	0-24 h			0-168 h	
	Dose group			Dose group	
	single oral intact m-f	single oral cannulated m	i.v. intact m-f	single oral intact m-f	i.v. intact m-f
Urine	62-63	20 ^a	67-62	65 ^a -65 ^a	70 ^a -65 ^a
Faeces	18-19	NC	25-30	19-21	25-31
Bile	NC	79	NC	NC	NC
Total excreted ^b	80-82	99	92-92	84-86	96-96

NC = not collected

Radiolabel recovered from plasma and liver was not reported as % of administered dose (only as concentration) and their weights were also not reported.

^a Including final cage wash

^b Due to round off slight differences may occur between the sums of the individual values and the totals listed.

Based on the comparison of urinary excretion after oral and i.v. doses, oral absorption was 89% in males and 97% in females¹.

Residual radiolabel in the liver, 168 h after oral or i.v. administration, did not differ significantly between routes and sexes and varied between 0.08 and 0.12 mg eq./kg. Residual radiolabel in plasma was beneath the limit of quantification (0.06 mg eq./kg) for all animals except 4 of the 5 i.v. dosed males, which demonstrated concentrations between 0.13 and 0.17 mg eq./kg.

Acceptability

No complete recovery was obtained, as residual carcass was not assayed for radioactivity. Therefore, the recovered radioactivity in the oral dose groups was somewhat lower than the minimum of 90% required by OECD guideline 417. Furthermore, only excretion was investigated and only single low doses used. Therefore, the value of this study for the evaluation of the toxicokinetics of Dichlobenil is limited. In section B.6.1.4 the overall acceptability of all submitted toxicokinetic studies with dichlobenil is discussed.

B.6.1.1.2 Distribution study

Characteristics of the study

Reference	: Kaldenberg and De Lange, 1988	Route	: oral
Year of execution	: 1987	Group size	: 2 sex/dose/time-point
Guideline	: OECD 417	Exposure	: gavage
GLP	: yes	Doses	: 2.5 mg/kg bw
Test substance	: [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87A30B; appearance not indicated, s.a. 692 MBq/g	Vehicle	: 1% tragacanth-gum
Purity	: 99.6 (radiochemical), 102.4%	Acceptable	: limited

¹ Calculated using the following formula $\frac{\% \text{radiolabel in urine (oral)}}{\% \text{radiolabel in urine (i.v.)}} \times 100$

Species (chemical)
rat, Sprague-Dawley

Study design

Animals were exposed as described above and held in excretion cages. Two animals at a time of each sex were sacrificed at 1, 2, 6, 24, 168 and 336 hours, and whole blood, plasma, liver, kidney, lung, heart, brain, spleen, muscle, bone, gonads, brown-fat and kidney-fat were collected. The urine and faeces from the rats killed at 168 and 336 hours was collected for 24 hours. After final collection, and for the rats killed at 336 hours also after 168 hours, cages were rinsed. Radioactivity in all samples and organs was measured by (combustion) LSC.

Results

Table 6.1.1.2a Cumulative excretion in rats of total radioactivity, 168 h after oral exposure to [phenyl-U-¹⁴C]-dichlobenil (Excretion expressed as percentage of the administered radiolabel $\pm$ SD (n=4))

Fraction	male	female
Urine ^a	75.1 $\pm$ 4.8	55.8 $\pm$ 7.1
Faeces	23.8 $\pm$ 10.5	20.6 $\pm$ 3.1
<i>Total excreted</i>	<i>98.7 $\pm$ 7.4</i>	<i>76.4 $\pm$ 7.3</i>

NC = not collected

^a Excluding cage washes

The authors of the report stated that the difference in urinary excretion observed between males and females was not statistically significant, without specifying the statistical test used. The reviewer applied a two-sided, unpaired, heteroschedastic t-test to the reported results, and did observe a statistical difference (p-value 0.006). So the difference does appear to be significant, and these results are not in line with the results of the excretion study reported above (B.6.1.1.1). In that study no difference was observed between males and females (urinary excretion 65% for both sexes, 168 h after administration). From the comparison of the concentrations of radioactivity observed in the tissues and organs of male and female rats at this time-point, it appears that at least part of the unexcreted radiolabel (24% of the administered radiolabel) may have been retained.

In all tissues and organs the highest concentrations of radioactivity were found between 1 and 3 hours after administration. Liver, kidney and kidney-fat retained the highest concentrations of radiolabel, peaking 1 hour after administration. No large differences in distribution were observed between males and females for most organs and tissues at most time-points, except plasma and brown-fat concentrations at 168 and 336 hours: In males plasma concentrations were ca. 3 times higher than in females, while for brown-fat the reverse was encountered.

The results show that dichlobenil is rapidly absorbed and distributed. After the highest concentrations have been reached, the concentrations rapidly decline showing no potential for accumulation.

Table 6.1.1.2b Concentration of radioactivity in some tissues and organs of male rats, after oral exposure to [phenyl-U-¹⁴C]-dichlobenil

Concentrations expressed mg eq./kg (n=2)

Tissue/organ	T=1 h	T=3 h	T=6 h	T=24 h	T=72 h	T=168 h	T=336 h
plasma	0.93	0.45	0.27	0.030	0.019	0.021	0.019
blood	0.64	0.30	0.18	0.021	0.011	0.008	0.007
liver	3.29	1.63	0.77	0.204	0.108	0.048	0.014
kidney	4.10	1.65	0.86	0.075	0.033	0.017	0.008
brown-fat	0.91	0.48	0.14	0.023	0.005	0.017	<0.006
kidney-fat	2.85	3.49	0.25	0.027	<0.011	0.053	0.014

Table 6.1.1.2c Concentration of radioactivity in some tissues and organs of female rats, after oral exposure to [phenyl-U-¹⁴C]-dichlobenil

Concentrations expressed mg eq./kg (n=2)

Tissue/organ	T=1 h	T=3 h	T=6 h	T=24 h	T=72 h	T=168 h	T=336 h
plasma	0.80	0.48	0.30	0.020	0.007	0.007	<0.006
blood	0.51	0.30	0.20	0.026	0.015	0.012	0.008
liver	2.13	0.98	1.06	0.252	0.111	0.038	0.016
kidney	3.33	2.68	1.58	0.097	0.037	0.022	0.013
brown-fat	0.89	0.33	0.41	0.023	0.016	0.048	0.014
kidney-fat	3.01	1.37	0.84	0.163	0.017	0.087	0.032

Acceptability

No complete recovery was obtained, as the absolute or relative amounts of radiolabel recovered from tissues, organs and residual carcass were not reported. Furthermore, only the excretion data of the animals exposed for 168 and 336 hours were presented. Only distribution was investigated and only a single low dose was used. Therefore, the value of this study for the evaluation of the toxicokinetics of dichlobenil is limited. In section B.6.1.4 the overall acceptability of all submitted toxicokinetic studies with dichlobenil is discussed.

B.6.1.1.3 Excretion and distribution study**Characteristics of the study**

Reference	: Cameron <i>et al.</i> , 1988	Route	: oral
Year of execution	: 1987	Group size	: 5 sex/dose
Guideline	: OECD 417	Exposure	: gavage, 11 d (day 1 and 11 radiolabelled a.s., day 2-10 cold a.s.)
GLP	: yes	Doses	: 3.75, 30 and 240 mg/kg bw/d
Test substance ^a	: [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87A30B; appearance not indicated, s.a. 692 MBq/g (low dose) [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87C25B; appearance not	Vehicle	: 1% tragacanth

	indicated, s.a. 74 MBq/g (mid dose) [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87C25A; appearance not indicated, s.a. 9.25 MBq/g (high dose) Dichlobenil, batch no. FUX003000/FUN81L08A, appearance not indicated labelled a.s.: >99% (radio-chemical), chemical purity not indicated	Acceptable	: limited
Purity	cold a.s.: 98.8%		
Species	rat, Charles River CD remote Sprague-Dawley		

^a Data copied from study B.6.1.1.4

Study design

Animals were exposed as described above and held in metabolism cages. Urine was collected at 6 h after each radioactive dose, and thereafter daily up to the time of sacrifice, 192 h after the last dose was administered. Faeces were collected daily up to the time of sacrifice. At sacrifice whole blood, plasma, residual carcass, liver and kidneys were removed for analysis. Radioactivity in all biological samples and cage washes was measured using (combustion) LSC.

Results

Table 6.1.1.3a Cumulative excretion of radioactivity in rats, 24 h after the first and the last dose of repeated oral exposure to dichlobenil (Excretion expressed as percentage of the administered radiolabel (single dose))

Fraction	0-24h			240-264h		
	First dose (mg/kg bw/d)			Last dose (mg/kg bw/d)		
	3.75 m-f	30 m-f	240 m-f	3.75 m-f	30 m-f	240 m-f
Urine	64-66	60-63	40-28	55-58	61-56	47-41
Cage wash	2.7-2.6	4.9-2.0	1.3-1.7	3.6-2.2	1.8-1.6	2.5-3.0
Faeces	16-15	11-9	3.5-4.1	22-17	15-20	18-16
Total excreted ^a	83-84	76-74	45-34	81-77	78-78	68-60

^a Due to round off slight differences may occur between the sums of the individual values and the totals listed.

Table 6.1.1.3b Retention and cumulative excretion of radioactivity in rats, 0-240 and 240-432h after repeated oral exposure to dichlobenil (Excretion expressed as percentage of the administered radiolabel (single dose))

Fraction	0-240h			240-432h		
	First dose (mg/kg bw/d)			Last dose (mg/kg bw/d)		
	3.75 m-f	30 m-f	240 m-f	3.75 m-f	30 m-f	240 m-f
Urine	67-69	65-69	64-55	59-62	64-57	52-47
Cage wash	3.3-3.2	5.7-2.6	3.1-3.5	4.2-4.6	2.8-2.4	3.3-4.0
Faeces	20-20	18-18	15-18	25-20	18-22	23-21
Total excreted ^a	90-92	89-90	83-77	87-87	85-81	78-72
Residual carcass	NC	NC	NC	0.33-0.33	0.32-0.22	0.22-0.22
Tissues/organs	NC	NC	NC	<0.01	<0.01	<0.01
Recovery ^a	90-92	89-90	83-77	87-87	86-83	78-72

^a Due to round off slight differences may occur between the sums of the individual values and the totals listed.

NC = not collected

No consistent differences appear to exist between the different dose groups and between sexes.

Some differences are observed between the amount of radiolabel recovered from urine in the various groups, but they seem to reflect the lower recovery in those groups. Based on radiolabel recovered from urine and cage wash, oral absorption is at least ca. 70% after the first dose and ca. 60% after the last dose, irrespective of dose or sex.

Table 6.1.1.3c Concentration of radiolabel in liver, kidney, whole blood and plasma in rats, 192h after the last dose repeated oral exposure to dichlobenil

Concentration expressed as mg eq./kg (L)

Organ/tissue	Dose (mg/kg bw/d)		
	3.75 m-f	30 m-f	240 m-f
Liver	0.06-0.07	0.31-0.39	2.65-2.62
Kidney	0.02-0.03	0.12-0.22	1.98-2.19
Whole blood	0.01-0.02	0.06-0.07	0.30-0.43
Plasma	<0.01	0.01-0.01	0.20-0.28

Acceptability

Recovery of especially the last dose of this repeated dose study was below the minimum of 90% required by the guideline. Furthermore no complete inventory of tissues and organs was separately assayed for radioactivity, and no expired air was analysed. Therefore this study is considered of

limited value for the assessment of dichlobenil toxicokinetic in rats. In section B.6.1.4 the overall acceptability of all submitted toxicokinetic studies with dichlobenil is discussed.

B.6.1.1.4 Metabolism study

Characteristics of the study

Reference	: Vincent <i>et al.</i> , 1989	Route	: oral
Year of execution	: 1987-1989	Group size	: 5 sex/dose
Guideline	: OECD 417	Exposure	: gavage, 11 d (day 1 and 11 radiolabelled a.s., day 2-10 cold a.s.)
GLP	: yes	Doses	: 3.75, 30 and 240 mg/kg bw/d
Test substance	: [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87A30B; appearance not indicated, s.a. 692 MBq/g (low dose) [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87C25B; appearance not indicated, s.a. 74 MBq/g (mid dose) [phenyl-U- ¹⁴ C]dichlobenil, batch no. AOI87C25A; appearance not indicated, s.a. 9.25 MBq/g (high dose)	Vehicle	: 1% tragacanth
Purity	: >99% (radiochemical), chemical purity not indicated	Acceptable	: limited
Species	: rat, Charles River CD remote Sprague-Dawley		

Study design

In this study metabolites from urinary and faecal samples from study B.6.1.1.3 were identified. Animals were exposed as described above and held in metabolism cages. Urine was collected at 6h after each radioactive dose, and thereafter daily up to the time of sacrifice, 192h after the last dose was administered. Faeces were collected daily up to the time of sacrifice. For metabolite fingerprints the 0-48h and 240-288h urine samples were pooled separately for males and females at the 3 dose levels. For faeces, the 0-24 or 0-48 and 240-264 or 240-288h samples were also pooled. For isolation and identification of metabolites, the 0-48h and 240-288h urine and faeces samples of the highest dose group were pooled. Radioactivity in urine and faeces samples was measured using (combustion) LSC.

Samples were tested for the presence of conjugates by incubation with β -glucuronidase and aryl sulphatase. Metabolic fingerprints were obtained via HPLC using a gradient programme composed of water and methanol. The radio-activity in the effluent collected in fractions was monitored with a Ramona detector. For identification, metabolite fractions were purified by rotary evaporation of methanol, then passing them through the HPLC column again and finally subjecting them to thin-layer chromatography. At the end of each step, a radio-activity balance was determined and recovery was calculated. Low recoveries were checked for selective losses by developing a metabolite pattern and comparing it with the preceding one. Identification was executed by ¹H-NMR, ¹³C-NMR, mass spectrometry and GC/MS and IR-spectrometry.

Results

Dose rising and multiple dosing tend to saturate glutathione conjugation. The amount of parent compound recovered from faeces increases with dose. At mid dose, there is a particularly great difference in recovery of parent compound from faeces between first and last dose, respectively ca. 22% and 61% of the recovered radiolabel (average of both sexes).

Below the metabolite patterns after the last dose of the repeated doses are presented.

Table 6.1.1.4 Metabolite patterns in rats, 192 h after the last dose of repeated oral exposure to dichlobenil (Values expressed as % of total radioactivity recovered from the respective excretion product)

Dose (mg/kg bw/d)	Urine			Faeces		
	3.75	30	240	3.75	30	240
Metabolite	m-f	m-f	m-f	m-f	m-f	m-f
A (conjugate)	3-3	4-5	5-6	--	--	--
B (conjugate)	11-6	5-4	3-4	--	--	--
C (conjugate)	2-4	3-3	8-3	--	--	--
D (conjugate)	20-12	29-25	30-14	19-8	5-7	--
E (conjugate)	6-2	4-2	2-1	--	--	--
F ₁ (conjugate)	2-2	4-3	3-1	--	--	--
F ₂ (conjugate)	18-13	14-10	9-14	14-5	5-9	--
G (conjugate)	16-24	11-13	5-7	--	--	--
H	13-24	16-27	22-37	28-25	8-11	5 ^a
I	2-3	3-4	4-6	--	--	--
K	--	--	--	--	--	(<3)
L	--	--	--	--	--	(<3)
Dichlobenil (parent compound)	--	--	--	6-4	60-62	93 ^a
Total	93-93	93-96	91-93	67-42	78-89	98^a

^a Male and female fractions combined

The authors did not provide the chemical names of the identified metabolites. In section B.6.1.3, the structural formulas are depicted as part of the proposed biochemical pathway.

Acceptability

Recovery of especially the last dose of this repeated dose study was below the minimum of 90% required by the guideline (see summary of study B.6.1.1.4). However, this is not considered to have influenced metabolite patterns in urine and faeces. According to the study reports by Cameron *et al.* (1988) and Vincent *et al.* (1989) the samples of the animal study were stored at -20 °C, but in the report of the metabolism study, which was executed up to 2 years later, it is not mentioned how the stability of the samples was ensured and monitored. However, as all identified metabolites are stable at -20 °C, quantification is considered reliable (based on expert judgement). As it does not cover all

aspects of toxicokinetics, this study is considered of limited value. In section B.6.1.4 (page 76 and further) the overall acceptability of all submitted toxicokinetic studies with dichlobenil is discussed.

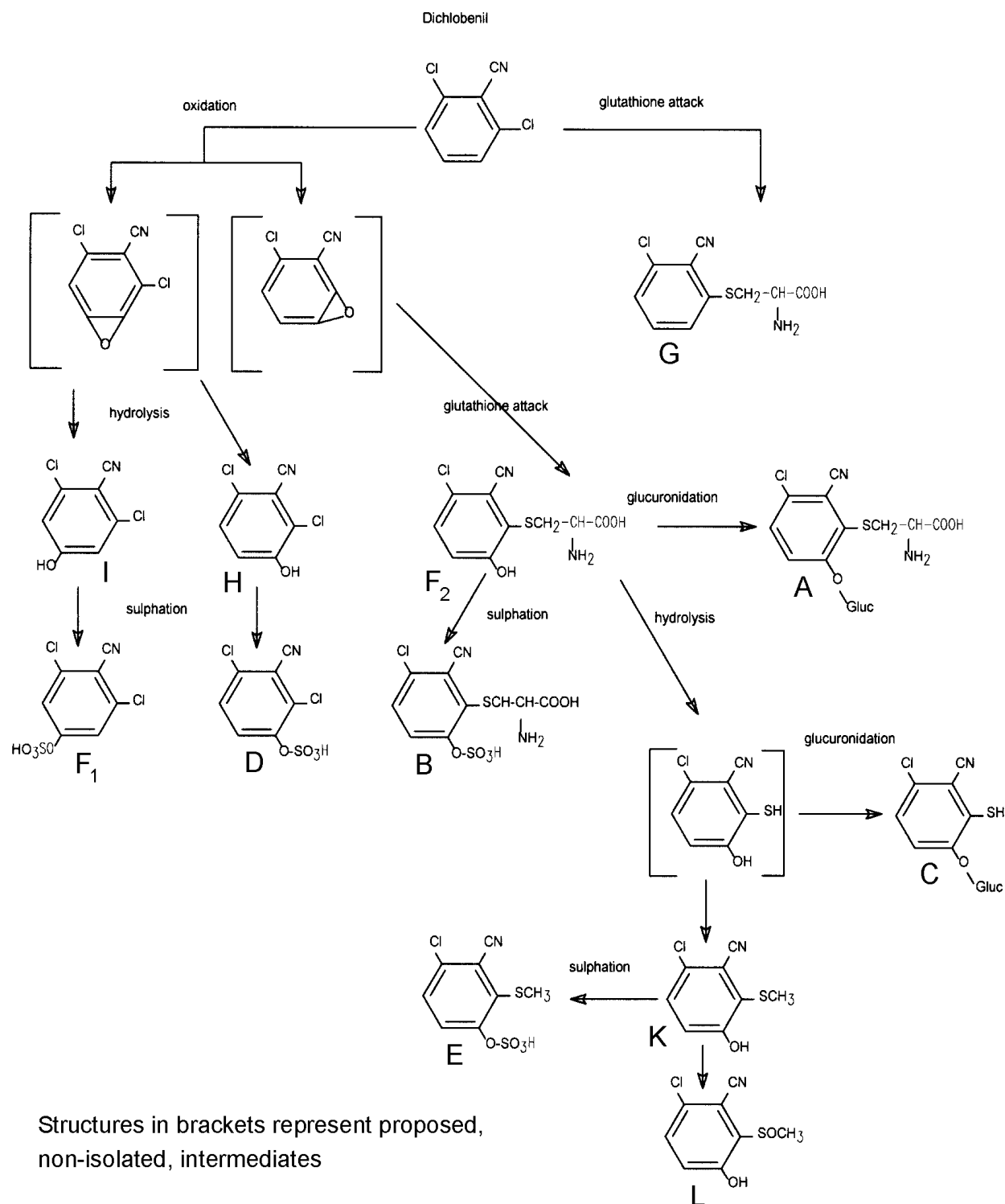
B.6.1.2 List of identified metabolites

All metabolites were identified in *in vivo* experiments with rats.

Code	Metabolite	Matrix
A	No chemical names provided in submitted reports	U
B		U
C		U
D		U, F
E		U
F ₁		U
F ₂		U, F
G		U
H		U, F
I		U
K		F
L		F

F = faeces; U = urine

B.6.1.3 Proposed metabolic pathway



the basis of this document.

WARNING: This doc

B.6.1.4 Summary and conclusions

Absorption

In one rat study with single low dose (ca. 6 mg/kg bw) in which oral and i.v. administration were compared, oral absorption was 89-97% (m-f).

In the rat, oral absorption after repeated exposure is at least approximately 60%, irrespective of dose and sex, based on radiolabel recovered from urine and cage wash. However, as the liver is the critical target organ for dichlobenil toxicity, it is the amount of compound which has passed the liver, that should be considered in risk assessment. After repeated low dose, the amount absorbed from the gut into the liver is more than 80% (see below under "Excretion").

Excretion

In rats, radiolabel is primarily excreted via the urine after oral exposure to [¹⁴C]-dichlobenil. Around 60% is excreted in the urine and around 20% in the faeces, irrespective of sex or dose regime, 9 to 10 days after initiation of exposure. Increasing dose or multiple dosing slows down the rate of excretion, without changing the final amount excreted. After repeated dosing, approximately 80% of the last administered dose is excreted within 24 h after low and mid dose (4 and 30 mg/kg bw/d), while for high dose (240 mg/kg bw/d) ca. 65% was excreted within 24 h.

After single oral low dose in rats, a substantial amount of the administered radioactivity is excreted in bile (80% in the first 24 h hours after administration). As the majority of the radiolabel ended up in the urine, enterohepatic cycling takes place with part of the radiolabel ultimately becoming systemically available and excreted via urine. As comparison with a concurrent i.v. experiment indicates that more than ca. 90% of the administered single oral dose becomes systemically available, also part of the faecally excreted radiolabel must have been excreted from systemic circulation. As with other dose regimes (multiple and/or increased) no i.v. and bile cannulation experiments were executed, the quantitative importance of biliary excretion and systemic absorption can not be definitely established for those dose regimes. However, with respect to low repeated dose, absorption from the gut is likely to be higher than 80% as only a low percentage of parent compound is found in faeces (less than 10%) and all metabolites identified are probably liver metabolites. In bile and urine no parent compound was detected at any dose regimen, and in faeces the relative amount of parent compound increases with increasing and multiple doses (see below "Metabolism"). Therefore, once it enters the liver dichlobenil appears to immediately be metabolised, while no metabolism takes place in faeces. Consequently ca. 90% of the faecal radiolabel (non-parent compound) will have originated from the liver via the bile, representing ca. 20% of the administered dose, which should be added to the radiolabel recovered from urine and the cage wash (respectively, ca. 60 and 4% at repeated low dose) in order to obtain the amount of administered radiolabel which has been absorbed from the gut.

In one study with single oral low dose (significant) differences in excretion between males and females were observed. However, two other studies, among which one with single and multiple low, mid and high doses, did not demonstrate these differences. Therefore this difference is considered to have occurred by coincidence.

Distribution

After single low dose (2.5 mg/kg bw/d) in rats, most radiolabel is recovered from liver, kidney, kidney-fat and brown-fat. In all tissues and organs investigated, concentrations peak between 1 and 3 hours after administration, in liver and kidney even after 1 hour. Therefore, radiolabel appears to be rapidly distributed and excreted. Seven days after administration little radioactivity was retained; the highest concentrations were observed in liver (0.048-0.038 mg eq./kg (m-f)), kidney (0.017-0.022 mg eq./kg), kidney-fat (0.053-0.087 mg eq./kg) and brown-fat (0.017-0.048 mg eq./kg). No potential for accumulation was observed.

After single low dose in rats, no large differences in distribution were observed between males and females for most organs and tissues at most time-points, except plasma and brown-fat concentrations at 168 and 336 hours: In males plasma concentrations were ca. 3 times higher than in females, while for brown-fat the reverse was encountered. This may be indicative of a somewhat higher tendency for accumulation in females as compared to males. However, in no other metabolic studies were sex differences observed.

In another study with rats various distributions after various oral dose regimes (single/multiple, low/mid/high) was studied. In all cases less than 0.01% of the last administered dose was recovered from selected tissues and organs (blood, plasma, liver and kidneys), while between 0.2 and 0.4% was encountered in residual carcass, eight days after administration of the last dose. After repeated oral low dose (ca. 4 mg/kg bw/d), 0.06-0.07 mg eq./kg were recovered from liver and 0.02-0.03 mg eq./kg from kidney, eight days after the last administered dose. When comparing this to the result of the study described above, it appears multiple dosing does not lead to accumulation in the liver or the kidneys. After repeated mid dose (30 mg/kg bw/d), concentrations in selected tissues and organs were a factor 5 to 7 higher than after repeated low dose, while after repeated high dose this was approximately a factor 40, reflecting the dose range applied.

Metabolism

In rats, ten metabolites were identified in urine and no parent compound. Most metabolites were conjugates such as sulphate, glutathione (and derivatives) and glucuronic acid conjugates. In faeces, three major metabolites (each representing 5% of the administered dose or more) were observed as well as parent compound (Dichlobenil). The proportion of conjugated metabolites decreases with increasing and multiple doses, while the amount of parent compound in faeces increases. No

chemical names were provided for the identified metabolites. Their structures are depicted in the metabolic pathway shown in section B.6.1.3.

Overall acceptability

Although the individual studies do not cover the complete scope of toxicokinetic parameters to be investigated, and they show some short-comings (e.g. with respect to reporting and recoveries), the combination of the four submitted studies is considered to provide sufficient reliable information on the toxicokinetics of dichlobenil and are therefore considered acceptable.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

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

B.6.2.1 Acute toxicity

B.6.2.1.1 Acute oral toxicity study

Characteristics of the study

Reference	: Graver, 1999	Route	: oral
Year of execution	: 1999	Group size	: 5/sex
Guideline	: OECD 401	Exposure	: once by gavage
GLP	: yes	Doses	: 2000 mg/kg bw
Test substance	: Dichlobenil, Technical lot FLE99A21C; white powder	Vehicle	: corn oil
Purity of a.s.	: 99.8%	Acceptability	: yes
Species	: rats, Wistar albino	LD ₅₀	: >2000 mg/kg bw

Study design

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

Results

Mortality: nine of ten animals survived the 2000 mg/kg oral dose. One male was sacrificed on day 2 due to moribund condition.

Symptoms of toxicity: lethargy, chromorhinorrhea, ataxia, sagging eyelids, soiling of the anogenital area, diarrhea, red staining of the nose/mouth, wetness of the anogenital area and localized alopecia.

Body weight: no treatment-related findings

Pathology: kidney abnormalities were observed in 7 out of 9 survivors.

Acceptability

Although the room temperature and humidity of the animal room are not reported, the study is considered acceptable.

Conclusions

The acute oral LD₅₀ of dichlobenil was estimated to be >2000 mg/kg bw for rats.

B.6.2.1.2 Various short-term toxicity investigations of dichlobenil

Reference	: Arnoldussen <i>et al.</i> , 1963	Route	: Acute: oral and dermal Sub-acute: dermal/subcutaneous
Year of execution	: not indicated	Group size	: -
Guideline	: OECD 401	Exposure	: See text below
GLP	: no	Doses	: See text below
Test substance	: Dichlobenil, batch number; appearance not indicated	Vehicle	: -
Purity of a.s.	: not indicated	Acceptable	: No/yes

Species	rabbits, rats, mice	acute oral LD ₅₀	:	4460 mg/kg bw
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Study design

In this report acute oral and dermal toxicity and sub-acute dermal toxicity studies in rats, mice and rabbits reported. Furthermore sub-acute subcutaneous/intraperitoneally toxicity study in rats is described. Due to various shortcomings, the limited number of parameters tested and very limited reporting of the study design and results only the relevant conclusions are presented.

Results

Acute oral toxicity in rats

Only from the acute toxicity trial with rats seems the study acceptable for LD₅₀ determination, although the reporting is limited. Dichlobenil was administered to the animals as a suspension in arachis oil by gavage to 10 groups of 10 Wistar rats (5 female and 5 male), doses of 2700 mg/kg bw till 8100 mg/kg bw. The main toxicological effects were histological abnormalities in the liver and kidney. The acute oral LD₅₀ of dichlobenil was estimated to be 4460 mg/kg bw for rats.

Acceptability

Only the acute oral toxicity study in rats is acceptable. The other studies are not acceptable, due various shortcomings, in view of the limit number of parameters tested and very succinct reporting of the study design and results.

Conclusions

The acute oral LD₅₀ of dichlobenil was estimated to be 4460 mg/kg bw for rats. No conclusions can be drawn from the other studies as they are not considered acceptable.

B.6.2.1.3 Acute dermal toxicity study

Study 1

Characteristics of the study

Reference	: Cerven, 2000	Route	: dermal
Year of execution	: 2000	Group size	: 5 (female)
Guideline	: OECD 402	Exposure	: 24 hours (occlusive)
GLP	: yes	Doses	: 2000 mg/kg bw
Test substance	: Dichlobenil, lot no. 41602018;	Vehicle	: distilled water
	white powder		
Purity of a.s.	: 100%	Acceptability	: yes
Species	: rabbits, New Zealand White	LD ₅₀	: >2000 mg/kg bw

Study design

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

Results

Mortality: none

Symptoms of toxicity: in one animal very slight erythema was observed at 24 hours, but was absent on days 7 and 14. No other signs of toxicity were observed.

Body weight: no treatment-related findings

Pathology: no treatment-related findings

Acceptability

Although the room temperature and humidity were not reported, the study is considered acceptable.

Conclusions

The acute dermal LD₅₀ of dichlobenil was estimated to be >2000 mg/kg bw for female rabbits.

Study 2

Characteristics of the study

Reference	: Johnson, 1994	Route	: dermal
Year of execution	: 1993/1994	Group size	: 5/sex
Guideline	: OECD 402	Exposure	: 24 hours (occlusive)
GLP	: yes	Doses	: 2000 mg/kg bw
Test substance	: Dichlobenil, batch no. FUN87E08A; white powder	Vehicle	: -
Purity of a.s.	: 98.8%	Acceptable	: no
Species	: rabbits, New Zealand White	LD ₅₀	: -

Study design

The study was not performed in accordance with OECD 402 (limit test), because only the application site was moistened and not the test substance itself.

Results

Mortality: two female rabbits were found dead on day 5

Symptoms of toxicity: under activity, pallor, cold to touch, pigmented staining of the snout and closed eyes. A gelatinous material was found in the cage under-trays of both decedents on days 3 and 4.

Body weight: no treatment-related findings

Pathology: necropsy of the decedents revealed blood staining around the mouth and nares of both animals, dark tracheal contents and dark areas on the left lung lobe of one animal and dark fluid in the thorax and abdomen of the other animal. The latter animal also had a thickened dermal application site with dark encrustations.

Necropsy of the surviving animals revealed no significant internal macroscopic lesions, although one animal had multiple dark areas in the musculature below the application site.

Acceptability

Because only the application site was moistened and not the test substance itself, the study is considered not acceptable.

Conclusions

The acute dermal LD₅₀ of dichlobenil for rabbits could not be determined.

B.6.2.1.4 Acute inhalation toxicity study

Study 1

Characteristics of the study

Reference	: Janssen and de Rooij, 1986	Route	: inhalation
Year of execution	: 1984	Group size	: 5/sex
Guideline	: OECD 403	Exposure	: 4 hours (nose only)
GLP	: yes	Concentration	: nominal: 0, 540 and 700 ¹ mg/m ³ , actual ² : >131 and >250 mg/m ³ . MMAD (±gsd): 2.59 (± 3.14) and 2.06 (± 2.90)
Test substance	: Dichlobenil, lot/batch no. FUX003000/FUN81L08A; appearance: white powder	Vehicle	: acetone
Purity of a.s.	: 98.8%	Acceptable	: no
Species	: rats, CPB-Wu Wistar	LC ₅₀	: -

¹ Highest attainable concentration

² Actual concentrations were estimated from total dust measurements and particle size distribution measurements.

Study design

The study was performed in accordance with OECD 403.

Results

Mortality: none

Symptoms of toxicity:

- During exposure: decreased respiratory frequency
- During observation period at 1.5 h after exposure:

High dose group (I): slight to moderate apathy, a moderate decreased alertness, a strongly decreased fear reaction, a slight to moderate decreased locomotor activity, a moderate abnormal touch reaction and moderate respiratory difficulties. Females showed a hunched back, an abnormal gait and a moderate pilo erection.

Low dose group (II): slight to moderate apathy, a moderately decreased alertness, fear and touch reaction, a slightly decreased locomotor activity and a slightly staggering gait.

Body weight: no treatment-related findings

Pathology: no treatment-related findings

Discussion

The test atmosphere was generated by nebulizing 3-5% solutions of dichlobenil in acetone. Although the generation efficiency of the dichlobenil particles obtained (30-60%) is high, the actual concentration of about 250 mg/m³ is low with regard to classification purposes. However, a 5% dichlobenil solution resulting in a nominal concentration of 700 mg/m³, also means that at least 100/5 x

700 mg/m³ = 14000 mg/m³ (6000 ppm) acetone has been generated in the test atmosphere (group I) and $100/3 \times 540 = 18000$ mg/m³ (7500 ppm group II). In other words, with such solutions at relatively low concentrations, it will never be possible to obtain high aerosol concentrations (i.e. at the limit concentration of 5000 mg/m³). It has not been indicated in the report whether other methods had been tried such as dry dispersion (eventually after grinding). Furthermore, although the control group (group III) had been exposed to acetone only, it was not indicated to which concentration of acetone.

Although the lowest LD50 value for acetone reported in literature was 24000 ppm, the high acetone concentrations present may have resulted in the slight decrease in breathing frequency. It should, however, be noted that breathing frequencies were not reported before exposure, so comparison with pre-exposure values is not possible.

Moreover, it is not correct to measure the actual concentration in the test atmosphere using a cascade impactor due to possible substantial losses (unless the whole cascade impactor is being rinsed, which was apparently not the case). Remarkable, though, concentrations measured by both methods (filter sampling and cascade impaction) were not very different from each other. Therefore, it is not understood why the average concentration was not given but only values larger than.

Furthermore, it has been indicated that the exposure chamber was kept under negative pressure, which may result in leak from outside to the inside alongside the animal in the restraining tube (in case of tubes which are not air-tight) which may result in dilution at the animal's nose, and therefore, concentrations in the animal's breathing zone may have been lower than actually measured.

From the data of the generation efficiency it can be concluded that approximately 50% of the Dichlobenil administered to the nebulizer is converted to an inhalable form (respirable particles, i.e. particles of sufficient low particle size). It is not expected that dichlobenil has been evaporated based on its vapour pressure (5×10^{-4}). The other 50% can be expected to have been lost in the generation set up (moreover, it was indicated that a filter had been placed).

Also, the analytical method used has not been described and it is not common to report particle size distributions in mg/m³.

Acceptability

Overall, although it was stated that the method used resulted in the highest attainable concentration, the method used is not considered to obtain sufficiently high concentrations in order to classify the test substance. Therefore, this study is not considered acceptable for the purpose of classification.

Conclusions

No conclusions can be drawn from this study as it is not considered acceptable.

Study 2

Characteristics of the study

Reference	:	Berczy, 1974	Route	:	inhalation
Year of execution	:	not indicated	Group size	:	5/sex

Guideline GLP	: 403 no	Exposure Concentration	: 4 hours (whole body) 5.0 mg/L (nominal, actual: 0.045 and 0.043 mg/L respectively), MMAD not indicated
Test substance	: Dichlobenil, lot/batch no. and appearance not indicated 2-chlorobenzonitrile, lot/batch no. and appearance not indicated	Vehicle	: none
Purity of a.s.	: >99%	Acceptable	: no
Species	: rats, Sprague-Dawley albino	LC ₅₀	: -

Study design

The study was not performed in accordance with OECD 403, however, the guideline was not available at the time the study was conducted. The most important deviations from the guideline are:

- The actual concentration of 5 mg/L is not achieved.
- the observation period was 7 days instead of 14 days
- the animals are not acclimatized prior to testing
- no data is given on room temperature, humidity and actual concentrations of the test substance in the breathing zone
- the study report was very concise

Acceptability

The study is considered not acceptable because of the deviations from guideline 403.

Conclusions

The study is considered not acceptable.

Overall conclusion with regard to acute inhalation toxicity

No adequate data on the acute inhalation toxicology of dichlobenil were provided. However, in view of the particle size (99.9% >100 µm; granule) and the application of the preparation (not in enclosed spaces), no acute inhalation studies are required.

B.6.2.2 Irritation and sensitisation

B.6.2.2.1 Skin irritation study

Characteristics of the study

Reference	: Koopman, 1985	Route	: dermal
Year of execution	: 1985	Group size	: 3 males
Guideline	: OECD 404	Exposure	: 4 hours (occlusive)
GLP	: yes	Doses	: 0.5 g
Test substance	: Dichlobenil, lot/batch no. FUX003000/FUN81L08A; appearance: white to yellowish brown powder	Vehicle	: 1%-tragacanth suspension
Purity of a.s.	: 98.8%	Acceptable	: yes
Species	: rabbit, New Zealand White	Effect	: not irritating to skin

Study design

The study was performed mostly in accordance with OECD 404. A patch of aluminium foil was used for application instead of a gauze patch, which makes the exposure occlusive. Also, irritating properties of the vehicle were not described.

Results

Table 6.2.2.1

Scores observed after	1 hour	24 hours	48 hours	72 hours
Erythema	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

Acceptability

Although application under a patch of aluminium foil is occlusive instead of semi-occlusive and the irritating properties of the vehicle were not described, no irritation was observed. Therefore, the study is considered acceptable.

Conclusions

Dichlobenil does not have to be classified as irritating to the skin.

B.6.2.2.2 Eye irritation study

Characteristics of the study

Reference	: Koopman, 1983	Route	: ocular
Year of execution	: 1983	Group size	: 3 males
Guideline	: OECD 405	Exposure	: single instillation in conjunctival sac
GLP	: yes	Doses	: 100 mg
Test substance	: Dichlobenil, lot/batch no. FUX003000/FUN83D14A; appearance not indicated	Vehicle	: none
Purity of a.s.	: 98.8%	Acceptable	: yes
Species	: rabbit, New Zealand White	Effect	: not irritating to eyes

Study design

The study was performed in accordance with OECD 405.

Results

Table 6.2.2.2

Scores observed after	24 hours	48 hours	72 hours	4 days	7 days
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Scores observed after	24 hours	48 hours	72 hours	4 days	7 days
Cornea/opacity	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
Conjunctiva redness	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Conjunctiva chemosis	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Conjunctiva discharge	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

Acceptability

Although no observations at 1 hour after application/instillation were reported, the study is considered acceptable.

Conclusions

The test substance does not have to be classified as irritating to the eyes.

B.6.2.2.3 Skin sensitisation study (Maximisation test)

Characteristics of the study

Reference	: Cuthbert and Carr, 1988	Route	: dermal
Year of execution	1987	Group size	: 20 controls, 20 test animals, 6 dose ranging animals (females only)
Guideline	: OECD 406	Exposure	: Intradermal and topical induction, topical challenge (occlusive, 48h)
GLP	yes	Doses	: 2% w/v intradermal induction; 10% SLS in distilled water; 10% w/v topical induction and 25% w/v challenge
Test substance	: Dichlobenil, lot/batch no. FUN87E08A; appearance: white powder	Vehicle	: Paraffin oil
Purity of a.s.	99.4%	Acceptable	: yes
Species	guinea pig, albino	Effect	: not sensitising to skin

Study design

The study was performed in accordance with OECD 406. The doses in the main study were based on the results of a range-finding study using four concentrations (10%, 5%, 2% and 1%) for the intradermal injections and four concentrations (25%, 10%, 5% and 2%) for the topical applications. The highest concentration which produced slight irritation at the injection site was 2% (w/v). It should be noted that the test material at concentrations of 10% and 5% could not be injected due to high viscosity. After topical application no irritation was noted to any of the concentrations of the test material. A concentration of 25% w/v in paraffin oil was selected for the topical application phase of induction in the test group. Due to a calculation error the actual concentration used for the topical phase of induction was 10% w/v in paraffin oil.

Results

Intradermal injection of 2% dichlobenil and topical induction of 10% dichlobenil +SLS caused slight-moderate reactions at sites treated with the test material. Slight reactions were noted at treated sites in the control group animals. Following challenge with 25% w/v, no dermal responses were observed in any of the test and negative control animals.

Sensitisation of this strain of animals was positively tested with 2,4-dinitro-chlorobenzene (DNCB).

Acceptability

Due to a calculation error the actual concentration used for the topical phase of induction was 10% w/v in paraffin oil instead of the by range-finding selected concentration of 25% w/v in paraffin oil.

However, since 10% w/v in paraffin oil induced slight-moderate irritation, it is considered not to have comprised the validity of the test results. Therefore, the study is considered acceptable.

Conclusions

The test substance is not sensitising to the skin of guinea pigs in this Maximisation test.

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, LD₅₀/LC₅₀ values

Test substance	LD ₅₀ /LC ₅₀	Species	Route	Vehicle	Reference
Dichlobenil	>2000 mg/kg bw	rat	oral	corn oil	Graver, 1999
Dichlobenil	4460 mg/kg bw	rat	oral	arachidis oil	Arnoldussen <i>et al.</i> , 1963
Dichlobenil	>2000 mg/kg bw	rabbit	dermal	distilled water	Cerven, 2000

Table 6.2.3.2 Skin and eye irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
Dichlobenil	not irritating to skin	rabbit	skin	1%-tragacanth suspension	Koopman, 1985
Dichlobenil	not irritating to eyes	rabbit	eye	none	Koopman, 1983

Table 6.2.3.3 Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
Dichlobenil	not sensitising to skin (Maximisation test)	guinea pig	dermal	Paraffin oil	Cuthbert and Carr, 1988

All studies of Arnoldussen et al., 1963 (except the acute oral toxicity study in rats), the acute dermal toxicity study of Johnson, 1994 and the acute inhalation toxicity study of Janssen and de Rooij, 1986 are not considered acceptable.

Dichlobenil does not need to be classified on the basis of its acute dermal toxicology in rats.

Dichlobenil is considered not irritating to skin and eyes according to the criteria mentioned in Annex VI of Directive 2001/59/EC. Dichlobenil does not have sensitising properties in a Maximisation test. No adequate data on the acute inhalation toxicology of dichlobenil were provided. However, in view of the particle size (99.9% >100 µm; granule) and the application of the preparation (not in enclosed spaces), no acute inhalation studies are required.

B.6.3 SHORT-TERM TOXICITY (ANNEX IIA 5.3)**B.6.3.1 Sub-acute oral toxicity****B.6.3.1.1 Oral toxicity study with dogs (range-finding)****Study 1****Characteristics of study**

Reference	: Pickersgill, 1994	Route	: oral
Year of execution	: 1994	Group size	: 2/sex/dose
Guideline	: OECD 409	Exposure	: 28 days, by gavage of capsules
GLP	: yes	Doses	: 1, 6, 36 and 72 mg/kg bw/d
Test substance	: Dichlobenil, batch no. FUN87E08A/ FUX011000, white to off-white powder	Vehicle	: none
Purity of a.s.	: 98.8%	Acceptable	: no, results can be used as supplementary information
Species	: dog, Beagle	NOAEL	: n/a

Study design

The study was performed largely in accordance with OECD 409 except that 2 animals per sex/dose were used instead 4 and no statistical analyses were performed.

Results

The results are presented in Table 6.3.1.1a.

Table 6.3.1.1a Results of the 28-d oral toxicity study with dichlobenil in dogs
(Between brackets relative decrease or increase compared to control, in percentage)

Dose (mg/kg bw/d)	1		6		36		72		dr
	m	f	m	f	m	f	m	f	
Mortality	None								
Clinical signs	No treatment-related findings								
Body weight gain									d (100)
Food consumption									d (23)
Water consumption	No treatment-related findings								
Ophthalmology	Not performed								
Haematology	No treatment-related findings								
Clinical chemistry									
- cholesterol			i (42)	i (32)	i (107)	i (40)	i (131)	i (100)	m,f
- triglycerides			i (72)	i (39)	i (336)	i (74)	i (414)	i (387)	m,f
- phospholipids			i (29)	i (33)	i (73)	i (53)	i (90)	i (76)	m,f
- glucose								d (30)	
- ALAT							i (56)		
- ALP					i (14)	i (36)	i (51)	i (289)	m,f

Dose (mg/kg bw/d)	1		6		36		72		dr
	m	f	m	f	m	f	m	f	
Urine analysis	No treatment-related findings								
Organ weights									
- liver			^{ar} (7,11)	^{ar} (4,6)	^{ar} (48,47)	^{ar} (35,46)	^{ar} (49,50)	^{ar} (54,86)	m,f
- adrenals							^{ar} (51,52)		
- thyroids			^{ar} (21,25)		^{ar} (37,37)		^{ar} (48,49)		m
Pathology	No treatment-related findings								
<u>macroscopy</u>									
<u>microscopy</u>									
<i>liver</i>									
-centrilobular homogeneous cytoplasm					1/2	1/2	2/2	1/2	
-mononuclear cell infiltration							1/2		
-cytoplasmic aggregation					1/2		2/2	2/2	

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

a/r absolute/relative organ weight/activity

Acceptability

This study is only acceptable for dose-range finding, but not for the evaluation of dichlobenil toxicity.

Conclusions

Oral exposure of beagle dogs to dichlobenil at doses of 6 mg/kg bw/d and higher for a period of four weeks resulted in increased liver weights in both sexes. This effect was accompanied by functional changes in lipid metabolism, and, at the higher dose levels, with impaired liver function. At the same dose levels, increased thyroid weights were observed in males only. In addition, decreased body weight gain, food consumption and glucose levels were observed in females and increased adrenal weights in males. At a dose level of 1 mg/kg bw/d, no treatment related effects were noted. In view of the range-finding character of this study, no NOAEL can be derived.

Study 2

Characteristics of study

Reference	: Til, <i>et al.</i> , 1967	Route	: oral
Year of execution	: 1967	Group size	: 2/sex/dose
Guideline	: OECD 409	Exposure	: 90 days, in diet
GLP	: no	Doses	: 0, 50, 150, 450 mg/kg food ¹
Test substance	: Dichlobenil, batch no. not indicated, light brown crystalline powder	Vehicle	: none
Purity of a.s.	: 95.5%	Acceptability	: not acceptable
Species	: dog, Beagle	NOAEL	: n/a

¹ Equal to approximately 0, 2, 5 and 15 mg/kg bw/d (males and females combined)

Study design

The study was performed largely in accordance with OECD 409. Deviations are described at 'Acceptability'.

Results

The results are presented in Table 6.3.1.1b

Table 6.3.1.1b Results of the 90-d oral toxicity study with dichlobenil in dogs

Dose (mg/kg food)	0		50		150		450		dr
	m	f	m	f	m	f	m	f	
Mortality	None								m+f
Clinical signs	No treatment-related findings								
Body weight gain	No treatment-related findings								
Food consumption	No treatment-related findings								
Water consumption	Not determined								
Ophthalmology	Not performed								
Haematology	No treatment-related findings								
Clinical chemistry							i (41%)		
- ALAT (6 w)							i (28,50%)		
- ALP (6 w, 12 w)									
Urine analysis	No treatment-related findings								
Organ weights							i (26,22%)		
- kidney					i ^{ar} (27,17%)		i (61,50%)		
- liver									
Pathology									
<u>macroscopy</u>	No treatment-related findings								
<u>microscopy</u>	No treatment-related findings								

dr dose related

d/i decreased/increased (no statistical analysis performed)

a/r absolute/relative organ weight/activity

Acceptability

The study was not performed completely in accordance with OECD 409, since:

- 2 instead of 4 animals per sex/dose were used
- the highest dose group contained 3 males and 1 female
- the stability of the test article is unknown,
- the dosing material was not analysed,
- no ophthalmologic examination was performed,
- incomplete haematology parameters,
- incomplete serum chemistry parameters,
- data summary tables did not distinguish between males and females
- no statistical analysis was provided
- the study report is incomplete,

Considering these deviations, this study is only acceptable for dose-range finding, but not for the evaluation of dichlobenil toxicity.

Oral exposure of beagle dogs to dichlobenil at a dietary level of 150 and 450 mg/kg food for 90 days resulted in a dose related increase of liver weight, in the highest dose accompanied by increased levels of alkaline phosphatase and ALAT indicating impaired liver function. In the highest dose group, kidney weights were increased as well. No adverse effects were observed at a dose level of 50 mg/kg food (equal to ca. 2 mg/kg bw/d). In view of the short-comings of this study (see 'Acceptability' above) no NOAEL can be derived.

In the study report of Arnoldussen *et al.*, 1963 a number of acute oral and dermal toxicity studies, sub-acute dermal in rabbits, rats and mice and subcutaneous and intraperitoneal toxicity studies in rats are performed. Due to various shortcomings this study is not acceptable (see for details B.6.2.1 acute toxicity).

Characteristics of the study

Reference	: Allan, 1995	Route	: dermal
Year of execution	: 1995	Group size	: 5/sex/dose
Guideline	: OECD 410	Exposure	: 21 days, 6 h/d, semi-occlusive ($\pm 10\%$ of the total body surface area)
GLP	: yes	Doses	: 0, 100, 300, and 1000 mg/kg bw/day
Test substance	: Dichlobenil, batch no. FUN87E08A/FUX011000; white agglomerated powder	Vehicle	: water
Purity of a.s.	: 98.8%	Acceptable	: yes
Species	: rabbit. New Zealand white	NOAEL	: > 1000 mg/kg bw/d

The study was performed in accordance with OECD 410.

The results are presented in Table 6.3.2.1.

Dose (mg/kg bw/d)	0	100	300	1000	dr
	m f	m f	m f	m f	
Mortality	none				

Dose (mg/kg bw/d)	0		100		300		1000		dr
	m	f	m	f	m	f	m	f	
Local dermal irritation									
Clinical signs									
Body weight									
Food consumption									
Haematology									
Clinical chemistry - cholesterol - creatinine								ic dc	
Organ weights - ovaries						i		ic	
Pathology									
Macroscopy									
Microscopy									

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 of rabbits to dichlobenil at a concentration of 1000 mg/kg bw/d for 21 days resulted in an increased cholesterol level, decreased creatinine level and increased ovary weights for female rats. However, the majority of individual values were within the expected background range. Additionally, there were no related biochemical or pathological changes in the liver or kidney observed. Therefore, these effects were not considered to be toxicologically relevant. The NOAEL for dichlobenil administered dermally for 21 days is set at 1000 mg/kg bw/d.

B.6.3.3 Sub-acute inhalation toxicity

Characteristics of the study

Reference	: Newton, 2002	Route	: inhalation
Year of execution	: 2002	Group size	: 10/sex/dose
Guideline	: OECD 412	Exposure	: 28 days (6-h/d, 5 d/w, nose only)
GLP	: yes	Doses	: 0, 2.3, 5.1, 12.0 mg/m ³ (actual level), MMAD ± gsd: 1.64±1.65, 2.11±1.75, 1.62±1.62 (at 2.3, 5.1 and 12.0 mg/m ³ respectively)
Test substance	: Dichlobenil, lot no. BFI-4138; off-white powder	Vehicle	: none
Purity of a.s.	: 98.1%	Acceptable	: yes
Species	: rats, Sprague-Dawley CD[CrI:CD® (SD) IGS BR]	NOAEL	: ≥12 mg/m ³

Study design

The study was performed mostly in accordance with OECD 412. A deviation from the guideline was that the highest dose used in the study did not show toxic effects. The high exposure levels in the current study were set at 12 mg/m³ based upon the results of a range-finding study (included in the report (Newton, 2002)). In that study, rats were exposed to 21, 77, or 200 mg/m³ for 7 days by nose-only inhalation. The low dose group of 21 mg/m³ showed toxic effects (liver weight increase, local effects nasal tissue); nevertheless, the high dose group in the current study was set at 12 mg/m³. In addition, all animals were submitted to Functional Observational Battery (FOB) and motor activity evaluations prior to termination of exposure to the test article (week 4).

Results

The results are presented in Table 6.3.3

Table 6.3.3 Results of the 28-day toxicity study with dichlobenil in rats

Dose (mg/m ³)	0		2.3		5.1		12		dr
	m	f	m	f	m	f	m	f	
Mortality									none
Clinical signs									No treatment-related findings
Body weight									No treatment-related findings
Food consumption									No treatment-related findings
Ophthalmoscopy									No treatment-related findings
Motor activity									No treatment-related findings
Functional observational battery									No treatment-related findings
Haematology									No treatment-related findings
Clinical chemistry									No treatment-related findings
Organ weights									No treatment-related findings
Pathology									
<u>Macroscopy</u> -spleen nodule				1/10					
<u>Microscopy</u> <i>Pancreas</i> -inflammation, subacute, trace								1/10	
<i>Prostate gland</i> -subacute inflammation, trace	1/10						1/10		
-chronic inflammation, severe							1/10		
<i>Spleen</i> -lymphoma, malignant				1/10					

Acceptability

The study is considered acceptable.

Conclusions

The nodule in the spleen of a 2.3 mg/m³ female was a malignant lymphoma, present only in the spleen. This was not considered to be treatment related, as it was not observed at higher doses. The inflammations found in the pancreas (high dose female) and in the prostate (high dose males and control group) were not considered to be treatment related, as these were incidental occurrences and no other cases with related effects were found in lower dose groups.

Therefore, respiratory exposure of rats to dichlobenil at concentrations of 2.3, 5.1 and 12 mg/m³ for 28 days did not result in toxic effects.

In conclusion, the NOAEL is set at ≥ 12 mg/m³.

B.6.3.4 Semi-chronic oral studies

B.6.3.4.1 90-day oral toxicity study with rats

Study 1

Characteristics of study

Reference	: van Esch and Arnoldussen, 1961	Route	: Oral
Year of execution	: not indicated	Group size	: 10/sex/dose
Guideline	: not indicated	Exposure	: 90 days, in diet
GLP	: no	Doses	: 0, 50, 100, 200, 300 and 500 mg/kg food ¹
Test substance	: H133, batch no. 5 II; appearance not indicated	Vehicle	: none
Purity of a.s.	: 93.5%	Acceptable	: no, results can be used as supplementary information
Species	: Rats, Wistar	NOAEL	: 50 mg/kg food (2 mg/kg bw/d)
¹ equivalent to 0, 2, 5, 10, 15, and 26 mg/kg bw/d for males and 0, 3, 6, 12, 18, and 29 mg/kg bw/d for females			

Study design

The study was not performed in accordance with OECD 408. A deviation from the guideline was the study design: three subsequent tests were conducted with 2, 3 and 3 groups of Wistar rats, respectively. In the first test rats were given 100 mg H133/kg food with their diet. In the second test two groups were given 200 and 300 mg H133/kg food and in the third test rats were given 50 and 500 mg H133/kg food. The three subsequent tests had their own control groups.

Further deviations from the guideline were: no data on clinical signs, ophthalmoscopy and clinical biochemistry. Furthermore, not all required parameters were measured for haematology (blood clotting time/potential). Limited (or no) information is given on housing and feeding conditions and the preparation of the animals. No individual animal data were submitted.

In addition, by mistake the control of the third test consisted of 11 female and 9 male rats.

Results

The results are presented in Table 6.3.4.1a.

Table 6.3.4.1a Overview of treatment-related effects in rats after semi-chronic oral exposure to H133 (numbers between brackets are percentages)

Dose (mg/kg food)	0		50		100		200		300		500		dr
	m	f	m	f	m	f	m	f	m	f	m	f	
Mortality		2/10 ¹	2/10	2/10									
Clinical signs					not determined								
Body weight			ic						dc				
Food consumption					no treatment-related findings								
Ophthalmoscopy					not determined								
Haematology													
-erythrocytes							ic					ic	
-leucocytes													
Clinical chemistry					not determined								
Organ weights													
-liver, absolute					ic (14)	ic (20)	ic (23)	ic (23)	ic (35)	ic (42)	ic (25)	ic (31)	m,f
-kidney, absolute							ic (13)		ic (15)	ic (10)		ic (11)	f
-uterus, absolute										ic		i	f
Pathology													
<u>macroscopy</u>					no treatment-related findings								
<u>microscopy</u>					see conclusions								

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

¹

2 animals died in the control group belonging to the test with the 50 mg/kg food dose group

Acceptability

The study is considered not acceptable due to deviations from the guideline as mentioned above. The results can only be used as supplementary information.

Conclusions

For unknown reasons, 2 female animals of the control group and 2 female animals and 2 male animals of the 50 mg/kg food group died at the beginning of the test.

The liver and kidneys were at all dosages statistically significant heavier compared to their control animals, with the exception of the group receiving 50 mg/kg food. The uterus weight was increased in females of the 300 and 500 mg/kg food groups.

The number of leucocytes was increased in the high dose group of male animals. The deviations of the erythrocyte number are still within the normal range and this becomes clear when compared to one of the other control groups.

The pathological examinations revealed typical lesions in the thyroids (cast-off epithelial cells in follicles and pycnosis of the nuclei of the tubuli-epithelial cells). These results were not mentioned in the table since the differences between male and female animals were not indicated. The toxicological relevance of these effects remains unclear.

In conclusion, the NOAEL is set at 50 mg/kg food (equivalent to 2 mg/kg bw/d), based on the increased liver weights in the 100 mg/kg food group. It is noted that this NOAEL is based only on the limited amount of parameters used in this study and can therefore only be used as supplementary information.

Study 2

Characteristics of study

Reference	: Hodge, 1961	Route	: Oral
Year of execution	: 1961	Group size	: 12/sex/dose
			: 6 males (highest dose)
Guideline	: not indicated	Exposure	: 90 days, in diet
GLP	: no	Doses	: 0, 100, 1000, 3000 and 10000 mg/kg food ¹
Test substance	: CASORON, tech., lot/batch no. MR A-306; appearance not indicated	Vehicle	:
Purity of a.s.	: 90.5%	Acceptable	: no, results can be used as supplementary information
Species	: rats, albino Rochester	NOAEL	: 100 mg/kg food (8 mg/kg bw/d)

¹ Equal to 0, 8, 77, 242, and 1246 mg/kg bw/d for males and 0, 9, 94, and 280 mg/kg bw/d for females.

Study design

The study was not performed in accordance with OECD 408. No ophthalmologic examinations were done and clinical chemistry and food consumption were not observed. The 10000 mg/kg food group consisted of only 6 male animals. Histopathological examinations were only performed on five animals per group, on the one remaining animal of the 10000 mg/kg food group and on two animals from this group which died during the first 30 days of the experiment. Histopathological examinations were not performed in all required organs (e.g. spinal cord, pituitary, parathyroid, thymus, oesophagus, salivary gland, pancreas, lymph nodes, etcetera).

Only averages were reported, without standard deviations. No statistical testing was executed.

Dose levels were based on the results of a previous three week feeding study done with mice in which a marked growth depression was observed in mice given 1000 and 3000 mg dichlobenil/kg food.

Results

The results are presented in Table 6.3.4.1b.

Table 6.3.4.1b Overview of treatment-related effects in rats after semi-chronic oral exposure to dichlobenil

Dose (mg/kg food)	0	100	1000	3000	10000	dr
	m f	m f	m f	m f	m	
Mortality	2/12	2/12	1/12 2/12	2/12	5/6	
Clinical signs	no treatment-related findings					

Dose (mg/kg food)	0		100		1000		3000		10000	dr
	m	f	m	f	m	f	m	f	m	
Body weight					d		d	d	d	m,f
Food consumption					not determined					
Urinalysis					no treatment-related findings					
Ophthalmoscopy					not determined					
Haematology										
RBC							d		d	
Hb							d		d	
Hct (%)							d		d	
Clinical chemistry					not determined					
Organ weights										
liver, relative					i	i	i	i	i	m,f
kidney, relative					i	i	i	i	i	m,f
testes, relative									i	m
Pathology										
<u>macroscopy</u>					no treatment-related findings					
<u>microscopy</u>										
liver										
-centrilobular degeneration							3/5	5/5	2/3	
-centrilobular necrosis									1/3	
-focal necrosis					1/6		1/5		2/3	
-focal bile duct proliferation									1/3	
kidney										
-focal chronic pyelonephritis, mild		1/5						3/5		
-protein in Bowmans capsule & filling tubules									1/3	
-increased brown pigment in tubules									1/3	

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 not acceptable due to the deviations from the guideline as mentioned above.

The study can be considered as supplementary.

Conclusions

The males from the high dose group gained almost no weight and a marked growth depression was observed in males given 3000 mg/kg food.

RBC counts, haemoglobin values and hematocrit percentages were depressed in the blood of the sole surviving rat given 10000 mg/kg food. A tendency toward reduction in these parameters was found also in the blood of rats that had been given 3000 mg/kg food.

Relative liver, kidney and testes weights increased with increasing percentages of dichlobenil in the diet, when diets contained 1000 mg/kg food and more. Even though the increased relative testes

weight was treatment-related, no (histo)pathological examinations were executed to investigate the cause of this effect. The livers of the rats given 3000 mg/kg food or more exhibited changes that were attributed to a toxic effect of dichlobenil. The liver of the sole surviving rat in the 10000 mg/kg food group exhibited necrosis, which was also attributed to the administration of dichlobenil. The gross changes on autopsy examination also included finding several lung abscesses and a nodule in the liver of one rat given 1000 mg/kg food. It was not indicated which animals had these symptoms (not presented in table). In addition, a number of abnormalities were found in the sole surviving rat given 10000 mg/kg food.

In conclusion, the NOAEL is set at 100 mg/kg food (equivalent to 8 mg/kg bw/d), based on increased relative liver and kidney weights in the 1000 mg/kg food group. It is noted that this NOAEL is based only on the limited amount of parameters used in this study and can therefore only be used as supplementary information.

B.6.3.4.2 90-day oral toxicity study with mice

Characteristics of study

Reference	: Kemp, 1987b	Route	: oral
Year of execution	: 1987	Group size	: 10/sex/dose, 20/sex in control group
Guideline	: OECD 408	Exposure	: 90 days, in diet
GLP	: yes	Doses	: 0, 25, 125, 625 and 3125 mg/kg food ¹
Test substance	: Dichlobenil, batch no. FUN86L19A, FUX010000; appearance not indicated	Vehicle	: none
Purity of a.s.	: 99.4%	Acceptable	: acceptable
Species	: mouse, Charles River CD1	NOAEL	: 125 mg/kg food (19 mg/kg bw/d)

¹ Equivalent to 0, 3.7, 19, 91 and 445 mg/kg bw/d for males and 0, 4.8, 24, 114 and 510 mg/kg bw/d for females

Study design

The study was performed in accordance with OECD 408.

Results

The results are presented in Table 6.3.4.2.

Table 6.3.4.2 Results of the 90-day toxicity study with dichlobenil in mice (numbers between brackets are percentages)

Dose (mg/kg food)	0	25	125	625	3125	dr
	m f	m f	m f	m f	m f	
Mortality	1/20	1/10				
Clinical signs¹						
-locomotor activity decr.					+	
-hunched posture					+	
					(10)	(50)
-pilo-erection					+	
					(20)	(70)

Dose (mg/kg food)	0		25		125		625		3125		dr
	m	f	m	f	m	f	m	f	m	f	
-abdominal tonus										+	
Body weight gain											
Week 1-4									dc (29)	d (9)	
Week 5-8									ic (100)	+	
Week 9-13										dc (52)	
Food consumption											
Week 1-4							d (5)	dc (8)	dc (15)	dc (19)	m,f
Week 5-8								dc (7)		dc (14)	f
Week 9-13										dc (14)	
Ophthalmoscopy											
											No treatment-related findings
Haematology											
											No treatment-related findings
Clinical chemistry											
Glucose							dc (16)		dc (10)		
Cholesterol									ic (48)	ic (43)	
Phospholipids									ic (46)	ic (40)	
Organ weights											
Liver, absolute									ic (17)	ic (18)	
Liver, relative									ic (20)	ic (23)	
Thymus, absolute									ic (28)		
Thymus, relative									ic (31)		
Testes, absolute				ic (13)			ic (14)		ic (8)		
Testes, relative				ic (10)			ic (13)		ic (10)		
Pathology											
<u>macroscopy</u>											No treatment-related findings
<u>Microscopy</u>											
<i>Liver</i>											
- centrilob. cells swollen, slight/moderate			1/10		1/10	2/10	6/10	2/10	4/10	5/10	f
- diffuse glycogen incr. ²		2/20						2/10	3/10	3/10	
- cells with glycogen incr.		8/20					1/10	2/10	2/10	6/10	
<i>Kidney</i>											
- pyelitis	1/19	1/20	3/10		1/10		3/10		4/10	2/10	
- interstitial nephritis, marginal/slight					1/10				3/10		

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 organ weight

+ present in one/a few animals

++ present in most/all animals

¹ clinical signs that were observed affected a larger number of females than males. The signs were only observed in the first 2 weeks. No incidences were reported, except for posture and pilo-erection in week 1, indicated in the table in percentage.

severity of effect increases with dose

Acceptability

The study is considered acceptable.

Conclusions

The reduced body weight gain observed at the 3125 mg/kg food group during the first week of treatment was associated with reduced food consumption. The body weight gain of the males returned to normal, while the body weight gain of the females remained reduced up until week 13 together with a reduced food consumption. The terminal body weight in week 13 displayed no differences for both sexes in all dose groups with regard to the control group.

Oral exposure of mice to dichlobenil at concentrations of 3125 mg/kg food for 90 days resulted in changes in liver function. The increased liver weight in both sexes was associated with swollen centrilobular hepatocytes at the 625 and 3125 mg/kg food levels. In addition, an indication of functional change in the liver was shown by the increased plasma levels of cholesterol and phospholipids at the highest dose group of both sexes, and the lower glucose level in plasma at the 625 and 3125 mg/kg food group of males. The kidneys of the males had marginal inflammatory and regenerative changes in the 625 and 3125 mg/kg food levels.

At a dose of 125 mg/kg food and above the testis weight was significantly increased, however, these results are not clearly dose-related and no histopathological effects were detected within the testis. Furthermore, two animals in the control group showed a lower absolute and relative testis weight. By leaving out these two outliers in the calculation an increase of relative testis weight lower than 10% (6-9%) and an increase of 4-10% for absolute testis weight were found.

In conclusion, the NOAEL is set at 125 mg/kg food (equivalent to 19 mg/kg bw/d for males and 24 mg/kg bw/d for females) based on the effects found at 91 mg/kg bw (reduced food consumption, histopathological liver and kidney effects).

B.6.3.4.3 90-day oral toxicity study with hamsters

Characteristics of study

Reference	: Kemp, <i>et al.</i> , 1987a	Route	: oral
Year of execution	: 1987	Group size	: 10/sex/dose, 20/sex in control group
Guideline	: OECD 408	Exposure	: 90 days, in diet
GLP	: yes	Doses	: 0, 60, 300, 1500 and 5000/7500 mg/kg food (Actual levels: 41, 209, 1289 and 4648 mg/kg diet) ¹
Test substance	Dichlobenil, batch no. FUX0100000; appearance not indicated	Vehicle	: none
Purity of a.s.	: 98.5%	Acceptable	: acceptable
Species	: hamster, bio F1 Alexander	NOAEL	: 60 mg/kg food (2.3 mg/kg bw/d)

¹ Equivalent to 0, 2.3, 12, 73 and 302 mg/kg bw/d for males and 0, 2.6, 13, 81 and 320 mg/kg bw/d for females

Study design

The study was largely performed in accordance with OECD 408. No functional observations were included and urinalysis (optional according to the guideline) was not performed. Organ weights of adrenals and epididymides were not determined. Histopathology on bone marrow was not performed. The dose of 7500 mg/kg food was reduced in week 3 to 5000 mg/kg food due to intolerance (body weight reduction after two weeks in both sexes) of the initial concentration.

Results

Table 6.3.4.3 Results of the 90-day toxicity study with dichlobenil in hamster (numbers between brackets are percentages)

Dose (mg/kg food)	0	60	300	1500	5000/7500	dr
	m f	m f	m f	m f	m f	
Mortality	None					
Clinical signs	No treatment-related findings					
Body weight gain -week 1-3					dc (-176)	dc (-168)
-week 4-13					ic (36)	ic (77)
Body weight (terminal)					dc (21)	dc (6)
Food consumption -week 1-6				dc (7)	dc (21)	dc (14)
-week 7-13			dc (8)	dc (8)	dc (10)	
Ophthalmoscopy	No treatment-related findings					
Haematology						
RBC					dc (4)	
MCV					ic (2)	ic (1)
MCH					ic (2)	
WBC						dc (21)
Clinical chemistry						
ALAT		ic (15)	ic (38)	ic (23)	ic (16)	ic (21)
Total protein						ic (4)
Serum albumin						ic (8)
Urea						ic (19)
Cholesterol		dc (13)	dc (19)	i (12)	dc (15)	ic (20)
Phospholipids				ic (17)	ic (18)	ic (25)
Creatinin					dc (9)	dc (12)
Glucose						dc (16)
						dc (18)
Organ weights						

Dose (mg/kg food)	0		60		300		1500		5000/7500		dr
	m	f	m	f	m	f	m	f	m	f	
Liver, absolute					ic (16)		ic (13)	ic (34)	ic (52)	ic (85)	m,f
Liver, relative					ic (8)		ic (17)	ic (32)	ic (107)	ic (97)	m,f
Spleen, relative										dc (18)	
Kidneys, relative									ic (20)	ic (13)	
Prostate, absolute					dc (19)		dc (23)		dc (53)		m
Prostate, relative					dc (19)		dc (21)		dc (36)		m
Sem. ves., relative									dc (31)		
Uterus, absolute										dc (25)	
Uterus, relative										dc (21)	
Pathology											
<u>Macroscopy</u>											
<u>Liver</u>											
-enlarged								5/10	5/9	10/10	m,f
-rough surface								3/10	1/9	8/10	f
<u>Testes</u>											
-small									3/9		
<u>Microscopy</u>											
<u>Prostate</u>											
-mineralization	1/20	-	1/10	-	3/10	-	4/10	-	3/9	-	
<u>Testis</u>											
-tubular degeneration	2/20	-	*	-	*	-	*	-	7/9	-	
<u>Epididymus</u>											
-decr. spermatocytes	1/20	-	*	-	*	-	0/10	-	5/9	-	
<u>Gall bladder</u>											
-lumen calculi	0/20	0/20	0/10	0/10	0/9	0/10	0/9	0/9	7/9	10/10	m,f
<u>Liver</u>											
-swollen hepatocytes	1/20	0/20	1/10	0/20	1/10	2/10	0/10	4/10	2/9	4/10	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

* not investigated

Acceptability

The study is considered acceptable.

Conclusions

Haematology showed significantly decreased RBC and increased MCV and MCH compared to the control. However, these were minor differences (1-4%) and therefore considered not toxicologically relevant. In addition, these minor effects were shown to be reversible during recovery.

Prostate mineralization appears to be a treatment-related effect, but when the severity of the effect is taken into account, no dose-related effect can be found.

Oral exposure of hamsters to dichlobenil at concentrations of 1500 and 5000 mg/kg food for 90 days resulted in numerous significant effects on various types of parameters analysed although the effects were more pronounced at 5000 mg/kg food. The results indicate liver and possibly kidney dysfunction (based on creatinin). Important changes were increased liver weight, and a dose-dependent decrease of prostate and uterus weight. A degeneration of testes tubules and a decrease of viable spermatocytes were observed in male hamsters. Nearly all animals of the highest dose group showed

calculi in the gallbladder, even after recovery. Various clinical biochemistry parameters were changed: ALAT, total proteins, albumin urea, creatinin, cholesterol and phospholipids. Many of these changes had returned to normal values after 4 weeks recovery.

At 300 mg/kg food the following effects were considered toxicologically relevant: decreased food intake in males, increased ALAT in females, increased cholesterol and phospholipids in females, increased liver weight in females, and decreased prostate weight in males.

At 60 mg/kg food, a marginal increase of ALAT activity (+15%) was observed in females, which was not associated with changes in organ weights. In addition, the increased ALAT was shown to be reversible during recovery. In males, a slight but significant decrease in cholesterol levels was observed which is not considered a direct adverse effect. Therefore, the NOAEL in this study is 60 mg/kg food (equivalent to 2.3 mg/kg bw/d), based on the effects found at the 300 mg/kg food dose level (effects on liver, kidney and male reproductive organs).

B.6.3.4.4 52-week oral toxicity study with dogs

Characteristics of study

Reference	: Pickersgill, 1995	Route	: oral
Year of execution	: 1994-1995	Group size	: 5/sex/dose
Guideline	: OECD 409	Exposure	: 52 weeks, by gavage or capsules
GLP	: yes	Doses	: 0, 1, 6, 36 mg/kg bw/d
Test substance	: Dichlobenil, batch no. FUX011000/FUN87E08A, white to off-white powder	Vehicle	: none
Purity of a.s.	: 98.8%	Acceptability	: acceptable
Species	: dog, Beagle	LOAEL	: 1 mg/kg bw/d

Study design

The study was performed in accordance with OECD 409. Dose levels were based on the results of a dose range-finding study, in which dose levels of 1, 6, 36 and 72 mg/kg/d were used, and clear systemic toxicity (mainly liver effects) was observed at 6 mg/kg bw/d and higher (Pickersgill, 1994).

Results

The results are presented in Table 6.3.4.4.

Table 6.3.4.4 Results of the 52-week toxicity study with dichlobenil in dogs

Dose (mg/kg bw/d)	0		1		6		36		dr
	m	f	m	f	m	f	m	f	
Mortality	None								m
Clinical signs	No treatment-related findings								
Body weight gain					d (9%)	dc (36%)		d (14%)	
Food consumption	No treatment-related findings								
Water consumption	No treatment-related findings								
Ophthalmology	No treatment related-findings								

Dose (mg/kg bw/d)	0		1		6		36		dr
	m	f	m	f	m	f	m	f	
Haematology¹									
- Hb					dc (8%)	d (7%)	dc (10%)	6 (6%)	m
- MCH						d (2%)		dc (5%)	
- PCV					dc (9%)	d (7%)	dc (11%)	d (4%)	m
- RBC					dc (11%)	d (5%)	dc (12%)		m
- RETI							dc (75%)		
Clinical chemistry¹									
- Cholesterol					ic (54%)	i (24%)	ic (108%)	ic (74%)	m, f
- Triglycerides			ic (34%)		ic (44%)	ic (29%)	ic (384%)	ic (163%)	m, f
- Phospholipids			ic (24%)		ic (39%)	i (18%)	ic (78%)	ic (46%)	m, f
- ALP					ic (74%)		ic (377%)	ic (225%)	m
Urine analysis					No treatment-related findings				
Organ weights									
- liver			ic ^r (17%)		ic ^{alr} (18, 25%)	ic ^{alr} (50, 34%)	ic ^{alr} (70, 97%)	ic ^{alr} (55, 67%)	m, f
- thyroids							ic ^{alr} (56, 84%)	ic ^{alr} (50, 63%)	
- kidney							ic ^{alr} (21, 41%)		
- ovaries								dc ^a (50%)	
- uterus								dc ^{alr} (78, 76%)	
- thymus				dc ^r (41%)		dc ^r (6%)		dc ^{alr} (31, 25%)	
Pathology									
<u>macroscopy</u>									
<i>lung</i>									
- pale areas							2/5		
<i>thymus</i>									
- involution	2/5	2/5	3/5	4/5		3/5	3/5	2/5	
<u>microscopy</u>									
<i>liver</i>									
- hepatocytic hypertrophy					2/5		5/5	5/5	
- myeloid bodies in periportal hepatocytes							3/5	2/5	
<i>adrenals</i>									
- increased cortical vacuolation in zona glomerulosa		1/5	1/5	2/5	1/5	1/5	5/5	3/5	
<i>spleen</i>									
- hemosiderin				1/5	2/5	1/5	1/5	2/5	
<i>uterus</i>									
- early maturity		1/5		3/5		2/5		5/5	

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 organ weight

+ present in one/a few animals

++ present in most/all animals

¹ in week 26 and 52

Acceptability

The study is considered acceptable.

Conclusions

Oral exposure of beagle dogs to dichlobenil at concentrations of 36 mg/kg bw/d for 52 weeks resulted in reduced body weight gain and elevated alkaline phosphate activities, increased liver weight and hepatocytic hypertrophy in both sexes. There were increased concentrations of serum cholesterol, triglyceride and phospholipid and to a lesser extent, the serum protein concentration was altered. Other changes at this dose level included vacuolation in the adrenals, considered to represent changes in mineralocorticoid production. Males had slightly lowered red cell parameters and there was some evidence of haemosiderin deposits in the spleen of animals of both sexes. Females showed decreased ovaries and uterus weights.

At a dose level of 6 mg/kg bw/d, animals had similar changes as described above but at a less severe degree.

At a dose level of 1 mg/kg bw/d, slightly higher triglyceride and phospholipid concentrations were observed in males.

At dose levels of 1 mg/kg bw/d and higher, females showed decreased thymus weights. Since this finding was not dose-related, the decrease was larger in the low dose group than in the higher dose groups, the effect was considered not to be treatment related.

At all dose levels involution of the thymus was noted in both sexes. Early maturity of the uterus was also noted. Since these findings were not dose-related, and the effects are also noted in the control group, this is not considered to be treatment related. However, a dose related increased liver weight in females was observed at 1 mg/kg bw/d. In conclusion, the LOAEL is set at 1 mg/kg bw/d.

The notifier proposed a NOAEL of 1 mg/kg bw. The notifier submitted a statement that the increased liver weight at a dose of 1 mg/kg bw is not an adverse effect in the absence of any supporting clinical or histopathological adverse effects; the increased relative liver weight should be viewed as an adaptive effect of the liver due to microsomal enzyme induction. The notifier refers in the statement to the PSD Guidance Document 'Interpretation of liver enlargement in regulatory toxicology studies' (Andrew, David (2005)).

The RMS does not agree with the statement. An adaptive effect of the liver is possible, however it is expected that an adaptive effect would have disappeared after a year. Furthermore, the notifier refers to the PSD Guidance Document. It should be noted that the purpose of this document is to derive a framework to promote consistency in the interpretation of increased liver weight in regulatory toxicity studies in **rodents**, based on mechanistic considerations. Part of the proposed framework in the PSD Guidance Document is:

- The toxicological significance of a statistically significant increase in liver weight of $\geq 10\%$ will be interpreted following consideration of the mechanism of action. Findings will be interpreted as

potentially adverse, with the specific exceptions of peroxisome proliferators and 'phenobarbitone-type' P450 inducers, as detailed below.

- If there is evidence that the hepatic effects of the compound are not caused purely by peroxisome proliferation or phenobarbitone-type' P450 induction, then a statistically significant increase in relative liver weight of $\geq 10\%$ will be interpreted as being adverse. Similarly, if the evidence provided for the mechanisms is incomplete or unconvincing, findings will be interpreted as adverse.
- Evidence for a 'phenobarbitone-type' response: Studies of enzyme induction are required to demonstrate a phenobarbitone-type' response.

Considering that the exact mechanism of the increased liver weight in the 52-week dog study is not known and that the statistically significant increase in liver weight $>10\%$, the RMS proposes a LOAEL of 1 mg/kg bw.

B.6.3.4.5 Two-year oral toxicity study with dogs

Characteristics of study:

Reference	: Til, <i>et al.</i> , 1969, Dijkstra, 1990	Route	: Oral
Year of execution	: 1966-1969	Group size	: 4/sex/dose
Guideline	: OECD 409	Exposure	: 104 weeks, in diet
GLP	: not applicable	Doses	: 0, 20, 50, 350 mg/kg food ¹
Test substance	: Dichlobenil, batch no. not indicated, light brown crystalline powder	Vehicle	: none
Purity of a.s.	: 95.5%	Acceptability	: acceptable
Species	: dog, Beagle	NOAEL	: 50 mg/kg food (1.3 mg/kg bw)

¹ Equal to 0, 0.5, 1.3, 9.1 mg/kg bw/d

Study design

The study was performed largely in accordance with OECD 409. Deviations are described at 'Acceptability'. Dose levels were based on the results of a dose range-finding study, in which dose levels of 150 and 450 mg/kg food were used. At 450 mg/kg food a slight increase in activity of serum enzymes and increased liver and kidney weights were observed. At 150 mg/kg food the liver weight was slightly increased (range-finding study not submitted).

Results

The results are presented in Table 6.3.4.5.

Table 6.3.4.5 Results of the 2-year toxicity study with dichlobenil in dogs

Dose (mg/kg food)	0	20	50	350	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				

Dose (mg/kg food)	0		20		50		350		dr
	m	f	m	f	m	f	m	f	
Water consumption	Not determined								data package and should not be read in isolation. Registration must not be granted on
Ophthalmology	Not performed								
Haematology	No treatment-related findings								
Clinical chemistry									
- ALAT	i (156%)								
- ALP	ic (182%) ic (130%)								
Urine analysis	No treatment-related findings								
Liver enzymology									
- glucose-6-phosphatase	ic ^r (42%) ic ^r (34%)								
- glucose-6-phosphate dehydrogenase	ic ^{a/r} (127, 196%) ic ^{a/r} (69, 151%)								
Organ weights									
- liver	i ^a , ic ^r (35, 31%) ic ^{a/r} (67, 44%)								
- thyroids	i ^{a/r} (58, 48%) ic ^{a/r} (104, 73%)								
Pathology									
<u>macroscopy</u>	No treatment-related findings								
<u>microscopy</u>									
<i>liver</i>									
- foamy appearance cytoplasm of hepatocytes (glycogen storage) with enlarged centrolobular hepatocytes	1/4 2/4								
- moderate numbers of inflammatory cells (mainly polymorphnuclear leukocytes)	2/4 3/4								

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/activity

Acceptability

The study was not performed completely in accordance with OECD 409, since:

- The stability of the test article is unknown,
- The dosing material was not analysed,
- Animals were divided into test groups according to age,
- No ophthalmologic examination was performed,
- Incomplete haematology parameters,
- Incomplete serum chemistry parameters,
- No data summary tables, no statistical analysis,
- The study report is incomplete,

- No internal auditor or GLP compliance statement.

In the addendum the deficiencies are rectified, and additional information is provided. Therefore, the study is considered acceptable.

Conclusions

Oral exposure of beagle dogs to dichlobenil at a dietary level of 350 mg/kg food for two years resulted in elevated alkaline phosphate (from week 12 and higher), glucose-6-phosphatase and glucose-6-phosphate dehydrogenase activities, increased weight of the liver and thyroid and histopathological liver changes in both sexes.

At dietary levels of 50 mg/kg food and less, no treatment related effects were noted.

Therefore, the NOAEL is set at 50 mg/kg food (equal to 1.3 mg/kg bw/d) based on liver effects and increased thyroid weights at the next higher level.

B.6.3.4.6 Statement 90-day inhalation and 90-day dermal

Two statements for not performing a 90-day inhalation and 90-day dermal toxicity study are provided.

In view of the application rate of 1-2 times per year the dermal and inhalation exposure will not occur on a repeated basis. Based on the results of a 28-day inhalation (NOAEL 12 mg/m³, maximum concentration tested) (7-day inhalation study (LOAEL 21 mg/m³), and 21-day dermal study (NOAEL 1000 mg/kg maximum dose tested) it is not expected that a 90-day study will provide extra information. The statements are considered acceptable. However, the remark 'no effects up to 200 mg/m³ were seen' is not acceptable, liver weight increase was observed from 21 mg/m³ in a 7-day inhalation study (Newton, 2002).

B.6.3.5 Summary

The results of the sub-acute and semi-chronic toxicity studies are summarised in Tables 6.3.5.1 and 6.3.5.2, respectively.

Table 6.3.5.1 Sub-acute studies

Duration	Species	Route	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
21 days	mice	dermal	> 1000	-	-	Allan, 1995
28 days	rats	inhalation	≥12 mg/m ³	-	-	Newton, 2002

Table 6.3.5.2 Semi-chronic studies

Duration	Species	Route	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
90 days	hamster	oral	2.3	12	decreased food intake, increased ALAT, increased cholesterol and phospholipids,	Kemp, 1987a

Duration	Species	Route	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
					increased liver weight, decreased prostate weight	
90 days	mice	oral	19	91	decreased food consumption, decreased glucose, increased testis weight, swollen centrilobular cells of the liver	Kemp, 1987b
52 weeks	dog, Beagle	oral	-	1	increased liver weight	Pickersgil, 1995
two years	dog, Beagle	oral	1.3	9.1	increased ALAT and ALP; increased glucose-6- phosphatase and glucose-6- phosphate dehydrogenase; increased liver and thyroids weight	Til, <i>et al.</i> , 1969, Dijkstra, 1990

The two 90-day rat toxicity studies are not acceptable and can be used as supplementary information (van Esch and Arnoldussen, 1961 en Hodge, 1961)

The 28-day and 90-day dose range findings studies in dogs can be used as supplementary (Pickersgil, 1994 and Til, *et al.*, 1967).

B.6.4 GENOTOXICITY (ANNEX IIA 5.4)**B.6.4.1 *In vitro*****B.6.4.1.1 Bacterial mutation test****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: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537 TA1538	point mut. point mut. point mut. point mut. point mut.	- - - - -	- - - - -	rat liver	Arochlor 1254	0, 40, 200, 1000 and 5000 µg/plate solvent: DMSO	Seket, 1983
Test substance: Dichlobenil, batch no. FUX003000/FUN82B07A, purity 98.8%, cream coloured powder Cytotoxicity observed at dose level: not indicated Precipitation observed at dose level: ≥ 5000 µg/plate, therefore the highest dose level scored was 1000 µg/plate. GLP statement: yes According to OECD 471(1983): yes							

Acceptability

The study was not performed according to OECD 471 adopted on 21st July, 1997 as no strain for detecting cross-linking mutagen activity was included. However, this study was performed according to the former version of the OECD guideline 471, adopted on May 26th, 1983, which was valid at the time of performance of the study.

Conclusions

The test substance did not induce point mutations in *S. typhimurium*.

B.6.4.1.2 Gene mutation**Study 1****Study design and results**Type of study: mammalian cells *in vitro*, gene mutation test

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
mouse lymphoma cells L5178Y	gene mutations (TK)	I ¹	I ¹	rat liver	Arochlor 1254	Experiment 1: 0, 160, 190, 220 and 250 µg/ml (- S9); 0, 20, 30, 40, 50 µg/ml (+ S9).	Richold <i>et al.</i> , 1983

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
						Experiment 2: 0, 190, 220, 250 and 280 µg/ml (-S9); 0, 10, 15, 20, 25 µg/ml (+S9). solvent: DMSO	
¹ I = inaccurate Test substance: Dichlobenil, batch no. FUX003000/FUN82B07A, purity 98.8%, off-white powder Cytotoxicity observed at dose level: ≥ 280 µg/ml (-S9) and ≥ 60 µg/ml (+S9) Precipitation observed at dose level: not indicated GLP statement: yes According to OECD 476 (1984): yes							

Acceptability

The study was not performed according to OECD guideline 476 adopted on 21 July 1997, but according to the former version of 4 April, 1984. In both the absence and presence of S9, concentration levels were used which are about equally (>75%) cytotoxic, whereas a toxicity range between maximum to little or no toxicity should be covered. Therefore, the study is not considered acceptable based on the chosen concentration range.

Conclusions

No conclusions can be derived as the test is not acceptable.

Study 2

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		
Chinese hamster ovary (CHO-K ₁) cells	gene mutations (HGPRT)	-		rat liver	Arochlor 1254	Experiment 1 (in duplo): 0, 5, 15, 25 and 50 µg/ml (-S9); 0, 1.5, 15, 25 and 50 µg/ml (+S9). Experiment 2 (in duplo): 0, 1.5, 5, 15, 25 and 50 µg/ml (-S9 and +S9). solvent: DMSO	Clarke, 2007
¹ I = inaccurate Test substance: Dichlobenil, lot no. MI-0779, purity 99.3%, white powder In experiment 1, relative cloning efficiency was 62% and 31% at the highest concentration tested, -S9 and +S9, respectively. In experiment 2, no cytotoxicity was observed. Precipitation observed at dose level: ≥ 50 µg/ml (+S9 and -S9) GLP statement: yes According to OECD 476: yes							

Acceptability

The study is considered acceptable.

Conclusions

B.6.4.1.3 Chromosome aberration

Study design and results

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
human blood lymphocytes	chromosome aberration	I ¹	I ¹	rat liver	Arochlor 1254	0, 0.1, 0.5 and 1.0 µg/ml (+ S9 and - S9) solvent: DMSO	Allen <i>et al.</i> , 1983

¹ I = inaccurate

Test substance: Dichlobenil, batch no. FUX003000/FUN82B07A, purity 98.8%, off-white powder

Cytotoxicity observed at dose level: -

Precipitation observed at dose level: > 1.0 µg/ml

GLP statement: yes

According to OECD 473: yes

The highest concentration tested was 1.0 µg/ml. Precipitation was not observed. From other *in vitro* mutagenic studies, it appears that the test substance does not precipitate at concentrations up to 51.2 µg/ml. Selecting the dose of 1.0 µg/ml as the highest dose in this study was therefore questionable. Based on this, the study is not considered acceptable.

No conclusions can be derived as the test is not acceptable.

Study design and results

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster ovary (CHO) cells	chromosome aberration	-	-	rat liver	Arochlor 1254	0, 45, 60, 80 and 100 µg/ml (- S9) 0, 25, 50, 75 and 100 µg/ml (+S9) solvent: DMSO	Murli, 1990
Test substance: Dichlobenil, batch no. FUN87E08A/FUX011000, purity > 99%, white powder Cytotoxicity observed at dose level: 100 µg/ml (severe cell cycle arrest; -S9 only) Precipitation observed at dose level: 100 µg/ml GLP statement: yes According to OECD 473: yes							

Acceptability

The study is considered acceptable.

Conclusions

The test substance did not induce chromosome aberrations in Chinese Hamster Ovary cells.

B.6.4.1.4 Unscheduled DNA synthesis**Study design and results**

Type of study: unscheduled DNA synthesis (UDS) assay in primary mammalian cells

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
human HeLa S3 epithelioid cells	DNA repair (unscheduled DNA synthesis)	-	-	rat liver	Arochlor 1254	0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2 and 102.4 µg/ml (+S9 and - S9) solvent: DMSO	Allen <i>et al.</i> , 1983
Test substance: Dichlobenil, batch no. FUX003000/FUN82B07A, purity 98.8%, off-white powder Cytotoxicity observed at dose level: - Precipitation observed at dose level: ≥ 51.2 µg/ml (+S9 and -S9) GLP statement: yes According to OECD 482: yes							

Acceptability

The study is considered acceptable.

Conclusions

Although at some concentrations an induction of UDS was observed in the absence of the S9, this effect was not reproducible. Furthermore, all dose levels showed a percentage of cells in repair below 20%. As such, the test substance did not induce a reproducible induction of unscheduled DNA synthesis in mammalian cells.

B.6.4.1.5 Transformation assay**Study 1****Study design and results**

Type of study: Transformation assay in mammalian cells

Indicator cells	Endpoint	Result	Dose range	Reference
BALB/c-3T3 mouse cells	Cell transformation	-	0, 240, 480, 720, 900 and 1125 µg/ml (- S9) solvent: culture medium	Rundell, 1984

Indicator cells	Endpoint	Result	Dose range	Reference
Test substance: Dichlobenil, batch no. FUX003000/FUN82B07A, purity 98.8%, off-white powder Cytotoxicity observed at dose level: $\geq 1125 \mu\text{g/ml}$ (-S9) Precipitation observed at dose level: $> 2000 \mu\text{g/ml}$ (-S9) GLP statement: yes According to EG 88/302/EEC method B.21: yes				

Acceptability

The study is considered acceptable.

Conclusions

The test substance did not induce cell transformation in the absence of S9 and was therefore considered to be inactive in this test.

Study 2

Study design and results

Type of study: Transformation assay in mammalian cells

Indicator cells	Endpoint	Result	Activation		Dose range	Reference
			Tissue	Inducer		
BALB/c-3T3 mouse cells	Cell transformation	-	rat liver	Arochlor 1254	0, 667, 2667, 4000, 6000 and 7500 $\mu\text{g/ml}$ (+S9) solvent: culture medium	Matthews, 1984
Test substance: Dichlobenil, batch no. FUX003000/FUN82B07A, purity 98.8%, off-white powder Cytotoxicity observed at dose level: $\geq 8000 \mu\text{g/ml}$ (+S9) Precipitation observed at dose level: $> 2000 \mu\text{g/ml}$ (+S9) GLP statement: yes According to EG 88/302/EEC method B.21: yes						

Acceptability

The study is considered acceptable.

Conclusions

The test substance did not induce cell transformation in the presence of S9 and was therefore considered to be inactive in this test.

B.6.4.2 *In vivo*

B.6.4.2.1 Chromosome aberration

Study design and results

Type of study: mouse micronucleus test

Species	Endpoint	Result	Dose range	Reference
Mouse, SPF Swiss 5/sex/dose	micronuclei (bone marrow)	-	0, 300, 600 and 1200 mg/kg bw/d, administered by gavage in two equal dosages with an interval of 24 hours; sacrifice at 6 h after last	Jens, 1983

Species	Endpoint	Result	Dose range	Reference
			dose vehicle: 1%-tragacanth suspension	
Test substance: Dichlobenil, batch no. FUX003000/FUN82B07A, purity 98.8%, off-white powder Toxicity observed at dose level: no change in the ratio PCE/NCE is observed as a result of exposure. The highest dose was chosen based on an LD ₅₀ of 1400 mg/kg bw from a study with mice (not submitted) GLP statement: yes According to OECD 474: yes † compound was suspended in 1%-tragacanth suspension				

Acceptability

The study is considered acceptable. No change in PCE/NCE ratio is observed as a result of exposure to the test substance. It is noted that it is not clear whether the test substance did reach the bone marrow as no toxic effect was found on the erythropoiesis and no increase in micronuclei in PCE of the bone marrow was seen. Considering the results of the ADME study, it can be concluded that the test substance reached the blood, and as such has reached the target cells.

Conclusions

The test substance did not induce micronuclei in mouse bone marrow cells.

B.6.4.3 Summary

The results from the *in vitro* and *in vivo* genotoxicity studies are summarized 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	
Dichlobenil	B: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537 TA 1538	point mut. point mut. point mut. point mut. point mut.	- - - - -	- - - - -	Seket, 1983
Dichlobenil	Chinese hamster ovary (CHO) cells	Gene mutation	-	-	Clarke, 2007
Dichlobenil	Chinese hamster ovary (CHO) cells	chromosome aberration	-	-	Murli, 1990
Dichlobenil	human HeLa S3 epithelioid cells	DNA repair (unscheduled DNA synthesis)	-	-	Allen <i>et al.</i> , 1983
Dichlobenil	BALB/c-3T3 mouse cells	Cell transformation	- -	- -	Rundell 1984 Matthews 1984

The gene mutation study of Richol *et al.*, 1983 and the chromosome aberration test of Allen *et al.*, 1983 are not considered acceptable.

Table 6.4.3.2 *In vivo* genotoxicity studies

Test substance	Type of study		Result	Reference
	Species	Endpoint		
Dichlobenil	Mouse, SPF Swiss	micronuclei (bone marrow)	-	Jens, 1983

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

Conform 91/414 it is mandatory to perform a carcinogenicity study with the mouse as test species. However, the hamster was shown to be more sensitive than the mouse (Kemp *et al.*, 1987a and 1987b). Therefore it is acceptable to perform the carcinogenicity study with the hamster instead of with the mouse.

B.6.5.1 Investigations to the toxicity of dichlobenil including a chronic experiment with rats

In the study report of van Esch and Verschuuren (1964) investigations to the toxicity of dichlobenil including a chronic experiment with rats are described. Due to various shortcomings the study is not considered acceptable (see B.6.8 Further toxicological studies).

B.6.5.2 Chronic toxicity and carcinogenicity

B.6.5.2.1 Chronic toxicity and carcinogenicity study in rats

Characteristics of study

Reference	: Inoue and Enomoto (1984) and Inoue and Enomoto (1998)	Route	: oral
Year of execution	1980-1982	Group size	: 50/sex/dose (and 10/sex/dose for interim kills after 52 and 78 weeks)
Guideline	: OECD 452	Exposure	: 110 weeks, in diet
GLP	yes	Doses	: 0, 50, 400 and 3200 mg/kg food ¹
Test substance	: Dichlobenil, lot no. No. FUN80C25A; white solid	Vehicle	: Olive oil (20 g/kg food)
Purity	: 96.7%	Acceptable	: yes
Species	: rat, Fischer F344	LOAEL	: 50mg/kg food (<3.2 mg/kg bw/d)

¹ Equal to 0, 3.2, 29, and 241 mg/kg bw/d for males and 0, 3.2, 26 and 248 mg/kg bw/d for females.

Study design

The study was performed in accordance with OECD 452.

An addendum containing historical data on kidney fibrosis was submitted in addition to the report (Inoue and Enomoto, 1998).

Results

The results are presented in Table 6.5.1.1.

Table 6.5.1.1 Results of the chronic and carcinogenicity study with dichlobenil in rats

Dose (mg/kg food)	0		50		400		3200		dr
	m	f	m	f	m	f	m	f	
Mortality (%)	17/50	17/50	24/50	13/50	18/50	16/50	50/50 ¹	22/50	
Clinical signs (total,							i ²		

Dose (mg/kg food)	0		50		400		3200		dr
	m	f	m	f	m	f	m	f	
incl. ophthalmoscopy)									
Body weight gain ³					d (11%)	d (5%)	dc (35%)	dc (53%)	m,f
Food consumption						dc (4%)	dc (8%)	dc (15%)	f
Water consumption			No dose related findings						
Haematology ⁵									
Hct							dc (13%)	dc (10%)	
Hb							d (9%)	dc (9%)	
MCV						dc (3%)	d (8%)	dc (12%)	f
MCH							d (4%)	dc (6%)	
Clinical chemistry									
Urea nitrogen					ic ⁴ (75%)		ic (210%)	ic (30%)	
Creatinine					ic ⁴ (42%)		ic (24%)		
Total cholesterol					ic ⁴ (69%)		ic (177%)	ic (157%)	
Albumin							dc (19%)	dc (18%)	
Inorg. P							ic (52%)	ic (24%)	
Ca							ic (11%)		
Glucose							dc (34%)		
Uric acid							ic (35%)		
Urinalysis (week 78) ⁶									
Volume							ic (150%)		
Density							dc (124%)		
Glucose							4/8	2/8	
Organ weights (week 78 male and 110 week female)									
liver					ic ^r (15%)	ic ^{a,r} (11%)	ic ^{a,r} (48%)	ic ^{a,r} (48%)	
kidney					ic ^r (14%)		ic ^{a,r} (16%)	ic ^r (9%)	
thymus							dc ^{a,r} (66%)	dc ^a (42%)	
spleen					ic ^r (135%)		ic ^r (8%)	ic ^r (week 78) (9%)	
Pathology									
<u>macroscopy</u>									
<u>microscopy</u>									
<i>non-neoplastic lesions</i>									
<i>Kidney</i>									
- nephrosis (grade 2+3)	7/47	8/47	15/47	10/47	32/47	13/47	48/50	43/47	m,f
- glomerulosclerosis	0/47	23/47	36/47	23/47	44/47	32/47	49/50	45/47	
- fibrosis (grade 1)	6/47		24/47		43/47		39/50		
- calcification (grade 2)	3/47	46/47	5/47	43/47	5/47	45/47	24/47	40/47	
<i>Liver</i>									
- Fatty metamorphosis	1/47	5/47	2/47	3/47	0/47	8/47	45/50	33/47	
<i>Parathyroid</i>									

No summary tables are presented

Dose (mg/kg food)	0		50		400		3200		dr
	m	f	m	f	m	f	m	f	
- hyperplasia	3/47		9/47	4/47	16/47	1/47	37/47	17/47	m
<i>Esophagus</i>									
- hyperkeratosis	3/47		15/47		23/46		40/50		
<i>Bone marrow</i>									
- telangiectasis	1/45		5/42		16/47		28/50		m
-fibrosis								++	
<i>Testes</i>									
- hyperplasia							++		
interstitial cells									
<i>Mammary</i>									
- atrophy							++		
<i>Prostate</i>									
-atrophy					+		++		m
<i>Bone</i>									
-atrophy					+	+	++	++	m
-fibrosis					+		++		m
<i>Vessels</i>									
- fibrosis	1/46		4/47		4/47		23/50		m
- calcification	1/46		3/50		6/47		28/50		m
<i>Pancreas</i>									
- oxyphilic cells in islets							+	+	
- atrophy exocrine tissue					+		++	+	m
<i>Stomach</i>									
-fibrosis	3/47	1/47	14/47	1/47	13/47	5/47	11/50	5/47	
<i>Microscopy</i>									
<i>neoplastic lesions</i>									
<i>Kidney</i>									
- adenocarcinoma							2/50	1/47	
- hamartoma							1/50		
<i>Liver</i>									
- adenoma						2/47	4/50	6/47	f
- carcinoma							1/50	1/47	
<i>Blood</i>									
- leukemia		1/47	3/47	1/47	2/47	1/47	2/50	5/47	
<i>Lymph</i>									
- malignant lymphoma	2/45	3/47	1/47	1/46	2/47	3/47		6/47	

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 absolute organ weight

r relative organ weights

+ present in one/a few animals

++ present in most/all animals

1 deaths in 3200 mg/kg food group increased from week 68 and all males died within 102 weeks

2 It should be noted that in the summary tables no incidence data are available on specific clinical signs, only a total clinical effects calculation.

3 % body weight gain relative to controls from week 0-110

4 effect determined after 110 weeks

5 due to the mortality in males in the highest dose group, only values from week 52 and week 78 are available for males. In the table only the effects to week 78 are reported, the tendency is idem after 52 weeks and after 110 weeks.

6 yellow coloured urine increased in both sexes

Acceptable

The study is considered acceptable.

Conclusions

The main effects of dichlobenil were seen on the liver and kidney. Blood biochemistry parameters (urea, cholesterol and electrolytes) were affected primarily in the highest dose group with urea and cholesterol also increased at 400 mg/kg food indicating changes in liver and/or kidney function.

Renal pathological effects were already noted at interim sacrifice after 52 weeks.

The pattern of histopathological changes observed in several organs (primarily in males) is most likely directly or indirectly related to this renal pathology.

Increases in nephrosis, glomerulosclerosis and renal fibrosis, parathyroid hyperplasia, calcification in the kidney and blood vessels, and atrophy of the bones represent a coherent pathological pattern induced by renal failure.

At the 50 mg/kg food level the increase in renal fibrosis is already highly statistically significant while related histopathological effects are all present at borderline importance. Because this histopathological pattern is coherent and well known in the literature and, moreover, is dose-dependently increased, the 50 mg/kg food level will be considered to be an effect level. Therefore, the NOAEL in this study cannot be determined, the LOAEL is 50 mg/kg food level (3.2 mg/kg bw/d).

At the highest dose, in both males and females an increase in malignant tumour incidence was observed (adenocarcinoma in kidney, carcinoma in liver, leukemia and malignant lymphoma). In this study, the highest dose clearly exceeds the so-called maximal tolerated dose (MTD) considering for example the high mortality in males. Therefore, the relevance of the increased tumour incidences at the highest dose should be questioned (however taking into account that there seems to be a dose-related increase in liver adenoma). At the dose of 400 mg/kg food two adenomas were observed in female liver (2 adenomas in 47 animals = 4.3%). However, no indications of liver cytotoxicity were observed which could constitute a possible indirect mechanism of carcinogenesis, caused by high toxicity.

The notifier submitted historical control data from:

- the conducting laboratory (AN-PYO = Biosafety Research Center, Foods, Drugs and Pesticides, in Japan) over a period of 9 years from 1981 – 1989
- the breeder, Charles River, for 13 chronic rat studies with in-life completion dates ranging from 1980-1986
- US National Toxicology Program (NTP) from in total 20 studies (with F-344 rats) with in-life completion dates ranging from 1971-1995

Table 6.5.1.2 Historical control data on the incidence of hepatic adenoma and carcinoma in male and female F-344 rats

	Adenoma		Carcinoma	
	Male	Female	Male	Female
Conducting laboratory (1981–1989)	5/569 animals = 0.9% range 0 – 7.7%	2/573 animals = 0.3% range 0 – 3.0%	3/569 animals = 0.5% range 0 – 2.7%	1/573 animals = 0.2% range 0 – 2.6%
Charles River Breeder (1980 – 1986)	8/956 animals = 0.8% range 0 – 4.3%	8/954 animals = 0.8% range 0 – 3.3%	6/956 animals = 0.6% range 0 – 3.3%	2/954 animals = 0.2% range 0 – 1.3%
NTP studies	23/1002 animals	4/1000 animals =	7/1002 animals =	1/1000 animals =

(1971 – 1995)	= 2.3% range 0 – 10%	0.4% range 0 – 4.0%	0.7% range 0 – 6.1%	0.1% range 0 – 2.0%
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The study was performed in the AN-PYO laboratory. The Fischer F-344 rats received dichlobenil from May 1980 until June 1982. The historical control data from the conducting laboratory are therefore relevant (although the time period, 1981-1989, is rather long). The historical control data from the breeder are less relevant, but are in the same range as from the conducting laboratory. The historical control data from NTP studies are not considered relevant, since the studies were performed in different laboratories and furthermore the time period is too extensive.

It can be concluded that the two adenomas observed in female liver at the dose of 400 mg/kg food (2 adenomas in 47 animals = 4.3%) are slightly above the historical control range of the conducting laboratory (and the breeder).

B.6.5.2.2 Oral carcinogenicity study in hamsters

Study 1

Characteristics of study

Reference	: Hooks <i>et al.</i> , 1990	Route	: oral
Year of execution	: 1988-1989	Group size	: 50/sex/dose (100/sex for control group)
Guideline	: OECD 451	Exposure	: 88 weeks (males) and 80 weeks (female), in diet
GLP	: yes	Doses	: 0, 5, 26, 132 and 675 mg/kg food ¹
Test substance	: 2,6-dichlorobenzonitrile, batch no. FUN87E08A/FUX011000, white crystals	Vehicle	
Purity	: 99.4%	Acceptable	: In combination with Chambers <i>et al.</i> , 1992a
Species	: hamsters, Alexander Syrian	NOAEL	: 132 mg/kg food (9.2 mg/kg bw/d)

¹ Equal to 0, 0.34, 1.7, 9.4, 46 mg/kg bw/d for males and 0, 0.35, 1.8, 9.2, 49 mg/kg bw/d for females.

Study design

The study was performed in accordance with OECD 451. Choice of species was based on the results of two 13-week dietary studies with hamsters and mice (Kemp *et al.*, 1987a and 1987b). In the mice study dose levels of 4, 19, 95 and 473 mg/kg bw/d and in the hamster study dose levels of 3, 16, 79 and 263 mg/kg bw/d were used. The hamster did not tolerate an initial diet concentration of 395 mg/kg bw/d. The liver was a target organ for the effects of dichlobenil in both species. In this respect the females of both species were more sensitive to the effect of dichlobenil than the males and the female hamster was about 6 times more sensitive than the female mouse. Because the hamster was the most sensitive of the two species and showed a wider spectrum of primary target organ toxicity it has been decided to select this species for testing further oncogenic potential of dichlobenil.

From the 13-week dietary study with hamsters, the highest dose level, 263 mg/kg bw/d showed a slight (reversible) macrocytic anaemia. Liver weight increase was present from 16 mg/kg bw/d in the females. Prostate weight was decreased, in males in all dose levels and this organ showed an increase of calcification (Kemp *et al.*, 1987a).

Results

The results are presented in Table 6.5.1.2a.

It should be noted that in the summary tables no incidence data are available on specific clinical signs.

The author reported that there were no apparent ante-mortum clinical signs of a reaction to treatment.

With regard to haematology, only differential white cell counts were performed instead of a detailed characterisation of all the blood cells.

Table 6.5.1.2a Results of the carcinogenicity study with dichlobenil in hamsters

Dose (mg/kg food)	0		5		26		132		675		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	50/ 100	58/ 100	20/50	28/50	26/50	29/50	18/50	35/50	21/50	22/50	
Clinical signs	No treatment related findings										
Body weight gain											
Food consumption											
Haematology	No treatment related findings										
Clinical chemistry	Not determined										
Organ weights											
Liver											
Pathology											
<u>macroscopy</u>											
<i>Liver</i>											
- pale											
- pitting of the liver											
<i>Adipose tissue reduction (minimal)</i>											
<i>Adrenals</i>											
- Pale focus											
<u>microscopy</u>											
<i>neoplastic lesions</i>											
<u>microscopy</u>											
<i>non-neoplastic lesions</i>											
<i>Liver</i>											
- Centrilobular hepatocyte enlargement and rarefaction											
<i>Adrenals</i>											
- cortical hyperplasia											

dr dose related

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

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

a/r absolute/relative organ weight

+ incidence ca. twice as high as in control

++ incidence more than twice as high as in control

Acceptable

From the study results it is not clear whether dichlobenil was tested up to the Maximum Tolerated Dose. However, in combination with the study of Chambers *et al.*, 1992a, the carcinogenic potential of dichlobenil in hamsters is sufficiently tested.

Conclusions

Treatment by prolonged oral administration of dichlobenil to hamsters at levels up to 675 mg/kg food (46 mg/kg bw/d) revealed no evidence of a carcinogenic potential. Treatment related changes were primarily confined to the high dosage level. In the 675 mg/kg food male group these changes included initial impairment of body weight gain with associated lower food consumption, and lower overall body weight change together with the histopathological finding of a reduction in the adipose tissue in the male decedents. In high dose females liver changes and cortical hyperplasia of the adrenals were observed. The pale focus in the adrenals observed in the female 132 mg/kg food group is considered not a critical effect. The NOAEL is set at the next lower dose level of 132 mg/kg food (equal to 9.2 mg/kg bw).

Study 2

Characteristics of study

Reference	: Chambers <i>et al.</i> , 1992a	Route	: oral
Year of execution	: 1989-1991	Group size	: 50/sex/dose (100/sex for control group)
Guideline	: OECD 451	Exposure	: 91 weeks (males) and 78 weeks ¹ (females), in diet
GLP	: yes	Doses	: 0, 675, 1500 and 3375 mg/kg food ²
Test substance	: 2,6-dichlorobenzonitrile, batch no. FUN87E08A/FUX011000, white crystals	Vehicle	: none
Purity	: 99.4%	Acceptable	: in combination with study Hooks <i>et al.</i> , 1990
Species	: hamsters, Alexander Syrian	NOAEL	: < 675 mg/kg food (51 mg/kg bw/d)

¹ Termination was effected when the respective control group mortality attained 50%

² Equal to 0, 51, 117 and 277 mg/kg bw/d for males and 0, 55, 121 and 277 mg/kg bw/d for females.

Study design

The study was performed in accordance with OECD 451. Choice of species was based on the results of two 13-week dietary studies with hamsters and mice a (Kemp *et al.*, 1987a and 1987b), as the hamster was more sensitive than the mouse (see study design of B.6.5.1.2). Dose levels were based on the results of a 13-week dietary study with hamsters, in which dose levels of 3, 16, 79 and 263 mg/kg bw/d were used (Kemp *et al.*, 1987a) and a 88-week oncogenicity study with hamsters, with dietary levels of 5, 26, 132 and 675 mg/kg food in the study in which dichlobenil had only minor effects on the hamster at any dietary level (Hooks *et al.*, 1990). With regard to haematology, only differential white cell counts were performed instead of a detailed characterisation of all the blood cells.

Results

The results are presented in Table 6.5.1.2b.

It should be noted that in the summary tables no incidence data are available on specific clinical signs.

The author reported that there were no signs of a reaction to treatment.

Table 6.5.1.2b Results of the carcinogenicity study with dichlobenil in hamsters

Dose (mg/kg food)	0		675		1500		3375		dr
	m	f	m	f	m	f	m	f	
Mortality	50/ 100	50/ 100	26/50	23/50	27/50	21/50	24/50	11/50	
Clinical signs			No treatment related findings						
Body weight									
Body weight gain			dc (68%)	dc (34%)	dc (136%)	dc (37%)	dc (107%)	dc (30%)	
Body weight (terminal)			d (7%)	d (8%)	d (13%)	d (8%)	d (17%)	d (7%)	
Food consumption			dc (6%)		dc (5%)		dc (9%)	dc (5%)	
Haematology			No treatment related findings						
Clinical chemistry			Not performed						
Organ weights									
Liver									
absolute						i ^a (14%)	i ^a (35%)	i ^a (50%)	
relative				ic (9%)	ic ^r (13%)	ic ^r (23%)	ic ^r (53%)	ic ^r (59%)	
Testes					dc ^r (12%)		dc ^r (13%)		
Heart			dc ^a (6%)		dc ^a (9%)		dc ^a (6%)	dc ^a (10%)	
Pathology									
<u>macroscopy</u>									
<i>Liver</i>									
- enlarged						++	+	++	f
- increased masses							+		
<i>Urinary bladder</i>									
<i>distension</i>					++		+		
<i>Penis protrusion</i>			++		++		++		m
<i>Spleen</i>									
- pallor							+		
- decreased size							+		
<u>microscopy</u>									
<i>neoplastic lesions</i>									
<i>Liver</i>									
- benign tumour					1/50		6/50		
- malignant tumour							1/50		
<u>microscopy</u>									
<i>non-neoplastic lesions</i>									
<i>Liver</i>									
- Minimal centrilobular hepatocyte enlargement	7/100	7/100	15/50	5/50	10/50	5/50	14/50	11/50	
- hepatitis	1/100		1/50		2/50	3/50	5/50	4/50	
- pigmented giant cells	10/100		4/50		5/50		20/50		
- pigmented sinusoidal cells	5/100	7/100	1/50	4/50	3/50	2/50	9/50	9/50	
- brown pigment in hepatocytes	9/100	1/100	7/50	1/50	16/50	3/50	19/50	10/50	

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
+ present in one/a few animals

++ present in most/all animals

Acceptable

The study is not considered acceptable as a carcinogenicity study as the mid and high doses were clearly higher than the Maximum Tolerable Dose. However, in combination with the study of Hooks *et al.*, 1990 the carcinogenic potential of dichlobenil in hamsters is sufficiently tested.

Conclusions

In all dose groups body weight decrease was observed. The body weight reduction was principally attributable to effects seen during the first two weeks of treatment when mean body weight loss was recorded amongst males receiving 1500 mg/kg food and both sexes receiving 3375 mg/kg food; whilst lower mean weight gain was recorded for males receiving 675 mg/kg food and females receiving 675 or 1500 mg/kg food, when compared with controls.

An increased incidence of benign liver tumours in males receiving 3375 mg/kg food was seen. A single malignant tumour was also recorded for males of this treatment level, whilst a single benign liver tumour was also recorded amongst males receiving 1500 mg/kg food.

Non-neoplastic changes were evident in the liver, principally amongst hamsters receiving 1500 or 3375 mg/kg food consisting of hepatitis in both sexes receiving 3375 mg/kg diet and also females receiving 1500 mg/kg diet; pigmented giant cells in males receiving 3375 mg/kg food; brown pigments in hepatocytes in both sexes receiving 1500 or 3375 mg/kg food and minimal centrilobular hepatocyte enlargement in all treatment groups.

In addition to hepatotoxicity, evident at all treatment levels, toxicity was also manifest as impaired body weight gain food intake amongst all treated males and for females receiving 3375 mg/kg food.

The doses 675 mg/kg food for males and 3375 mg/kg food for females was considered to be the no adverse observed effect level with respect to tumorigenicity. Regarding the non-neoplastic effects, no NOAEL could be established. The LOAEL is considered to be 675 mg/kg food or 51 mg/kg bw/d.

In this study, the mid and highest dose levels exceed the so-called maximal tolerated dose (MTD), e.g. the high increase of relative liver weight. Therefore, the relevance of the tumours observed at doses of 1500 and 3375 mg/kg food should be questioned.

B.6.5.2.3 Background tumour incidence study in hamsters

Study design

The principal objective of this study (Chambers *et al.*, 1992b) was to obtain data on the incidences of spontaneously occurring tumours in hamsters of the F1D Alexander Syrian strain. The information was submitted in support of two previous studies (Hooks *et al.*, 1990 and Chambers *et al.*, 1992a) in this strain of hamster to assess the potential tumorigenic effects of dichlobenil. The conditions and procedures used in this study were designed to duplicate those of the aforementioned investigations. The study consisted of one untreated group each of 50 male and 50 female hamster monitored from about 6 weeks of age until approximately 50% survival was reached in each group at which time all

surviving animals were sacrificed. This occurred after 77 weeks for females and 90 weeks for males. All animals were subjected to histopathological examination to assess tumour incidences. Additional data were also generated for survival, body weight change and food consumption with limited haematological and organ weight parameters.

Results/Conclusions

Values for all parameters investigated in this study were essentially similar to the respective control groups of the previously mentioned tumorigenicity studies.

With regard to neoplastic findings:

- Benign cortical tumours were reported as cortical adenoma and cortical adenoma (fusiform cell type). In male and female hamsters the incidence was 11/50 and 2/50, respectively.
- The incidence of tumours reported in other tissues was comparatively low and ranged between 1/50 and 4/50. In male hamsters the following tumours were reported: malignant lymphoma, malignant islet cell carcinoma in pancreas, malignant parafollicular cell carcinoma in thyroids, malignant cortical carcinoma in adrenals, benign pheochromocytoma in adrenals, malignant osteosarcoma in skeletal muscle. In female hamsters the following tumours were reported: malignant lymphoma, benign islet cell adenoma in pancreas, benign thecoma in ovaries, benign polypoid adenoma in uterus, malignant cortical carcinoma in adrenals, benign ganglioneuroma in adrenals, benign adenoma in pituitary and malignant intestinal adenocarcinoma.
- No benign or malignant tumours in liver were observed in this study.

B.6.5.3 Summary

In Table 6.5.3 the results of the chronic toxicity and carcinogenicity studies with dichlobenil are summarised.

Table 6.5.3 Oral chronic toxicity and carcinogenicity studies

Duration (weeks)	Species	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
110	rat	-	3.2	Kidney: nephrosis (grade 2+3), glomerulosclerosis, fibrosis; hyperplasia of Parathyroid, telangiectasis of the bone marrow. Esophagus: hyperkeratosis. Stomach: fibrosis; vessels (fibrosis and calcification). Tumors: adenocarcinoma kidney and adenoma and carcinoma liver in the 26 and 241 mg/kg bw/d groups	Inoue and Enomoto (1984) and Inoue and Enomoto (1998)
88 (males) and 80 (females)	hamsters	9.2	46	Liver: slightly higher weights, pitted liver and centrilobular hepatocyte enlargement and rarefaction and cortical hyperplasia of the adrenals	Hooks <i>et al.</i> , 1990
91 (males, and 78	hamsters	-	51	body weight and food consumption decrease, liver weight gain, heart weight	Chambers

Duration	Species	NOAEL	LOAEL	Critical effects	Reference
(weeks)		(mg/kg bw/d)	(mg/kg bw/d)		
females)				decrease, penis protrusion, liver effects (among centrilobular hepatocyte enlargement). Tumors: In males benign and malignant tumours were found in liver, at dose levels ≥ 117 mg/kg bw	<i>et al.</i> , 1992a

In the rat study, the highest dose clearly exceeds the so-called maximal tolerated dose (MTD) considering the high mortality in males. Therefore, the relevance of the increased tumour incidences at the highest dose should be questioned (however taking into account that there seems to be a dose-related increase in liver adenoma). However, in the rat also liver adenomas were observed at a dose level below the MTD. It was concluded that the two adenomas observed in female liver at the dose of 400 mg/kg food (2 adenomas in 47 animals = 4.3%) are slightly above the historical control range of the conducting laboratory (and the breeder).

In the second hamster study (Chambers *et al.*, 1992a), the mid and highest dose levels exceed the so-called maximal tolerated dose (MTD), e.g. the high increase of relative liver weight. Therefore, the relevance of the tumours observed at doses of 1500 and 3375 mg/kg food should be questioned. The background tumour incidence study in hamsters showed no benign or malignant tumours in liver. In fact, the study of Hooks *et al.* (1990) might also be regarded as background tumour incidence study showing no tumours in 600 animals.

In conclusion, the relevance of the observed tumours in rats and hamsters at levels exceeding the MTD, for the assessment of the carcinogenic potential of dichlobenil, should be questioned. However, it seems that dichlobenil has some carcinogenic potential in the rat, not explainable by cytotoxicity in liver as this was not observed. It is proposed to regard dichlobenil as a potential carcinogen according to the criteria mentioned in Annex VI of Directive 2001/59/EC and to classify with R40 (category 3; Limited evidence of a carcinogenic effect). As dichlobenil is not genotoxic, a threshold mechanism of its carcinogenicity is likely.

The notifier does not agree with this proposal and submitted a statement against the proposed classification (Oevelen, 2006). However, as explained above, the incidence of the adenomas observed in the rat study in female liver at the dose of 400 mg/kg food is slightly above the historical control range. Furthermore, it should indeed be questioned what the relevance is of the tumours observed at dose levels exceeding the MTD. However, according to the classification and labelling Directive 67/548/EC, Annex VI, also tumours at dose levels exceeding the MTD should be taken into account for classification and labelling:

For a distinction between categories 2 and 3 the arguments listed below are relevant which reduce the significance of experimental tumour induction in view of possible human exposure. These arguments, especially in combination, would lead in most cases to classification in category 3, even though tumours have been induced in animals:

- carcinogenic effects only at very high dose levels exceeding the "maximal tolerated dose". The maximal tolerated dose is characterised by toxic effects which, although not yet reducing lifespan, go along with physical changes such as about 10 % retardation in weight gain,
- appearance of tumours, especially at high dose levels, only in particular organs of certain species known to be susceptible to a high spontaneous tumour formation,
- appearance of tumours, only at the site of application, in very sensitive test systems (e.g. i.p. or s.c. application of certain locally active compounds), if the particular target is not relevant to man,
- lack of genotoxicity in short-term tests in vivo and in vitro,
- existence of a secondary mechanism of action with the implication of a practical threshold above a certain dose level (e.g., hormonal effects on target organs or on mechanisms of physiological regulation, chronic stimulation of cell proliferation),
- existence of a species-specific mechanism of tumour formation (e.g. by specific metabolic pathways) irrelevant for man.

For a distinction between category 3 and no classification arguments are relevant which exclude a concern for man:

- a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man,
- if the only available tumour data are liver tumours in certain sensitive strains of mice, without any other supplementary evidence, the substance may not be classified in any of the categories,
- particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence.

The proposed classification with R40 should be discussed in a PRAPeR meeting and by ECB.

B.6.6.1 Reproductive toxicity

Characteristics of study:

Reference	: Leeming, 1989 Barker, 1992a Barker, 1992b Barker, 1993	Route	: oral (diet)
Year of execution	: 1987-1988	Group size	: F0: 30/sex/dose F1: 25/sex/dose
Guideline	: OECD 416	Exposure	: continuously through the study period
GLP	: yes	Doses	: 0, 60, 350 and 2000 mg/kg food ¹
Test substance	: Dichlobenil, batch no.FUN87E08A/FUX011000, lot no. 4-5, white powder	Vehicle	: powdered diet
Purity	: 99.4%	Acceptable	: acceptable
Species	: rat, CrI:CD(SD)BR	NOAEL _{par}	: 60 mg/kg food (4 mg/kg bw/d)
		NOAEL _{dev}	: 60 mg/kg food (4 mg/kg bw/d)
		reproductive effects:	: >2000 mg/kg food (133 mg/kg bw/d)

[†] Equivalent to 0, 4, 23 and 133 mg/kg bw/d, using a default food conversion factor of 15

The study was performed in accordance with OECD 416. In contrast to the guideline, organ weights and sex ratios were not reported. The study was recorded in one study report and three separate pathology addenda.

The results are presented in Table 6.6.1.1

Table 6.6.1.1: Results of the 2-generation study with dichlobenil in rats

Dose (mg/kg food)	0	60	350	2000	dr
	m f	m f	m f	m f	
<u>F0 animals</u>					
Mortality				1/30 ¹	
Clinical signs		no relevant treatment-related findings			
Body weight				dc (16%)	dc (16%)
Food consumption				dc (14%)	dc (17%)
Mating/fertility/gestation		no treatment-related findings			
Organ weight		not determined			
Pathology					

Dose (mg/kg food)	0		60		350		2000		dr
	m	f	m	f	m	f	m	f	
<u>macroscopy</u>	no treatment-related findings								
<u>microscopy</u>									
<i>liver</i>									
-lobar necrosis	0/30						1/30		
<i>kidney</i>									
-focal nephropathy	0/30						1/30		
-nephroblastoma	0/30							1/30	
<i>pituitary</i>									
-altered cell focus	0/30						2/30		
-cyst	0/30						3/30		
<i>tail</i>									
-dermatitis	0/30						3/30		
<u>F1 pups</u>									m,f
Litter size	no treatment-related findings								
Survival index	no treatment-related findings								
Sex ratio	not reported								
Body weight			dc (4%)		dc (10%)		dc (23%)	dc (24%)	
Pathology									
<u>macroscopy</u>	no treatment-related findings								
<u>F1 animals</u>									m,f
Mortality							1/25 ²		
Clinical signs	no treatment-related findings								
Body weight			dc (5%)		d (6%)		dc (20%)	dc (19%)	
Food consumption			d (5%)		d (5%)		dc (17%)	dc (19%)	
Mating/fertility/gestation	no treatment-related findings								
Organ weight	not determined								
Pathology									
<u>macroscopy</u>									
-no. of implantation sites								dc (16%)	
<u>microscopy</u>									
<i>kidney</i>									
-hyaline droplets	0/25						3/25		
-basophilic tubules	0/25						3/25		
-casts	0/25						3/25		
-cyst	0/25						1/25		
<u>F2 pups</u>									m,f
Litter size							dc (20%)		
Survival index	no treatment-related findings								
Sex ratio	not reported								
Body weight			dc (8%)		dc (10%)		dc (22%)	dc (22%)	

Dose (mg/kg food)	0	60	350	2000	dr
	m f	m f	m f	m f	
Pathology					
<u>macroscopy</u>					
-dilated ureter(s)	18%	38%	27%	30%	
<i>Kidney</i> ³					
-renal pelvic cavitation	0/152			6/178	

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

¹ death caused by liver lesion

² death caused by upper respiratory infection/stomatitis

³ incidence given in percentages

m,f males, females

Acceptability

The study is considered acceptable.

Conclusions

The one death in the F0 high dose group, due to a liver lesion, was considered unlikely to be due to the test substance, since no liver problems were seen in the other high dose animals. The death in the F1 high dose group was caused by of an upper respiratory tract infection and therefore considered not to be treatment related.

The lower litter size in the F1 high dose group may well be due to the lower maternal body weight at conception and is therefore not considered to be a reproduction effect. The kidney anomalies (hyaline droplet nephropathy, basophilic tubules and casts) observed in the F1 high dose group were considered to be treatment related.

A slight increase of dilated ureters was found in culled F2 animals. The control values were, however, extremely low in comparison with historical control data (mean incidence normally about 40%). Since this finding was neither dose-related, being higher in the low dose group than in the higher dose groups, nor seen the other weaned offspring, the effect was considered not to be treatment related. Weaned offspring from three litters in the high dose group showed increased renal pelvic cavitation. Since renal pelvic cavitation is commonly observed in this strain of rat and the incidence seen in this study was low, this finding is not considered to be treatment related.

In conclusion, the NOAEL for parental toxicity in this study is 60 mg/kg food, which corresponds to 4 mg/kg bw/d. Above this NOAEL, a decreased body weight and a decreased food consumption in adults and offspring in both F0 and F1 generations was observed. The NOAEL for developmental toxicity is 60 mg/kg food (4 mg/kg bw/d). Above this NOAEL, a decreased pup body weight was observed.

Reproductive effects were not observed in this study. The NOAEL for reproductive toxicity is therefore ≥ 2000 mg/kg food (133 mg/kg bw/d).

B.6.6.2 Teratogenicity studies**B.6.6.2.1 Teratogenicity study with dichlobenil in rats****Characteristics of study:**

Reference	: Koëter, 1984	Route	: oral (by gavage)
Year of execution	: 1983	Group size	: 25 females/dose
Guideline	: OECD 414 (1981)	Exposure	: days 6-16 of gestation
GLP	: yes	Doses	: 0, 20, 60 and 180 mg/kg bw/d (10 mL/kg bw)
Test substance	: Dichlobenil, batch no. FUX 003000, sample no. FUN 82B07A, off-white crystalline solid	Vehicle	: 1.0% gum tragacanth suspension
Purity	: 98.8%	Acceptable	: acceptable
Species	: rat, Wistar Cpb:WU, SPF	NOAEL _{mat}	: 20 mg/kg bw/d
		NOAEL _{dev}	: >180 mg/kg bw/d
		teratogenic effects	: not observed

Study design

The study was performed in accordance with OECD 414 (1981).

Results

The results are presented in Table 6.6.2.1.

Table 6.6.2.1: Results of the oral embryotoxicity/teratogenicity study with dichlobenil in rats

Dose (mg/kg bw/day)	0	20	60	180	dr
<u>Maternal effects</u>					
Mortality	0	0	0	0	
Clinical signs		no treatment-related findings			
Pregnant animals	23	23	22	21	
Body weight gain (day 6-16) ¹			dc (29%)	dc (36%)	f
Food consumption (day 6-16)			dc (15%)	dc (21%)	f
Organ weight -empty uterus weight (a)			dc (16%)		
Pathology					
macroscopy		no treatment-related findings			
<u>Litter response</u>					
Litters	23	22	22	21	
Live foetuses	279	272	248	266	
Foetal weight		no treatment-related findings			
Post implantation loss		no treatment-related findings			

Dose (mg/kg bw/day)	0	20	60	180	dr
Sex ratio	no treatment-related findings				
Examination of the foetuses					
External observations (no. of foetuses)	0/279	2/272	3/248	3/266	
Skeletal findings (no. of foetuses, no. of litters affected)		not examined			
-thoracic rib no. 14 bilateral present (% difference in degree of ossification ²)	(1/134)			ic (6/129, 4)	
-degree of ossification of cervical vertebral bodies			dc (23%) dc (32%)		
absent					
incomplete					
-degree of ossification of forelimb phalanges			dc (18%)	ic (11%)	
absent					
-degree of ossification of hindlimb phalanges				ic (27%)	
incomplete					
-degree of ossification of sternbrae				ic (1092%) ic (69%)	
absent					
incomplete					
Visceral findings (no. of foetuses, no. of litters affected)					
-minor visceral anomalies	(14/145, 10)	ic (27/140, 13)	i (23/131, 10)	ic (26/135, 17)	
-unilateral cavitation in kidneys	(7/145, 6)		ic (18/131, 14)		

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

¹ no difference in body weight gain was observed during days 16-21² degree of ossification = no. of bones with absent or incomplete ossification ÷ no. of bones examined

f females

Acceptability

The study is considered acceptable.

Conclusions

In this teratogenicity study in rats, the maternal NOAEL is 20 mg/kg bw/d. Above this NOAEL, decreased body weight gain during gestation day 6-16 and a decreased food consumption were observed.

All observed external, skeletal and visceral findings are common in this strain of rats and do not indicate embryotoxic or teratogenic effects that could be related to the exposure of dichlobenil as there was clearly no dose relationship found. The developmental NOAEL is therefore ≥ 180 mg/kg bw/d.

B.6.6.2.2 Teratogenicity study with dichlobenil in rabbits

Characteristics of study:

Reference	: Barker, 1989b, Barker, 1991	Route	: oral (by gavage)
Year of execution	: 1988	Group size	: 18 females/dose
Guideline	: OECD 414 (1981)	Exposure	: days 7-19 of gestation
GLP	: yes	Doses	: 0, 15, 45 and 135 mg/kg bw/d (2mL/kg bw)
Test substance	: Dichlobenil, batch no. FUX 010000, lot no. 3 and 6, white powder	Vehicle	: 1% gum tragacanth suspension
Purity	: 98.5	Acceptable	: acceptable
Species	: rabbit, New Zealand White	NOAEL _{mat}	: 45 mg/kg bw/d
		NOAEL _{dev}	: 45 mg/kg bw/d
		teratogenic effects:	: yes, at 135 mg/kg bw/d

This study is a repeat of a previous study (Barker, 1989a) which had insufficient surviving animals due to an infection and therefore did not meet the criteria. This unaccepted study is not summarised in this dossier.

Study design

The study was performed in accordance with OECD 414 (1981).

Results

The results are presented in Table 6.6.2.2.

Table 6.6.2.2: Results of the oral embryotoxicity/teratogenicity study with dichlobenil in rabbits

Dose (mg/kg bw/day)	0	15	45	135	dr
<u>Maternal effects</u>					
Mortality	0	2	1	1	
Clinical signs		no treatment-related effects			
Pregnant animals	17	18	17	17	
Abortions	1	0	0	2	
Body weight gain (day 7-19)				dc ¹	
Food consumption (day 7-19)				dc (30%)	
Organ weight -gravid uterus weight (a)				d (13%)	
<u>Pathology</u>					
macroscopy					
liver					
-minor liver changes ²				i ³	
<u>Litter response</u>					
Live foetuses/litters	142/16	154/16	146/16	115/14	
Foetal weight		d (6%)	d (2%)	dc (11%)	
Post implantation loss				ic (100%)	
Sex ratio		no treatment-related effects			
<u>Examination of the</u>					

Dose (mg/kg bw/day)	0	15	45	135	dr
foetuses					
Skeletal findings				ic	dr
	(1/142)	(0/154)	(0/164)	(26/115)	
-fontanelle grossly enlarged	0	0	0	23	
-cleft palate	0	0	0	1	
-other severe cranial defects	0	0	0	5	
-major fusion of sternebrae	0	0	0	3	
External and visceral observations				ic	dr
	(0/142)	(1/154)	(1/164)	(11/115)	
-open eye(s)	0	0	0	4	
-hydrocephaly	0	0	0	1	

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

1 1% loss of weight was observed in the high dose group whereas all other dose groups gained 4-5% weight

2 pale, mottled and/or enlarged lobes

3 group incidence not reported

Acceptability

The study is considered acceptable.

Conclusions

The majority of the observed abnormalities, either in the dams or foetuses, were concentrated in (the litters of) the three animals showing the most marked reaction to the treatment with dichlobenil. If these three animals are excluded from the assessment, the overall incidence of the different major abnormalities is still higher in the high dose group than in the control group.

It is noted that the nature of major abnormalities observed in the high dose group in this study have been found as well in a control group of a previous rabbit study conducted in this laboratory, indicating that some deliveries of rabbits may exhibit hereditary abnormalities.

In this teratogenicity study in rabbits, both the maternal and developmental NOAEL are 45 mg/kg bw/d. Above the maternal NOAEL, decreased weight gain (day 7-19), decreased foetal weight and food consumption are observed. Above the developmental NOAEL (45 mg/kg bw/d), major abnormalities, such as grossly enlarged fontanelles, body weight decrease and other major cranial defects and open eyes, are seen in the foetuses. Since these teratogenic defects only occur at a level of maternal toxicity, dichlobenil does not have to be labelled as teratogenic.

B.6.6.3 Summary

The results of the reproduction toxicity and teratogenicity studies are summarised in Table 6.6.3.

Table 6.6.3 Summary of reproduction toxicity and teratogenicity studies

Type of study	Species		NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
<i>Reproduction toxicity</i>						
2-generation toxicity study	rat	Parental	4	23	- decreased body weight and food consumption - decreased pup body weight	Leeming, 1989 Barker, 1992a Barker, 1992b Barker, 1993
		developmental	4	23		
		reproduction effects	not observed	-		
<i>Embryo/foetal toxicity and teratogenicity</i>						
teratogenicity study	rat	maternal	20	60	- decreased body weight gain (day 6-16) and food consumption	Koëter, 1984
		developmental	>180	-		
		teratogenicity	no			
teratogenicity study	rabbit	maternal	45	135	- decreased body weight gain (day 7-19) and food consumption - major abnormalities in the foetuses (grossly enlarged fontanelles, body weight decrease other major cranial defects, open eyes; decreased body weight)	Barker, 1989b Barker, 1991
		developmental	45	135		
		teratogenicity	yes at 135			

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

No specific studies are submitted. In some subacute and semichronic repeated dose toxicity studies FOB and motoractivities are determined. In these studies no neurotoxicological effects are observed. As such, studies on neurotoxicity are not required.

B.6.8 FURTHER TOXICOLOGICAL STUDIES (ANNEX IIA 5.8)

In this chapter first an old study of dichlobenil is summarized. Subsequently a statement from the notifier is presented with regard to the reported effect of dichlobenil on the olfactory nasal mucosa of rodents. From the third paragraph studies with the metabolite 2,6-dichlorobenzamide (BAM) are summarized. BAM is a soil metabolite of dichlobenil and becomes available as residue in plants. BAM is not a metabolite formed in rats based on the submitted metabolism studies.

B.6.8.1 Investigations to the toxicity of dichlobenil including a chronic experiment with rats

Characteristics of study

Reference	: Esch, van and Verschuuren (1964)	Route	: oral
Year of execution	: not indicated	Group size	: see table below
Guideline	: not applicable	Exposure	: see table below
GLP	: yes	Doses	: see table below
Test substance	: H 133 (2,6-dichloro benzonitrile)	Vehicle	: in study 7, croton oil
Purity	: not indicated	Acceptable	: no
Species	: rat/mice/rabbit	NOAEL	: -

Study design

In the study report the following studies are included.

1. Chronic toxicity investigations of H 133 with rats, including generation test.
2. Repeated generation test with rats
3. A 90-days toxicity investigation with F1-generation of rats
4. Liver function test in rats
5. A 90-days toxicity investigation of H133 with rabbits (oral)
6. Investigation into possible initiating properties of H 133 in combination with croton oil in mice.
7. Investigation into teratogenic effects in mice.

Results

1.	Group size 25/sex/dose	Doses 0, 20, 50, 100 ppm	Duration 112 weeks	Critical effects No clear dose related effects (incl. tumours) of influence H 133
2.	10/sex/dose (P-generation)	0, 20, 50, 100 ppm, food	32 weeks	No clear dose related effects (incl. breeding results) of influence H 133
3.	10/sex (F1-generation)	0, 20, 50, 100(5/sex) ppm, food	90 days	Two highest dose groups (decreased body weight gain during test period) number of litters in the 100 ppm group was markedly lower. Increase kidney weights both sexes highest dose group. In males liver, pituitary gland, pancreas and test weight in the two highest dose groups
4.				
a.	21 B.S.P. test	3 g/bw , single dose repeated with 0.6 g/bw	-	2/21 died, The B.S.P. shows two maxima, one after 2 and 3 days, and one after 7 days. After 9 all the values have returned to normal. The results of the second study are comparable with the previous test, but the deviations are less pronounced.
b.	S.G.P.T. determination	3 g/bw , single dose repeated with 0.6 g/bw	-	The high dose level shows about the same picture as the B.S.P. retention (maxima 3 rd and 7 th day). The results of the low dose are less clear.
5.	5/sex/dose	0, 20, 100, 500 ppm pellets	90 days	decreased heart weight and trend liver enlargement in male of highest dose group.
6.	22 female&23 male (control) 15/sex/dose	0 (croton oil 5% on skin), 100 ppm (with and without croton oil 5% on skin)	6 months	No marked deviations. In the group with test substance and without croton oil an increased number of liver deviations were observed.
7.	32 control and 33 test group	60 mg dichlobenil in 10 ml olive oil/ kg bw	3 rd till 14 th day	no effects number of pregnant animals and the number of

	Group size	Doses	Duration	Critical effects
1.	25/sex/dose	0, 20, 50, 100 ppm	112 weeks	No clear dose related effects (incl. tumours) of influence H 133
				dead embryos

Acceptability

The study is not acceptable, in view of the limited number of parameters tested and very limited reporting of the results.

Conclusions

No conclusions can be drawn from this study as it is not considered acceptable.

B.6.8.2 Effect of dichlobenil on the olfactory nasal mucosa of rodents

The notifier submitted a statement with regard to the effect of dichlobenil on the olfactory nasal mucosa of rodents (Story, 2006). The statement is considered acceptable:

It has been reported in the literature that intra-peritoneal administration of dichlobenil to mice at doses of 12 mg/kg and greater has been shown to cause destruction of the olfactory mucosa and necrosis of the Bowman's glands, whereas an intra-peritoneal dose of 6 mg/kg did not produce these effects (refs. 1, 2, 4). A single intra-peritoneal dose of 50 mg/kg dichlobenil was found to cause extensive damage to the olfactory epithelium and Bowman's glands of rats (ref. 12). A single dermal application of dichlobenil caused olfactory damage in mice at doses of 50 mg/kg and greater; however, five sequential applications of 25 mg/kg had no effect on the olfactory mucosa (ref. 5).

It has also been reported that dichlobenil is metabolised in mouse olfactory mucosa homogenates to a greater degree than in liver homogenates (ref. 7). This is considered to be a cytochrome P450 dependent mechanism (refs. 1, 2, 7, 8, 17, 18) that results in the formation of an electrophilic intermediate in the olfactory mucosa, which covalently binds to tissue proteins (refs. 1, 6, 8, 9). Administration of 3-aminobenzene, a probable inhibitor of cytochrome P450, decreased the toxicity of dichlobenil in mice and the addition of glutathione or the P450 inhibitor, metyrapone, prevented the binding of [¹⁴C]-dichlobenil to olfactory microsomal proteins (ref. 10). [¹⁴C]-Dichlobenil binds irreversibly to the Bowman's glands to an even greater extent than in the olfactory epithelium (ref. 1).

Covalent binding to the olfactory mucosa after intra-peritoneal administration of 12 mg/kg [¹⁴C]-dichlobenil was six times greater than the binding seen after administration of 6 mg/kg (ref. 3), establishing a threshold for the covalent binding that corresponds with the NOEL of 6 mg/kg that was determined in previous studies (refs. 1, 2, 4).

However, depletion of glutathione with phorone prior to intra-peritoneal treatment with 6 mg/kg dichlobenil resulted in increased covalent binding and extensive toxicity in the olfactory mucosa (ref. 3).

3). These data demonstrate:

- the protective role of glutathione in the olfactory mucosa and
- that the levels of glutathione in the mucosal tissue are adequate to support detoxification of tissue levels of dichlobenil associated with an intra-peritoneal dose of up to 6 mg/kg.

Studies have been done to evaluate the effect of olfactory mucosal destruction on actual sensory function. No effects were seen in an odour discrimination test in rats after a single intra-peritoneal dose of 50 mg/kg dichlobenil. Two weeks later the rats were given 100 mg/kg and again there were no effects on sensory function. After a single injection of 200 mg/kg dichlobenil it was found that performance on the odour trials dropped to 50%; however performance on the odour task was nearly normal five days after exposure (ref. 12). Histological evaluations showed that the Bowman's glands in rats were reduced or eliminated four days after the intraperitoneal administration of 50 mg/kg dichlobenil, but began to re-appear at day eleven and were fairly normal 25 days after treatment (ref. 12). In another study, dichlobenil (100 mg/kg, i.p.), given to rats previously trained in an olfactory task of finding food buried in bedding, caused a transient increase in the mean latency time to find the food pellet. Treated animals were normal by day 28 (ref. 11). This type of olfactory sensory recovery has also been seen after methyl bromide exposure. Inhalation exposure to 200 ppm methyl bromide, which caused complete olfactory epithelial destruction, was followed by recovery of olfactory function, also assessed by the buried food pellet test, when only a layer of rapidly proliferating basal cells was present throughout the ethmoid turbinate region of the nasal cavity (ref. 13). These studies demonstrate that the nasal mucosa has the ability to regenerate after chemical induced destruction and that sensory function returns with this tissue regeneration.

There appear to be no published studies evaluating the effects of inhalation exposure to dichlobenil. Crompton Corporation has completed a 28-day inhalation study at dosage levels of 2.3, 5.1 and 12 mg/m³ (ref. 15, and see B.6.3.3). Histo-pathology on the nasal tissue showed that there was no effect of dichlobenil at doses up to 12 mg/m³. A previously conducted one week range finding study was conducted at dosage levels of 21, 77 and 200 mg/m³. Degeneration of the olfactory epithelium in the dorsal meatus of the nose was seen in males and females from all groups (ref. 16). These results show that 12 mg/m³ is a no observable effect level for dichlobenil induced nasal toxicity for an exposure period up to 28 days. However, an exposure of 21 mg/m³ for 7 days appears to exceed the detoxification capabilities of the nasal mucosa and induces tissue degeneration. The range of toxicity and margin of safety seen in these studies is similar to that seen with dermal exposure to dichlobenil, described previously, in which a single dose of 50 mg/kg causes mucosal tissue damage, but five sequential doses of 25 mg/kg were tolerated with no detrimental effects (ref. 5). In addition, it should be noted that no effects were observed upon histopathological evaluation of nasal tissue in a one-year dog oral (capsule) toxicity study up to a dose level of 36 mg/kg b.w./day (ref. 16).

Therefore, from the above review, it can be concluded that a safe level of human exposure to dichlobenil can be established. This is supported by the lack of adverse findings in a review of the published literature and in workers involved in the manufacture of dichlobenil. A recently conducted search of the published literature by Crompton Corporation to determine the human exposure/poisoning incidents with dichlobenil did not produce any positive findings. With regard to workers, in more than forty years of manufacturing and handling dichlobenil there have not been any reported cases of adverse effects in workers (see statement from Plant Manager & company physician; annex I).

CONCLUSION

Regardless of the route of exposure, there appears to be a safe level of exposure to dichlobenil and even after that level is exceeded it has been shown that animals can recover from the harmful effects of dichlobenil on the nasal mucosa. With this type of mechanism in operation, using the NOEL determined in the 28-day inhalation study (ref. 15) and standard safety factors, a safe level for operator exposure can be determined.

References mentioned in the statement with regard to the effect of dichlobenil on the olfactory nasal mucosa of rodents (Story, 2006).

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B.6.8.3 Metabolism study with BAM

Characteristics of the study

Reference	: Wit, 1967	Route	: oral
Year of execution	: not indicated	Group size	: 5/dose
Guideline	: no	Exposure	: gavage, once
GLP	: no	Doses	: ca. 100 mg/kg bw (rabbit) between 45 and 75 mg/kg bw (rats)
Test substance	: 2,6-dichlorobenz[¹⁴ C]amide, lot/batch no. not indicated; appearance not indicated, s.a. 250 mCi/g	Vehicle	: water
Purity	: not indicated	Acceptable	: no
Species	: rat, Wistar rabbit, first generation Danish Rabbit x Flemish Giant		

Study design

Metabolism of 2,6-dichlorobenzamide was studied in rats and rabbits exposed as described above. As the study is not considered acceptable, no further description is offered here.

Results

As the study is not considered acceptable, no results are described.

Acceptability

The submitted document is an unpublished article, which offers a very limited and incomplete description of the materials and methods used. Furthermore, the author indicated that a large fraction

of especially the urinary radioactivity was not identified and that the recovery was poor. In view of these short-comings, the study is considered not acceptable.

B.6.8.4 Acute toxicity studies on BAM

B.6.8.4.1 Acute oral toxicity study in rat

Characteristics of the study

Reference	: Schüngel, 2003	Route	: oral
Year of execution	: 2003	Group size	: 3 or 6/sex/dose
Guideline	: OECD 423	Exposure	: once by gavage
GLP	: yes	Doses	: 300 and 2000 mg/kg bw
Test substance	: AE C653711 (metabolite of AE C638206) ¹ , batch no. 8808018; appearance: beige powder	Vehicle	: demineralised water with 2% Cremophor EL
Purity of content of a.s.	: 97.0%	Acceptable	: yes
Species	: rats, Wistar	LD ₅₀	: 500 mg/kg bw

¹ metabolite: 2,6-dichlorobenzamide

Study design

The study was performed in accordance with OECD 423.

Results

Mortality: in the first 2000 mg/kg oral dose group two of three males died on day 6. In the second 2000 mg/kg oral dose group all three male rats survived.

In the first and second 300 mg/kg oral dose group all six female rats survived. All three female rats died in the 2000 mg/kg dose group between 6 hours and day 3 after administration.

Symptoms of toxicity:

In the 300 mg/kg bw dose group: decreased motility, uncoordinated gait and narrowed palpebral fissure.

In the 2000 mg/kg bw dose group: abdominal position, lateral position, decreased motility, poor reflexes, reactivity decreased, spasmodic state, uncoordinated gait, labored breathing, tachypnea, chromodacryorrhea, lacrimation/increased lacrimation, closed eyelids, narrowed palpebral fissure and piloerection.

Body weight: no treatment-related findings in the 300 mg/kg bw dose group. There was a vigorous depression in body weight and in body weight gain in the surviving rats treated with 2000 mg/kg bw.

Pathology: in animals that died or were killed in a moribund state: pale/spotted discoloration in the liver and pale discoloration in the spleen. The necropsies performed at the end of the study revealed no treatment-related findings.

Acceptability

The study is considered acceptable. However, an additional group with 2000 mg/kg was used in the males due to a deviation to study plan. According to the stepwise procedure in Annex 2c of OECD

guideline 423, the estimated LD₅₀ for male rats should be 1000 mg/kg bw. If for males both groups dosed with 2000 mg/kg were used for assessment in reverse order, then the acute oral LD₅₀ of the test substance can be estimated to be 2000 mg/kg bw for male rats.

Conclusions

According to Annex 2c of OECD guideline 423 the acute oral LD₅₀ of the metabolite 2,6-dichlorobenzamide was estimated to be 2000 mg/kg bw for male rats and 500 mg/kg bw for female rats.

B.6.8.4.2 Acute oral toxicity study in rat

Characteristics of the study

Reference	: Kemp, 1967	Route	: oral
Year of execution	: not indicated	Group size	: 5/sex/dose
Guideline	: OECD 401	Exposure	: once by gavage
GLP	: not applicable	Doses	: 1000, 2150, 4640 and 10000 mg/kg bw
Test substance	: 2,6 dichlorobenzamide, batch no. 133/2/4/104; appearance not indicated	Vehicle	: suspended in 1% w/v aqueous tragacanth
Purity of a.s.	: not indicated	Acceptable	: no
Species	: rats, Wistar	LD ₅₀	: -

Study design

The study was not performed in accordance with OECD 401. Only clinical observations were performed. No changes in body weight were determined. No raw data were given. The calculations of the LD₅₀ were not elucidated.

Acceptability

The study is considered not acceptable due to the deviations from the guideline as mentioned above.

Conclusions

No LD₅₀ could be determined.

B.6.8.5 90-day oral toxicity study with BAM in rats

Characteristics of study

Reference	: Boschman <i>et al.</i> , 1967	Route	: oral
Year of execution	: not indicated	Group size	: 10/sex/dose
Guideline	: OECD 408	Exposure	: 13 weeks, in diet
GLP	: no	Doses	: 0, 50, 180, 600, and 2300 ppm ¹
Test substance	: 2,6-dichlorobenzamide, batch no. 133/2/4/104; appearance not indicated	Vehicle	: -
Purity	: not indicated	Acceptable	: yes
Species	: rat, Wistar	NOAEL	: 180 mg/kg food (14 mg/kg bw/d)

¹ Equal to 0, 4, 14, 49 and 172 mg/kg bw/d for males and females.

The study was partly performed in accordance with OECD 408. Ophthalmological examinations and functional observations battery were not performed. No detailed pathology report was submitted.

Additionally, a liver function test (a bromosulphthalein test according to Seligen) was conducted. The method was not further described in the report.

Furthermore, skeletal muscle tone was estimated because a preliminary experiment with the compound indicated a reduction in skeletal muscle tone on handling as an effect (range-finding study in rats – daily oral application for eight days, Kemp, 1967). The effect was measured at four time-points during the study (on day 4, 21, 91 and 92) by two observers ranking the degree of muscle hypotonus displayed by the rats when handled. The degree of muscle tone was divided into four levels: normal tonus (0), slight hypotonus (1), moderate hypotonus (2) and marked hypotonus (3).

The results are presented in Table 6.8.5.

Dose (mg/kg food)	0	50	180	600	2300	dr
	m f	m f	m f	m f	m f	
Mortality	1/10			1/10	1/10	
Clinical signs						
-hair loss				3/10	2/10	
-skeletal muscle hypotonus ¹	(0.3) (0.3)	(0.6) (0.2)	(0.6) (0.5)	ic (1.1)	ic (1.3)	
Body weight²						
-terminal					dc (20)	m,f
-gain				dc (18)	dc (30)	f
Food consumption²				dc (7)	dc (16)	f
Ophthalmoscopy			not determined			
Haematology²						
-PT				dc (23)	dc (19)	
Clinical chemistry²						
-total protein					ic (11)	
-cholesterol					ic (35)	ic (40)
-urea				ic (53)	ic (69)	m
Urinalysis			no treatment-related findings			
Organ weights^{2,3}						

Dose (mg/kg food)	0		50		180		600		2300		dr
	m	f	m	f	m	f	m	f	m	f	
-liver (rel)							i (17)	i (9)	i (26)	i (20)	m,f
-kidney (rel)							i (22)	i (11)	i (21)	i (4)	
-adrenals (rel)							i (19)		i (16)		
Pathology											
<u>macroscopy</u>											
<u>microscopy</u>											

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

+ present in one/a few animals

++ present in most/all animals

¹ average muscle hypotonus score is indicated between brackets

² % change with regard to the control is indicated between brackets

³ it is not clear in the study report whether changes of relative organ weight are statistically evaluated by the author, therefore changes were only indicated with 'i' or 'd'.

Acceptability

Although there were some deviations from the guideline, the study is considered acceptable, as at the time the study was performed, no guidelines were available.

Conclusions

Three rats from different dose groups were accidentally killed by an overdose of anaesthetic (ether) during blood collection, which is not considered related to treatment with BAM.

Oral exposure of rats to 2,6-dichlorobenzamide at concentrations of 2300 and 600 mg/kg food for 13 weeks resulted in numerous effects on various types of parameters analysed. At 600 mg/kg food and at 2300 mg/kg food a decreased body weight (gain), reduced food consumption, hair loss and a reduction in skeletal muscle tone was observed in the female animals. The male animals of these groups displayed an increased level of urea, which is possibly related to the increased relative kidney weight in these dose groups. Furthermore, the relative liver weight was not statistically increased in both sexes and relative adrenal weight was increased in males only.

In the 2300 mg/kg food group a decreased body weight (gain), decreased food consumption and a reduction in skeletal muscle tone was also observed for male animals. Male animals in the 2300 mg/kg food group showed a decrease in prothrombin time, while females only showed a decreased prothrombin time in the 600 mg/kg food group. In view of this absence of a clear dose-relationship, this effect is considered to be not toxicologically relevant. Furthermore, the elevated cholesterol level in both sexes and a higher protein level in the males may be associated with the increased relative liver weight, indicating possible functional changes in the liver.

At a dose level of 50 and 180 mg/kg food no effects were found.

In conclusion, the NOAEL is set at 180 mg/kg food (equal to 14 mg/kg bw/d), based on the decreased body weight (gain), reduced food consumption, hair loss and a reduction in skeletal muscle tone in the

female animals, the increased relative adrenal weight in males and the liver- and kidney effects in both sexes found at the next higher dose (600 mg/kg food, equal to 49 mg/kg bw/d).

B.6.8.6 Genotoxicity studies on BAM

B.6.8.6.1 In vitro studies

B.6.8.6.1.1 Bacterial mutation test with BAM

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: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537	point mut. point mut. point mut. point mut.	- - - -	- - - -	rat liver	Arochlor 1254	0, 625, 1250, 2500 and 5000 µg/plate (+S9 mix and -S9 mix) solvent: DMSO	Koorn, 1993
Test substance: 2,6-dichlorobenzamide, batch no. FUX001000/FUN81G02C, purity 100%, white powder Cytotoxicity observed at dose level: > 5000 µg/plate Precipitation observed at dose level: not indicated GLP statement: yes According to OECD 471 (1983): yes							

Acceptability

The study was not performed according to OECD 471 adopted on 21st July, 1997 as no strain for detecting cross-linking mutagen activity was included. However, this study was performed according to the former version of the OECD guideline 471, adopted on May 26th, 1983, which was valid at the time of performance of the study.

Conclusions

The test substance did not induce point mutations in *S. typhimurium*.

Study 2

Study design and results

Type of study: Ames test, plate incorporation and preincubation method

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range	Reference
				Tissue	Inducer		
B: <i>S. typh.</i>				rat liver	Arochlor	0, 16, 50, 158, 500, 1581 and	Herbold, 2003a

Indicator cells	Endpoint	Res. - act.	Res. + act.	Activation		Dose range	Reference
				Tissue	Inducer		
TA 98 TA 100 TA 1535 TA 1537 TA102	point mut. point mut. point mut. point mut. point mut.	- - - - -	- - - - -		1254	5000 µg/plate (+S9 mix and -S9 mix) solvent: DMSO	
Test substance: 2,6-dichlorobenzamide, batch no. 08018ET, purity 96.2%, fine light-brown powder Cytotoxicity observed at dose level: ≥ 5000 µg/plate Precipitation observed at dose level: not indicated GLP statement: yes According to OECD 471: yes							

Acceptability

The study was considered acceptable.

Conclusions

The test substance did not induce point mutations in *S. typhimurium*.

B.6.8.6.1.2 Gene mutation with BAM

Study design and results

Type of study: mammalian cells *in vitro*, gene mutation test

Indicator cells	Endpoint	Res. - act.	Res. + act.	Activation		Dose range	Reference
				Tissue	Inducer		
Chinese hamster V79 cells	gene mutations (HPRT)	-	-	rat liver	Arochlor 1254	0, 125, 250, 500, 1000, 3000 and 5000 µg/mL (+S9 mix and -S9 mix) solvent: DMSO	Herbold, 2003b
Test substance: 2,6-dichlorobenzamide, batch no. 08018ET, purity 96.2%, beige powder Cytotoxicity observed at dose level: > 5000 µg/mL (-S9 mix); ≥ 250 µg/mL (+S9 mix) Precipitation observed at dose level: ≥ 2500 µg/mL GLP statement: yes According to OECD 476: yes							

Acceptability

The study is considered acceptable.

Conclusions

The test substance did not induce gene mutations in Chinese hamster V79 cells.

B.6.8.6.1.3 Unscheduled DNA synthesis with BAM

Study design and results

Type of study: unscheduled DNA synthesis (UDS) assay in primary mammalian cells

Indicator cells	Endpoint	Result	Dose range	Reference
primary rat hepatocytes	DNA repair (unscheduled DNA synthesis)	-	0, 1, 3, 10, 33, 100, 333 and 1000 µg/mL solvent: DMSO	Waart van de, 1993a
Test substance: 2,6-dichlorobenzamide, batch no. FUX001000/FUN81G02C, purity 100%, white solid Cytotoxicity observed at dose level: > 1000 µg/mL Precipitation observed at dose level: ≥ 1000 µg/mL GLP statement: yes According to OECD 482: yes				

Acceptability

The study is considered acceptable.

Conclusions

The test substance did not induce unscheduled DNA synthesis in mammalian cells.

B.6.8.6.2 In vivo studies

B.6.8.6.2.1 Chromosome aberration with BAM

Study design and results

Type of study: mouse micronucleus test

Species	Endpoint	Result	Dose range	Reference
mouse, Swiss OF-1 5/sex/dose	micronuclei (bone marrow)	-	250 mg/kg bw/d ¹ , administered orally once; sampling at 24,48 and 72 h after dosing. vehicle: corn oil	Waart van de, 1993b
Test substance: 2,6-dichlorobenzamide, batch no. FUX001000/FUN81G02C, purity 100%, white solid Toxicity observed at dose level: no change in the ratio PCE/NCE is observed as a result of exposure. GLP statement: yes According to OECD 474: yes				

¹ maximum tolerated dose

Acceptability

The study is considered acceptable. No change in the ratio of PCE/NCE was observed as a result of exposure to the test substance. It is however noted that although no toxic effect was found on the erythropoiesis, systemic effects were observed in the repeated dose toxicity studies. It is therefore considered that the test substance becomes systemic available and had reached the target cells in the micronucleus test in vivo.

Conclusions

The test substance did not induce micronuclei in mouse bone marrow cells.

Dose (mg/kg food)	0		60		100		180		500		dr
	m	f	m	f	m	f	m	f	m	f	
<u>macroscopy</u>	No treatment related findings ¹										
<u>microscopy</u>											
<i>neoplastic lesions</i>											
<i>Liver</i>											
- hepatocellular adenoma	1/26		1/28		1/32				1/34	5/35	
<u>microscopy</u>											
<i>non-neoplastic lesions</i>											
<i>Liver</i>											
Vacuolation, fat deposition and hepatocyte degeneration			1/10	3/10	1/10		2/10		1/15	9/20	
- Eosinophilic hepatocytes					ic (240)		ic (220)		ic (320)	ic (360)	
<i>frequency</i>	5/26	5/25	12/28	4/28	17/32	7/28	11/25	16/32	21/34	23/35	
- Eosinophilic hepatocytes areas										ic (800)	
<i>frequency</i>	1/26	2/25	3/28	2/28		1/28	2/25	5/32	4/34	18/35	
- Basophilic hepatocytes-focal										ic (155)	
<i>frequency</i>	7/26	9/25	11/28	10/28	5/32	6/28	6/25	14/32	9/34	23/35	
- Hepatocyte vacuolation centrilobular									ic (220)	i (120)	
<i>frequency</i>	5/26	5/25	7/28	7/28	10/32	5/28	5/25	8/32	16/34	11/35	

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/activity

¹ It should be noted that no summary tables are available on specific clinical signs and macroscopy.

Acceptability

The study is considered acceptable.

Conclusions

From the analyses of the dietary formulations (Johnson, 1996) it can be concluded that the homogeneity was confirmed for low and high doses (60 and 500 mg/kg food). The stability of the rodent diet formulations was confirmed during storage at ambient temperature for 18 days for the 500 ppm nominal concentration, however a loss in concentration of approximately 12% was observed for the 60 ppm nominal concentration. The 18-day storage period exceeds the maximum period of time (7 days) between preparation and completion of dosing during the main study (Weldon *et al.*, 1971). In view of the small deviation and as the storage period is longer than the test period, the dosing in the study is considered acceptable. Oral exposure of rats to 2,6-dichlorobenzamide (BAM) at a dietary level of 500 mg/kg food for two years resulted in liver effects (liver weight increase, hepatocellular adenomas, hepatocyte vacuolation centrilobular and Eosinophilic/Basophilic hepatocytes). The Eosinophilic hepatocytes observed in males at 100 mg/kg food and in females at 180 mg/kg food are considered not dose related. Therefore, the NOAEL is set at 180 mg/kg food (equal to 6.5 mg/kg bw/d).

The increase in benign hepatocellular adenoma in females at 500 mg/kg food is not statistically significant. However, taking into account the small group size (only 26 animals in the control group and 35 in the test group) the hepatocellular adenomas can be biologically relevant.

However, according to OECD guideline 451, the highest dose level in a carcinogenicity study should be sufficiently high to elicit signs of minimal toxicity, such as alterations in certain serum enzyme levels or slight depression of body weight gain (<10%). This implicates that the highest dose level in this study exceeds the MTD. Therefore, the relevance of the increased tumour incidences at the highest dose should be questioned. However, in line with the classification and labelling Directive (for more explanation, see B.6.5.3) the RMS proposes to classify BAM with R40 (category 3; Limited evidence of a carcinogenic effect). It should be noted that this is not in line with the evaluation of BAM in the DAR for fluopicolide.

The notifier does not agree with this proposal.

B.6.8.8 Summary

Table 6.8.8.1 Acute toxicity study with 2,6-dichlorobenzamide (BAM)

Test substance	LD ₅₀ /LC ₅₀	Species	Route	Vehicle	Reference
2,6-dichlorobenzamide (BAM)	500 mg/kg bw	rat	oral	demineralised water with 2% Cremophor EL	Schüngel, 2003

Table 6.8.8.2 semi-chronic toxicity study with 2,6-dichlorobenzamide (BAM)

Duration	Species	Route	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
90 days	rat	oral	14	49	Reduced body weight (gain), reduced food consumption, hair loss and a reduction in skeletal muscle tone in the female animals, the increased relative adrenal weight in males and the liver- and kidney effects in both sexes	Boschman <i>et al.</i> , 1967

Table 6.8.8.3 chronic toxicity study with 2,6-dichlorobenzamide (BAM)

Duration	Species	Route	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects	Reference
2-year	rat	oral	6.5	18	Body weight decrease, liver weight increase (female), non-neoplastic effects on the liver Tumors: hepatocellular adenoma highest dose group (5/20 and 1/15 (f,m))	Wheldon <i>et al.</i> , 1971, Johnson, 1996 and Connick <i>et al.</i> , 1996

Table 6.8.8.4 In vitro genotoxicity studies with 2,6-dichlorobenzamide (BAM)

Test substance	Type of study		Result		Reference
	Indicator cells	Endpoint	without activation	with activation	
2,6-dichlorobenzamide	B: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537	Point mutations	-	-	Koorn, 1993
2,6-dichlorobenzamide	B: <i>S. typh.</i> TA 98 TA 100 TA 1535 TA 1537 TA102	point mutations	-	-	Herbold, 2003a
2,6-dichlorobenzamide	Chinese hamster V79 cells	gene mutations (HGPRT)	-	-	Herbold, 2003b
2,6-dichlorobenzamide	Primary rat hepatocytes	DNA repair (unscheduled DNA synthesis)		Not relevant	Waart van de, 1993a

Table 6.8.8.5 In vivo genotoxicity studies with 2,6-dichlorobenzamide (BAM)

Test substance	Type of study		Result	Reference
	Species	Endpoint		
2,6-dichlorobenzamide	mouse	micronuclei (bone marrow)	-	Waart van de, 1993b

The LD₅₀ of the metabolite 2,6-dichlorobenzamide (BAM) (500 mg/kg bw) is lower than the LD₅₀ (>2000 mg/kg bw) of the parent compound. The metabolite is considered harmful after acute oral exposure.

With regard to genotoxicity, semi-chronic toxicity and chronic toxicity, the toxicity of the metabolite BAM is comparable to or less than the toxicity of the parent compound dichlobenil.

In the two year rat study, dietary administration of 2,6-dichlorobenzamide resulted in an increase in hepatocellular adenomas in females at the highest dose group. The highest dose level in this study exceeds the MTD. Therefore, the relevance of the increased tumour incidences at the highest dose should be questioned. However, considering that these tumours:

- are increased only at the highest dose,
- in a single species,
- occur also spontaneously in this species and sex,
- are at the border of statistical significance,
- occur under conditions of clear local cytotoxicity (i.e. in the liver), and
- only occur at a dose level that may be considered to clearly exceed the maximum tolerated dose criterion, and that this substance clearly has no genotoxic potential,

application of the criteria mentioned in Annex VI of Directive 67/548/EC allows to conclude that this substance should be classified with R40 (category 3; Limited evidence of a carcinogenic effect).

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

No reports of any adverse effects on workers producing dichlobenil as active substance or formulations, and no adverse incidents applying plant protection products were reported.

B.6.9.1 Medical surveillance on manufacturing

No information submitted by the notifier

B.6.9.2 Clinical cases of poisoning incidents

No information submitted by the notifier

B.6.9.3 Observations on exposure of the general population

No information submitted by the notifier

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

No information submitted by the notifier

B.6.9.5 First aid measures and therapeutic regimes

The notifier proposed the following first aid measures in document H (Safety Data Sheet):

Swallowing: Do not induce vomiting. Rinse mouth with water. Obtain medical attention.

Inhalation: Remove to fresh air. Obtain medical attention as symptoms persist.

Skin contact: Remove contaminated clothing. Wash thoroughly with warm water using a mild soap. Obtain medical attention if irritation persists.

Eye contact: Immediately flush eyes with water and continue washing for several minutes. Obtain medical attention if irritation persists.

B.6.9.6 Expected effects of poisoning

No information submitted by the notifier

B.6.10 SUMMARY OF MAMMALIAN TOXICOLOGY AND PROPOSED ADI, AOEL, ARFD AND DRINKING WATER LIMIT (ANNEX IIA 5.10)**B.6.10.1 Toxicokinetics**Absorption

In one rat study with single low dose (ca. 6 mg/kg bw) in which oral and i.v. administration were compared, oral absorption was 89-97% (m-f).

In the rat, oral absorption after repeated exposure is at least approximately 60%, irrespective of dose and sex, based on radiolabel recovered from urine and cage wash. However, as the liver is the critical target organ for dichlobenil toxicity, it is the amount of compound which has passed the liver, that

should be considered in risk assessment. After repeated low dose, the amount absorbed from the gut into the liver is more than 80% (see below under "Excretion").

In order to calculate internal doses after dermal exposure a value of 1.4% was used, based on an *in vitro* dermal absorption study with human skin.

Excretion

In rats, radiolabel is primarily excreted via the urine after oral exposure to [¹⁴C]-dichlobenil. Around 60% is excreted in the urine and around 20% in the faeces, irrespective of sex or dose regime, 9 to 10 days after initiation of exposure. Increasing dose or multiple dosing slows down the rate of excretion, without changing the final amount excreted. After repeated dosing, approximately 80% of the last administered dose is excreted within 24 h after low and mid dose (4 and 30 mg/kg bw/d), while for high dose (240 mg/kg bw/d) ca. 65% was excreted within 24 h.

After single oral low dose in rats, a substantial amount of the administered radioactivity is excreted in bile (80% in the first 24 h hours after administration). As the majority of the radiolabel ended up in the urine, enterohepatic cycling takes place with part of the radiolabel ultimately becoming systemically available and excreted via urine. As comparison with a concurrent i.v. experiment indicates that more than ca. 90% of the administered single oral dose becomes systemically available, also part of the faecally excreted radiolabel must have been excreted from systemic circulation. As with other dose regimes (multiple and/or increased) no i.v. and bile cannulation experiments were executed, the quantitative importance of biliary excretion and systemic absorption can not be definitely established for those dose regimes. However, with respect to low repeated dose, absorption from the gut is likely to be higher than 80% as only a low percentage of parent compound is found in faeces (less than 10%) and all metabolites identified are probably liver metabolites: In bile and urine no parent compound was detected at any dose regimen, and in faeces the relative amount of parent compound increases with increasing and multiple doses (see below "Metabolism"). Therefore, once it enters the liver dichlobenil appears to immediately be metabolised, while no metabolism takes place in faeces. Consequently ca. 90% of the faecal radiolabel (non-parent compound) will have originated from the liver via the bile, representing ca. 20% of the administered dose, which should be added to the radiolabel recovered from urine and the cage wash (respectively, ca. 60 and 4% at repeated low dose) in order to obtain the amount of administered radiolabel which has been absorbed from the gut.

In one study with single oral low dose (significant) differences in excretion between males and females were observed. However, two other studies, among which one with single and multiple low, mid and high doses, did not demonstrate these differences. Therefore this difference is considered to have occurred by coincidence.

Distribution

After single low dose (2.5 mg/kg bw/d) in rats, most radiolabel is recovered from liver, kidney, kidney-fat and brown-fat. In all tissues and organs investigated, concentrations peak between 1 and 3 hours after administration, in liver and kidney even after 1 hour. Therefore, radiolabel appears to be rapidly distributed and excreted. Seven days after administration little radioactivity was retained. No potential for accumulation was observed.

In another study with rats various distributions after various oral dose regimes (single/multiple, low/mid/high) was studied. In all cases less than 0.01% of the last administered dose was recovered from selected tissues and organs (blood, plasma, liver and kidneys), while between 0.2 and 0.4% was encountered in residual carcass, eight days after administration of the last dose.

Metabolism

In rats, ten metabolites were identified in urine and no parent compound. Most metabolites were conjugates such as sulphate, glutathione (and derivatives) and glucuronic acid conjugates. In faeces, three major metabolites (each representing 5% of the administered dose or more) were observed as well as parent compound (Dichlobenil). The proportion of conjugated metabolites decreases with increasing and multiple doses, while the amount of parent compound in faeces increases. No chemical names were provided for the identified metabolites.

B.6.10.2 Toxicodynamics

The acute toxicity of dichlobenil is low. In the acute oral toxicity studies, liver and kidney histopathological effects were observed. Oral repeated dose studies clearly indicate the liver and kidney as the critical target organ. The hamster is more sensitive for dichlobenil than mice based on the 90-days semi-chronic oral toxicity studies. Therefore, the second carcinogenicity study was performed with the hamster instead of with the mouse.

Acute toxicity

Dichlobenil does not need to be classified on the basis of its acute oral and dermal toxicity in rats. Dichlobenil is considered not irritating to skin and eyes according to the criteria mentioned in Annex VI of Directive 2001/59/EC. Dichlobenil does not have sensitising properties in a Maximisation test. No adequate data on the acute inhalation toxicology of dichlobenil were provided. However, in view of the particle size (99.9% >100 µm; granule) and the application of the preparation (not in enclosed spaces), no acute inhalation studies are required.

Short-term toxicity

In a 21-day dermal mice study exposure of mice to dichlobenil at doses of 100, 300 and 1000 mg/kg bw/d did not result in toxic effects. Therefore, the NOAEL was set at >1000 mg/kg bw/d.

In a 28-day inhalation rat study respiratory exposure of rats to dichlobenil at concentrations of 2.3, 5.1 and 12 mg/m³ for 5 days/week did not result in toxic effects. Therefore, the NOAEL was set at ≥ 12 mg/m³.

A 90-day oral toxicity study was performed on mice administered 3.7, 19, 91, 445 mg/kg bw/d. The critical effects were reduced body weight gain, reduced food consumption and liver effects (swollen hepatocytes, increased organ weight), observed at doses of 91 mg/kg bw/d and higher. Based on these effects, the NOAEL is set at 19 mg/kg bw/d.

A 90-day oral toxicity study was performed on hamsters administered 2.3, 12, 73 and 302 mg/kg bw/d for males and 2.6, 13, 81 and 320 mg/kg bw/d for females. The following effects were considered toxicologically relevant: increased liver weight, a decrease of prostate weight, testes weight and uterus weight, a degeneration of testes tubules and a decrease of viable spermatocytes, calculi in the gallbladder, decreased food intake, increased ALAT and increased cholesterol and phospholipids. Therefore, the NOAEL in this study is 2.3 mg/kg bw/d, based on the effects found at the 12 mg/kg bw/d dose level (effects on liver, kidney and male reproductive organs).

Two repeated dose toxicity studies in dogs are submitted. In an oral 52-week and a 2-years study, the dogs were exposed to 1, 6 and 36 mg/kg bw/d and 0.5, 1.3, and 9.1 mg/kg bw/d, respectively.

The 2-year oral toxicity study in dogs showed in the highest dose group elevated alkaline phosphatase (from week 12 and higher), glucose-6-phosphatase and glucose-6-phosphate dehydrogenase activities, increased weight of the liver and thyroid and histopathological liver changes in both sexes. At dietary levels of 1.3 mg/kg bw/d and less, no treatment related effects were noted.

Therefore, the NOAEL is set at 1.3 mg/kg bw/d based on liver effects and increased thyroid weights at the next higher level.

In the two highest dose groups in the 52-week oral toxicity study, reduced body weight gain and elevated alkaline phosphatase activities, increased liver weight and hepatocytic hypertrophy and effects in the clinical chemistry were observed. Other changes at this dose level included vacuolation in the adrenals, considered to represent changes in mineralocorticoid production. Males had slightly lowered red cell parameters and there was some evidence of haemosiderin deposits in the spleen of animals of both sexes. Females showed decreased ovaries and uterus weights. A dose related increased liver weight in females was observed from the lowest dose group at 1 mg/kg bw/d. In conclusion, the LOAEL is set at 1 mg/kg bw/d.

Genotoxicity

In vitro, dichlobenil tested negative in the point mutation tests with *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538, in a gene mutation test with Chinese hamster ovary cells (CHO), in a chromosome aberration test with Chinese hamster ovary cells (CHO), in an unscheduled DNA synthesis test with human HeLa S3 epithelioid cells and in two transformation assays in BALB/c-3T3 mouse cells. *In vivo*, dichlobenil tested negative in a micronucleus test in mice, with the bone marrow as observed target organ. Based on the available data the substance dichlobenil does not

need to be classified according to the classification criteria of Directive 93/21/EC. The test results of an *in vitro* gene mutation test with mouse lymphoma cells (L5178Y) and the chromosome aberration test with human blood lymphocytes were considered unacceptable due to wrong concentration ranges tested. As the *in vitro* chromosome aberration test performed with CHO cells is considered acceptable, the chromosome aberration test performed with human lymphocytes is not considered relevant for the evaluation of the genotoxic potential of the substance.

Long-term toxicity and carcinogenicity

In an oral combined chronic and carcinogenicity study with dichlobenil rats were exposed to 3.2, 26 and 241 mg/kg bw/d. The main effects of dichlobenil in this study were seen on the liver and kidney. Blood biochemistry parameters (urea, cholesterol and electrolytes) were affected primarily in the highest dose group with urea and cholesterol also increased at 26 mg/kg bw/d indicating changes in liver and/or kidney function. Renal pathological effects were already noted at interim sacrifice after 52 weeks.

The pattern of histopathological changes observed in several organs (primarily in males) is most likely directly or indirectly related to this renal pathology. Increases in nephrosis, glomerulosclerosis and renal fibrosis, parathyroid hyperplasia, calcification in the kidney and blood vessels, and atrophy of the bones represent a coherent pathological pattern induced by renal failure.

At the 3.2 mg/kg bw/d level the increase in renal fibrosis is already highly statistical significant while related histopathological effects are all present at borderline importance. Because this histopathological pattern is coherent and well known in the literature and, moreover, is dose-dependently increased, the 3.2 mg/kg bw/d level will be considered to be an effect level. Therefore, the NOAEL in this study cannot be determined, and the LOAEL is set at 3.2 mg/kg bw/d.

At the highest dose, in both males and females an increase in malignant tumour incidence was observed (adenocarcinoma in kidney, carcinoma in liver, leukemia and malignant lymphoma). At the dose of 241 mg/kg bw/d two adenomas were observed in female liver. In this study, the highest dose clearly exceeds the so-called maximal tolerated dose (MTD) considering the high mortality in males. Therefore, the relevance of the increased tumour incidences at the highest dose should be questioned (however taking into account that there seems to be a dose-related increase in liver adenoma). At the dose of 400 mg/kg food two adenomas were observed in female liver. However, no indications of liver cytotoxicity were observed which could constitute a possible indirect mechanism of carcinogenesis, caused by high toxicity. Furthermore, the incidence of the adenomas observed in the rat study in female liver at the dose of 400 mg/kg food is slightly above the historical control range. Therefore, a direct effect of dichlobenil on the tumor incidence cannot be excluded.

Two oral carcinogenicity studies with hamster were performed.

In the first (80-88-week) and the second (78-91 week) carcinogenicity study in hamsters, the hamsters were exposed to respectively 0.34, 1.7, 9.2, and 46 mg/kg bw/d and 51, 117 and 277 mg/kg bw/d.

In the first study treatment by prolonged oral administration of dichlobenil to hamsters at levels up to 46 mg/kg bw/d revealed no evidence of a carcinogenic potential. Treatment related changes were

confined to the high dosage level. In the 46 mg/kg bw/d male group these changes included initial impairment of body weight gain with associated lower food consumption, and lower overall body weight change together with the histopathological finding of a reduction in the adipose tissue in the male decedents. In high dose females liver changes (slightly higher weights, pitted liver and centrilobular hepatocyte enlargement and rarefaction) and cortical hyperplasia of the adrenals were observed. Based on the effects above, the NOAEL is set at the next lower dose level of to 9.2 mg/kg bw/d.

In the second study with hamster in all dose groups decrease of body weight was observed. An increased incidence of benign liver tumours in males receiving 277 mg/kg bw/d was seen. A single malignant tumour was also recorded for males at this treatment level, whilst a single benign liver tumour was recorded amongst males receiving 117 mg/kg bw/d.

Non-neoplastic changes were evident in the liver, principally amongst hamsters receiving 117 or 277 mg/kg bw/d consisting of hepatitis in both sexes receiving 277 mg/kg bw/d and also females receiving 117 mg/kg bw/d; pigmented giant cells in males receiving 277 mg/kg bw/d; brown pigments in hepatocytes in both sexes receiving 117 or 277 mg/kg bw/d and minimal centrilobular hepatocyte enlargement in all treatment groups. In addition to hepatotoxicity, evident at all treatment levels, toxicity was also manifest as impaired body weight gain food intake amongst all treated males and for females receiving 277 mg/kg bw/d.

The doses of 51 mg/kg bw/d for males and 277 mg/kg bw/d for females was considered to be the NOAEL with respect to tumorigenicity. Regarding the non-neoplastic effects, no NOAEL could be established. The LOAEL is considered to be 51 mg/kg bw/d.

In this study, mid and highest dose levels exceed the so-called maximal tolerated dose (MTD), e.g. the high increase of relative liver weight. Therefore, the relevance of the tumours observed at doses of 1500 and 3375 mg/kg food should be questioned.

In the rat study, the highest dose clearly exceeds the so-called maximal tolerated dose (MTD) considering the high mortality in males. Therefore, the relevance of the increased tumour incidences at the highest dose should be questioned (however taking into account that there seems to be a dose-related increase in liver adenoma). However, in the rat also liver adenomas were observed at a dose level below the MTD. It was concluded that the two adenomas observed in female liver at the dose of 400 mg/kg food (2 adenomas in 47 animals = 4.3%) are slightly above the historical control range of the conducting laboratory (and the breeder).

In the second hamster study (Chambers *et al.*, 1992a), the mid and highest dose levels exceed the so-called maximal tolerated dose (MTD), e.g. the high increase of relative liver weight. Therefore, the relevance of the tumours observed at doses of 1500 and 3375 mg/kg food should be questioned.

The background tumour incidence study in hamsters showed no benign or malignant tumours in liver.

In fact, the study of Hooks *et al.* (1990) might also be regarded as background tumour incidence study showing no tumours in 600 animals.

In conclusion, the relevance of the observed tumours in rats and hamsters at levels exceeding the MTD, for the assessment of the carcinogenic potential of dichlobenil, should be questioned. However, it seems that dichlobenil has some carcinogenic potential in the rat, not explainable by cytotoxicity in liver as this was not observed. It is proposed to regard dichlobenil as a potential carcinogen according to the criteria mentioned in Annex VI of Directive 2001/59/EC and to classify with R40 (category 3; Limited evidence of a carcinogenic effect). As dichlobenil is not genotoxic, a threshold mechanism of its carcinogenicity is likely.

Reproductive and developmental toxicity

From the 2-generation study in rats with doses of 4, 23 and 133 mg/kg bw/d, the NOAEL for parental toxicity is 4 mg/kg bw/d. Above this NOAEL, a decreased body weight and decreased food consumption in adults and offspring in both F0 and F1 generations is observed. The NOAEL for developmental toxicity is also 4 mg/kg bw/d. Above this NOAEL, a decreased pup body weight is observed.

Reproductive effects were not observed in this study. The NOAEL for reproductive toxicity is therefore set at 133 mg/kg bw/d.

Two teratogenicity studies in rats and rabbits are performed. The rats and rabbits were exposed to respectively 20, 60, and 180 mg/kg bw/d and 15, 45 and 135 mg/kg bw/d.

The maternal NOAEL of the study in rats is 20 mg/kg bw/d. Above this NOAEL, decreased body weight gain during day 6-16 and decreased food consumption were observed.

All observed external, skeletal and visceral findings are common in this strain of rats and do not indicate embryotoxic or teratogenic effects that could be related to the exposure of dichlobenil. The developmental NOAEL is therefore >180 mg/kg bw/d. In the study in rabbits both the maternal and developmental NOAEL are 45 mg/kg bw/d. Above the maternal NOAEL, decreased weight gain (day 7-19) and food consumption were observed. Above the developmental NOAEL, major abnormalities, such as grossly enlarged fontanelles and other major cranial defects and open eyes, are seen in the foetuses. Since these teratogenic defects only occur at a maternally toxic level, dichlobenil does not have to be labelled for developmental effects.

Delayed neurotoxicity

The chemical structure of dichlobenil does not resemble that of the organophosphates or carbamates, nor do the results of the submitted repeated dose studies suggest a neurotoxic effect of dichlobenil. Therefore, a delayed neurotoxicity study is not deemed necessary.

Further toxicological studies

The notifier submitted a statement with regard to the effect of dichlobenil on the olfactory nasal mucosa of rodents (reported in the literature). The statement is considered acceptable.

The metabolite of dichlobenil tested is 2,6-dichlorobenzamide (BAM). BAM is a soil metabolite of dichlobenil and becomes available as residue in plants. BAM is not formed in rats based on the submitted metabolism studies.

An acute oral toxicity study in rats with BAM showed that it should be classified as Harmful (Xn, R22). The acute oral LD₅₀ was 500 mg/kg in rats. No acute dermal or inhalation toxicity studies with BAM were provided (not necessary since BAM is a soil metabolite).

In vitro, BAM tested negative in two point mutation tests with *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 102, in a gene mutation test with Chinese hamsters V79 cells, and in an unscheduled DNA synthesis test with rat primary hepatocytes. *In vivo*, BAM tested negative in a micronucleus test in mice, with the bone marrow as observed target organ. Based on these tests, BAM does not possess genotoxic potential.

Two repeated dose toxicity studies with BAM were performed in rats. In a 90-day and 2-years diet study, the rats were exposed to respectively 4, 14, 49 and 172 and 2, 3.5, 6.5, and 18 mg/kg bw/day. The 90-day oral toxicity study in rats showed, in the 49 mg/kg bw/d dose group, reduced body weight (gain), reduced food consumption, hair loss and a reduction in skeletal muscle tone in the female animals, increased relative adrenal weight in males and liver- and kidney effects in both sexes. The NOAEL is set at 14 mg/kg bw/d based on these effects at the next higher dose.

In the 2-year oral toxicity study in rats in the highest dose group liver weight increase, hepatocellular adenoma, hepatocyte vacuolation centrilobular and Eosinophilic/Basophilic hepatocytes were observed. The NOAEL is set at 6.5 mg/kg bw/d.

In the two year rat study, dietary administration of 2,6-dichlorobenzamide resulted in an increase in hepatocellular adenomas in females at the highest dose group. The highest dose level in this study exceeds the MTD. Therefore, the relevance of the increased tumour incidences at the highest dose should be questioned. However, considering that these tumours:

- are increased only at the highest dose,
- in a single species,
- occur also spontaneously in this species and sex,
- are at the border of statistical significance,
- occur under conditions of clear local cytotoxicity (i.e. in the liver), and
- only occur at a dose level that may be considered to clearly exceed the maximum tolerated dose criterium, and that this substance clearly has no genotoxic potential,

application of the criteria mentioned in Annex VI of Directive 67/548/EC allows to conclude that this substance should be classified with R40 (category 3; Limited evidence of a carcinogenic effect).

It should be noted that this is not in line with the evaluation of BAM in the DAR for fluopicolide.

B.6.10.3 ADI

The ADI was 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. Dichlobenil was tested in several subacute, semi-chronic, and chronic toxicity studies in dogs, rats, hamster, and mice, providing the basis for the establishment of the ADI. The dog is the most sensitive species, and as such was used for the derivation of the ADI. The lowest LOAEL (1 mg/kg/day) was obtained from a 52 weeks study in dogs. The NOAEL from the 2-year dog study is 1.3 mg/kg bw/d. In view of almost equal NOAEL of the 2-years study and the LOAEL from the 52-weeks dog study, only a factor of 2 for extrapolation from a LOAEL to NAEL is used. This NAEL (0.5 mg/kg bw/d) is used as a starting point for the establishment of the ADI. Application of a safety factor for inter- and intraspecies differences of 100 results in an ADI of 0.005 mg/kg bw/d. Based on the two dog studies the NOAEL does not decrease with increased exposure duration. Therefore no correction factor for exposure duration is used. Furthermore, no additional factor is used for carcinogenic effects, caused via a threshold mechanism, as they are observed well above the LOAEL for non-carcinogenic systemic effects.

For the main soil metabolite of dichlobenil, 2,6-dichlorobenzamide (BAM), a separate ADI is proposed. This metabolite has been included in the residue definition, whereas it is not observed as a metabolite in toxicokinetic studies conducted with dichlobenil. The proposed ADI is based on the chronic NOAEL of 6.5 mg/kg bw/d from the 2-year chronic study with rats and safety factor for inter- and intraspecies differences of 100. Furthermore, as tumours were formed, be it via threshold mechanism, at the LOAEL for non-carcinogenic systemic effects, an uncertainty factor of 3 is proposed in view of the serious nature of this effect. Applying these safety factors leads to an ADI for BAM of 0.022 mg/kg bw/d. This results in a factor of 800 between the ADI for BAM and the dose level at which tumours were observed in the 2-year study with BAM.

B.6.10.4 ARfD (acute reference dose)

For dichlobenil no ARfD is derived. No classification is required based on the acute toxicity studies and there are no indications for acute effects from the repeated dose toxicity studies.

For the main soil metabolite of dichlobenil, 2,6-dichlorobenzamide (BAM), a separate ARfD is proposed. This metabolite has been included in the residue definition, whereas it is not observed as a metabolite in toxicokinetic studies conducted with dichlobenil. Based on the submitted data on BAM, the following considerations were made:

- The acute oral LD₅₀ of BAM was estimated to be 2000 mg/kg bw in male rats and 500 mg/kg bw in females rats.
- In the 90-day toxicity study, a significant reduction in skeletal muscle tone was observed from day 4 – day 92, at dose levels of ≥ 600 ppm (49 mg/kg bw/d).

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. Furthermore, an acute toxic effect for BAM was observed in the 90-day study, and it was concluded that allocation of an ARfD is considered justified.

It is proposed to consider the 90-day oral toxicity study with BAM in rats as the most suitable study to derive an ARfD. The NOAEL for skeletal muscle hypotonus is 14 mg/kg bw/d. Application of a safety factor of 100 for inter- and intraspecies differences results in an ARfD for BAM of 0.14 mg/kg bw.

B.6.10.5 AOEL

Dichlobenil is the active ingredient of Casoron G, at a concentration of 6.75% in granules. Casoron G is used as an herbicide. Dichlobenil is applied by mechanical or manual scattering of granules around buildings, on industrial sites, alongside paths and roads, under fence lines and hedges around well-established trees and shrubs in parks and recreational areas and around grape vines.

The application frequency is for all applications once a year, pre-emergence in spring only. Therefore, the operator exposure will not exceed a period of 3 months, and it is considered semichronic. The lowest NAEL of 0.5 mg/kg bw/d is derived from the 52-week dog toxicity study. The oral absorption is derived from the metabolic studies and is assumed >80%. Application of a safety factor for inter- and intraspecies differences of 100 results in a systemic AOEL of 0.005 mg/kg bw/day. As the derived bioavailability is >80%, no correction for bioavailability will be implemented, and the systemic AOEL will remain 0.005 mg/kg bw/d.

B.6.10.6 Drinking water limit

According to Council Directive 97/57/EC, exposure to dichlobenil through 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 $((60 \times 0.005) / 10) / 2$ 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 dichlobenil.

According to Council Directive 97/57/EC, exposure to 2,6-dichlorobenzamide (BAM) through 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 $((60 \times 0.022) / 10) / 2$ mg/L, i.e. 0.07 mg/L can be established. According to Document 8064/VI/79 of the European Commission, the EU drinking water limit for relevant metabolites of 0.1 µg/l is applicable for 2,6-dichlorobenzamide (BAM).

B.6.11 ACUTE TOXICITY INCLUDING SKIN SENSITISATION OF PREPARATIONS (ANNEX IIIA 7.1)

B.6.11.1 Acute toxicity

B.6.11.1.1 Acute oral toxicity study**STUDY 1****Characteristics of study**

Reference	: Koopman, 1983	Route	: oral
Year of execution	: 1983	Group size	: 5/sex
Guideline	: OECD 401	Exposure	: once by gavage
GLP	: no	Doses	: 5000 mg/kg bw
Test substance	: CASORON G, lot/batch no. FUN83A27; appearance not indicated	Vehicle	: 1%-tragacanth
Content of a.s.	: 6.75% dichlobenil	Acceptability	: acceptable
Species	: rats, Wistar	LD ₅₀	: >5000 mg/kg bw

Study design

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

Results

Mortality: none

Symptoms of toxicity: dispersion in cage, apathy, decreased alertness and startle response, decreased locomotor activity, pilo-erection, passiveness, ptosis, lacrimation. All signs were slight in intensity and disappeared within six hours after dosing.

Body weight: in the first two days after dosing a weight loss in both sexes occurred but the animals recovered within 14 days.

Pathology: no treatment-related findings

Acceptability

Although the study is not performed under GLP, it is considered acceptable.

Conclusions

The acute oral LD₅₀ of the test substance was estimated to be >5000 mg/kg bw for rats.

STUDY 2**Characteristics of study**

Reference	: Berryman <i>et al.</i> , 1989	Route	: oral
Year of execution	: 1989	Group size	: 5/sex/dose
Guideline	: OECD 401	Exposure	: twice by gavage within 24 h
GLP	: yes	Doses	: 4000, 4400, 5000, 5800 and 7500 mg/kg bw (total)
Test substance	: CASORON G, lot/batch no. FUN89G31A; appearance: grey granular solid	Vehicle	: propylene glycol
Content of a.s.	: not indicated	Acceptability	: acceptable
Species	: mice, CD-1	LD ₅₀	: >7500 mg/kg bw

Study design

The study was performed mostly in accordance with OECD 401. A deviation from the guideline was that the body weight range for the males at the start of treatment was 28-35 grams and not 15-25 grams and the content of a.s. is not indicated.

Initially one group was dosed at 5000 mg/kg bw. Three animals died and the study was extended to include four further groups with dose levels of 4000, 4400, 5800 and 7500 mg/kg bw.

The suspension was highly viscous and in order to facilitate dosing, it was diluted and administered in two equal doses within 24 hours (on days 1 and 2).

Results

Mortality: males: 2, 2, 2, 0, 0 (from high to low dose group; 7500, 5800, 5000, 4400, 4000 mg/kg bw), females: 1, 0, 1, 0, 0 (from high to low dose group). Both females and three of the males died on days 1, 2 or 3 of the study. The other three males died on days 5, 6 or 8.

Symptoms of toxicity: lethargy, pilo-erection, ataxia. All surviving animals had recovered by day 11 of the study

Body weight: no treatment-related findings

Pathology: Animals that died during the study revealed haemorrhages in the glandular stomach, an enlarged bladder, haemorrhages in the bladder wall and a dark red color in lungs. Animals sacrificed at termination revealed no abnormalities, apart from one animal that had an enlarged spleen and white spots on the surface of all liver lobes.

Acceptability

Although the body weight range of the male animals at the start of treatment was too high, the study is considered acceptable.

Conclusions

The LD₅₀ value of CASORON G in mice of both sexes was established to exceed 7500 mg/kg bw.

B.6.11.1.2 Acute dermal toxicity study

Characteristics of study

Reference	Koopman, 1985a	Route	: dermal
Year of execution	1985	Group size	: 5/sex
Guideline	: OECD 402	Exposure	: 24 hours (occlusive)
GLP	no	Doses	: 2000 mg/kg bw
Test substance	: CASORON G-6.75, lot no. FUN85G03A; granular formulation	Vehicle	: moistened with 1%-tragacanth
Content of a.s.	6.75% dichlobenil	Acceptability	: acceptable
Species	rats, Wistar	LD ₅₀	: >2000 mg/kg bw

Study design

The study was performed in accordance with OECD 402.

Results

Mortality: none

Symptoms of toxicity: no treatment-related findings

Body weight: there was a slight weight-loss in the first two days after application. The rats recovered after two days.

Pathology: no treatment-related findings

Acceptability

Although no GLP statement is given, the study is considered acceptable.

Conclusions

The acute dermal LD₅₀ of the test substance was estimated to be >2000 mg/kg bw for rats.

B.6.11.1.3 Acute inhalation toxicity study

No acute inhalation toxicity studies with the preparation were submitted. An acute inhalation study is not required for this preparation.

B.6.11.2 Irritation and sensitisation

B.6.11.2.1 Skin irritation

Characteristics of study:

Reference	: Koopman, 1985b	Route	: dermal
Year of execution	: 1985	Group size	: 3 males
Guideline	: OECD 404	Exposure	: 4 hours (occlusive)
GLP	: no	Doses	: 0.5 g
Test substance	: CASORON G-6.75, lot/batch no. FUN85G03A; appearance: granular formulation	Vehicle	: moistened with 1%-tragacanth
Content of a.s.	: 6.75% dichlobenil	Acceptability	: acceptable
Species	: rabbit, New Zealand White	Effect	: not irritating to skin

Study design

The study was performed in accordance with OECD 404.

Results

The results are presented in Table 6.11.2.1

Table 6.11.2.1

Scores observed after	0.5-1 hours	24 hours	48 hours	72 hours
Erythema	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

Scores observed after	0.5-1 hours	24 hours	48 hours	72 hours
Oedema	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0

Acceptability

Although no GLP statement is given, the study is considered acceptable.

Conclusions

The test substance does not have to be classified as irritating to the skin.

B.6.11.2.2 Eye irritation

Characteristics of study:

Reference	: Daamen, 1989	Route	: ocular
Year of execution	: 1989	Group size	: 6 females
Guideline	: OECD 405	Exposure	: single instillation in conjunctival sac
GLP	: yes	Doses	: 100 mg
Test substance	: CASORON G, lot/batch no. FUN89G31B; appearance: grey powder	Vehicle	: none
Content of a.s.	: not indicated	Acceptability	: acceptable
Species	: rabbit, New Zealand White	Effect	: not irritating to eyes

Study design

The study was performed mostly in accordance with OECD 405. Too many animals are used.

According to the guideline only up to three animals have to be used.

Results

The results are presented in Table 6.11.2.2.

Table 6.11.2.2

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	1, 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	1, 1, 1, 1, 1, 1	1, 1, 1, 1, 1, 1	0, 1, 1, 1, 1, 1	0, 0, 0, 0, 0, 0
Conjunctiva chemosis	1, 1, 1, 1, 1, 1	0, 1, 1, 1, 1, 1	0, 0, 0, 0, 1, 0	0, 0, 0, 0, 0, 0
Conjunctiva discharge	0, 0, 0, 0, 0, 0	0, 1, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

Acceptability

Although too many animals are used, the study is considered acceptable.

Conclusions

The test substance does not have to be classified as irritating to the eyes.

B.6.11.2.3 Skin sensitisation (Buehler test)

Characteristics of the study:

Reference	: Huygevoort (2003)	Route	: dermal
Year of execution	: 2003	Group size	: 10 controls, 20 test animals (females only)
Guideline	: OECD 406	Exposure	: 3 topical inductions and 1 topical challenge (occlusive, 6h)
GLP	: yes	Doses	: 50% w/v induction and 50% w/v challenge ¹
Test substance	: CASORON G, lot/batch no. MI0395; appearance: fine greyish granules	Vehicle	: water
Content of a.s.	: 6.65% dichlobenil	Acceptability	: acceptable
Species	: guinea pig, Dunkin Hartley Albino	Effect	: not sensitising to skin

¹ Based on the results of a range-finding study using 50% w/v topical applications. The highest concentration that did not cause significant irritation by topical application was 50% w/v.

Study design

The study was performed partly in accordance with OECD 406. OECD 406 requires the use of the highest possible concentration that produced mild irritation for the induction exposure. Since none of the doses used in the range-finding study (50, 20, 10 and 5% w/v) caused any irritation, the 50% test substance concentration was selected for the induction phase.

Results

Topical induction caused slight erythema in 12 out of 20 test animals. Following challenge with 50% w/v, no dermal responses were observed in any of the test and negative control animals.

Sensitisation of this strain of animals was positively tested with Alpha-hexylcinnamic aldehyde, tech. 85%.

Acceptability

The study is considered acceptable. However, it should be noted that for a correct classification and labelling in accordance with EC regulations, a maximisation test is preferred with regard to the assessment of dermal sensitisation potential.

Conclusions

The test substance is not sensitising to the skin of guinea pigs in this Buehler test. It is noted that no Buehler test had to be performed in 2003; a maximisation test (or LLNA) is the preferred method.

B.6.11.3 Summary

The results of the acute toxicity, irritation and sensitisation studies are presented in Tables 6.11.8.1, 6.11.8.2 and 6.11.8.3, respectively.

Table 6.11.8.1 Acute toxicity, LD₅₀/LC₅₀ values

Test substance	LD ₅₀ /LC ₅₀	Species	Route	Vehicle	Reference
CASORON G	>5000 mg/kg bw	rat	oral	1%-tragacanth	Koopman, 1983
CASORON G	>7500 mg/kg bw	mouse	oral	Propylene glycol	Berryman, 1989
CASORON G	>2000 mg/kg bw	rat	dermal	1%-tragacanth	Koopman, 1985a

Table 6.11.8.2 Skin and eye irritation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
CASORON G	not irritating to skin	rabbit	skin	1%-tragacanth	Koopman, 1985b
CASORON G	not irritating to eyes	rabbit	eye	none	Daamen, 1989

Table 6.11.8.3 Sensitisation studies

Test substance	Effect/Classification	Species	Route	Vehicle	Reference
CASORON G	not sensitising to skin (Buehler test)	guinea pig	dermal	water	Huygevoort, 2003

B.6.12 DERMAL ABSORPTION (ANNEX IIIA 7.3)**B.6.12.1 Dermal absorption, *in vitro*****STUDY 1****Characteristics**

Reference	: Kane, 2006	exposure	: 6 h, non-occlusive
type of study	: <i>in vitro</i> dermal absorption	doses	: 763 µg/cm ² (undiluted)
year of execution	: 2006	vehicle	: Casoron G (containing 6.75% dichlobenil) formulation
test substance	: Radiolabelled test substance: [¹⁴ C]-dichlobenil, radiochemical purity >98%, specific activity 1.96 GBq/mmol.	GLP statement	: Yes
	: Unlabelled test substance: dichlobenil, purity 98.8%		
Route	: Dermal	guideline	: OECD guideline 428
Species	: Human	acceptability	: Acceptable
group size	: 8 replicates	Result	: Human skin: 1.4% (for an area dose of 763 µg/cm ²)

Study design

The rate and extent of absorption of [¹⁴C]-labelled dichlobenil was investigated following topical application to excised human skin in an aqueous slurry of Casoron G ready-to-use formulation (containing 6.75% dichlobenil), at one dose concentration.

The percutaneous absorption of [^{14}C]-dichlobenil was studied *in vitro*, using flow-through diffusion cells. Dermatomed skin membranes from humans were exposed to a mean dose of dichlobenil of 763 $\mu\text{g}/\text{cm}^2$. This dose level was representative of the concentration of dichlobenil in the commercially available granule.

The integrity of the skin membranes was established prior to the application of the test substance, by determination of the penetration of tritiated water. Aliquots of 6.4 μl were applied to an area of 0.64 cm^2 . The test substance remained in contact with the unoccluded skin for 6 hours. Receptor fluid samples were collected at hourly intervals until 24 h after application. At the end of the exposure period, the skin was swabbed with 1% v/v Tween 80 in distilled water on cotton wool buds. Subsequently, the skin surface was dried with a dry cotton wool bud. After 24 hours, the skin membranes were tape-stripped. The initial tape strips (1 – 2) were collected separately. Subsequent tape strips containing the stratum corneum were pooled and analysed in batches of three. The remaining skin was retained and analysed separately.

The exposure chambers were washed. The receptor fluid, skin wash, skin membranes, tape strips and the exposure chamber washes were analysed using LSC.

Results

Mean recovery and distribution of radioactivity expressed as % of the applied dose and μg equivalents of dichlobenil are shown in Table 6.12.1.1.

Total mean recovery was 96%. The Figures in the study report (Figure 2 and 3) show that at 24 hours after application still increasing levels of radiolabel were recovered from the receptor fluid. Therefore, radiolabel from the skin membranes should be included into the potentially absorbed amount. Furthermore, it is not clear whether the amount in the tape strips could be absorbed. During EPCO 23 in May 2005 it was decided to regard the amount of AR in tape strips 1 and 2 as exfoliated and to regard the amount of AR in the following tape strips as potentially absorbed. This results in a mean total absorption of 1.4%.

In human skin, the mean flux constant for the absorption of dichlobenil formulated as Casoron G was 0.512 μg equivalents dichlobenil/ cm^2/h .

Table 6.12.1.1 Distribution and recovery of radioactivity following application of [^{14}C]-dichlobenil to human skin

Absorption is expressed as both percentage of the administered radiolabel and μg equivalents of dichlobenil.

	%	μg
receptor fluid 0-24 h (including dose in receptor)	0.79	3.86
Receptor chamber	nd	nd
skin	0.20	0.98
tape strips 1 and 2	2.61	12.74
tape strips 3 – 13	0.37	1.81

skin wash/cotton swabs	87.56	427.46
donor chamber	0.67	3.27
Remaining in volatile trap chamber	3.57	17.43
total absorbed ¹	0.79	3.86
potentially absorbed ²	1.36	6.65
dislodgeable dose ³	94.41	460.90
recovery	95.77	467.55

nd non detectable

¹ receptor fluid, receptor chamber

² receptor fluid, receptor chamber, skin, tape strips 3 - 13

³ skin wash/cotton swabs, donor chamber, remaining in volatile trap chamber, tape strips 1 and 2

Acceptability

The study was performed in accordance with OECD 428 and is considered acceptable.

Conclusion

The objective of the study was to determine dermal absorption through human skin *in vitro*. Considering the results from the *in vitro* dermal absorption study, for occupational risk assessment a dermal absorption of 1.4% should be considered.

Since Casoron G is a ready-to-use granular formulation, this value for dermal absorption can be used for dermal exposure during mixing/loading and application.

B.6.13 TOXICOLOGICAL DATA ON NON ACTIVE SUBSTANCES (ANNEX IIIA 7.4 AND POINT 4 OF THE INTRODUCTION)

Information with regard to the non active substances was submitted by the notifier in MSDSs. Based on this information no further information is required.

B.6.14 EXPOSURE DATA (ANNEX IIIA 7.2)

B.6.14.1 Calculations

For the present applications, mechanical and manual scattering of granules, no exposure models are available. The current available models, like the German model, UK-POEM, Dutch model, or EUROPOEM do not have a module to estimate exposure during application of granules. Therefore, an assessment of the data available in the North-American model PHED was made, using the PHED Surrogate Exposure Guide (1998).

For personal protection, also the data of PHED were used.

The calculations for operator exposure, bystander exposure and worker exposure are presented in Appendix 3a.

In order to calculate internal doses after dermal exposure a value of 1.4% was used, based on an *in vitro* dermal absorption study with human skin. 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 Casoron G are further specified in Appendix 3a.

B.6.14.2 Internal exposure and risk assessment

Internal operator exposures and risk assessments are specified in Table 6.14.2.1.

Table 6.14.2.1 Operator internal exposure and risk assessment

Activity	Route	Estimated internal exposure		AOEL- systemic *	% AOEL	
		(mg a.s./day)				
		without PPE	with PPE	without PPE		
Mechanical scattering in grapes with a solid broadcast spreader with open cab						
Mix/loading	Respiratory	0.16	0.016	0.35	46	4.6
Mix/loading	Dermal	0.04	<0.01	0.35	11	1.1
Application	Respiratory	0.11	0.011	0.35	31	3.1
Application	Dermal	0.05	<0.01	0.35	14	1.4
Total		0.36	0.03	0.35	103	10
Mechanical scattering in grapes with a solid broadcast spreader with closed cab						
Mix/loading	Respiratory	0.16	0.016	0.35	46	4.6
Mix/loading	Dermal	0.04	<0.01	0.35	11	1.1
Application	Respiratory	0.02	<0.01	0.35	6	0.6
Application	Dermal	0.003	<0.01	0.35	1	0.1
Total		0.22	0.02	0.35	64	6
Manual scattering alongside paths and roads and under fence lines and hedges, and around buildings and on industrial sites, with 5 kg ready-to-use container						
Mix/loading		n.a.	n.a.	-	n.a.	n.a.
Application		negligible	negligible	-	negligible	negligible
Manual scattering alongside paths and roads and under fence lines and hedges, and around buildings and on industrial sites, with 25 kg 'knap sacked' applicator						
Mix/loading	Respiratory	0.01	<0.01	0.35	3	0.3
Mix/loading	Dermal	<0.01	<0.01		negligible	negligible

Activity	Route	Estimated internal exposure (mg a.s./day)		AOEL- systemic * (mg a.s./day)	% AOEL	
		without PPE	with PPE		without PPE	with PPE
Application		negligible	negligible	-	negligible	negligible
Total		0.01	<0.01	0.35	3	0.3
<i>Manual scattering around well established trees and shrubs in parks and recreational areas, with 1 or 5 kg ready-to-use container</i>						
Mix/loading		n.a.	n.a.	-	n.a.	n.a.
Application		negligible	negligible	-	negligible	negligible

* AOEL (mg/d) was calculated using a body weight of 70 kg
n.a. = not applicable

B.6.14.3 Conclusions

- A safe use was identified for operators without PPE for mechanical application of Casoron G in grapes, using the PHED model. The exposure estimate for operators using a solid broadcast spreader with open cab is 103% of the AOEL and for operators using a solid broadcast spreader with closed cab 64% of the AOEL. It should be taken into account that these are worst-case estimates. Casoron G is a nearly dust-free formulation (see physical/chemical properties) and the dust-fractions of the formulations used in the underlying data-base of PHED are unknown, but are likely to be higher. Furthermore, it should be noted that a spreader with closed cab is used in current practice.
- Safe uses were identified for operators without PPE for manual application of Casoron G alongside paths and roads, under fence lines and hedges, around buildings and on industrial sites, and around well established trees and shrubs in parks and recreational areas.
- No risk assessment was performed for bystanders, as it is assumed that no bystander exposure will occur.
- No risk assessment was performed for workers, as it is assumed that no re-entry exposure will occur.

B.6.15 REFERENCES RELIED ON**Section B.6 Toxicology and metabolism (Annex IIA, point 5, Annex IIIa, point 7)**

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
5.1/01	Koorn, E., Kaldenberg, K., Lange, N.de	1987	Dichlobenil: excretion in rats after oral and intravenous administration Report [REDACTED] No.56654/14/1987 CA - 2851 GLP, Unpublished	Y	CHEM
5.1/02	Kaldenberg, K., Lange, N.De	1988	Dichlobenil distribution in rats after oral administration. A study with [14C]dichlobenil Report [REDACTED] No.56654/02/1988 CA - 2850 GLP, Unpublished	Y	CHEM
5.1/03	Cameron, B.D., Mutch, P.J., Scott, G.	1988	The excretion and retention of [14C]-dichlobenil in the rat following single and multiple oral administration Report [REDACTED] [REDACTED] No.4458 (int. Doc.No.56654/04/1988) CA - 2776 GLP, Unpublished	Y	CHEM
5.1/04	Vincent, W.R., Ruijten, H.M., Eck, M.P.E. van et al	1989	The metabolism of dichlobenil in the rat Report [REDACTED] No.56630/213/1989 CA - 2854 GLP, Unpublished	Y	CHEM
5.2.1/01	Arnoldussen, A.M., Verschuuren, H.G., Esch, G.J.Van	1963	Various acute and short-term toxicity investigations of dichlobenil Report [REDACTED] [REDACTED] No.U4/63 tox CA - 509 Non-GLP, Unpublished	Y	CHEM
5.2.1/02	Graver, K.	1999	Dichlobenil technical - acute oral toxicity / LD50 in rats Report [REDACTED] No. [REDACTED] 99-7507.01 CA - 3879 GLP, Unpublished	Y	CHEM
5.2.2/02	Cerven, D.R.	2000	Acute dermal toxicity in rabbits/LD50 in rabbits - technical dichlobenil Report [REDACTED] No. [REDACTED] 00-8155.02 CA - 3920 GLP, Unpublished	Y	CHEM
5.2.4/01	Koopman, T.S.M.	1985	Primary irritation study of dichlobenil technical to the skin of the male rabbit Report [REDACTED] No.56645/70/1985 CA - 2364 GLP, Unpublished	Y	CHEM
5.2.5/01	Koopman, T.S.M.	1983	Primary irritation of dichlobenil technical to the rabbit eye Report [REDACTED] No.56645/38/1983	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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5.2.6/01	Cuthbert, J.A., Carr, S.M.A.	1988	Dichlobenil: magnusson-kligman maximisation test in guinea pigs Report [REDACTED] No. 238368 CA - 2648 GLP, Unpublished	Y	CHEM
5.3.2/03	Kemp, A., Haan, M. de, Jager, F.C.	1987a	A 13-week toxicity study with in male and female hamster followed by a 4-week recovery period Report [REDACTED] No.56645/04/1987 CA - 2654 GLP, Unpublished	Y	CHEM
5.3.2/04	Kemp, A., Haan, M. de, Dawes, R.L.F.	1987b	A 13-week (dietary) toxicity study with dichlobenil in male and female mice Report [REDACTED] No.56645/03/1987 CA - 2650 GLP, Unpublished	Y	CHEM
5.3.2/06	Til, H.P., Feron, V.J., Huismans, J.W. et al.	1969	Chronic (two-year) toxicity study with dichlobenil (h 133) in beagle dogs Report [REDACTED] [REDACTED] No.R2813 CA - 530 Non-GLP, Unpublished	Y	CHEM
5.3.2/06	Dijkstra, A.C.	1990	Chronic (two-year) toxicity study with dichlobenil (h 133) in beagle dogs- addendum to [REDACTED] Report No. R 2813 Report [REDACTED] [REDACTED] CA - 530 Non-GLP, Unpublished	Y	CHEM
5.3.2/07	Pickersgill, N.	1995	Dichlobenil - 52 week oral (capsule) toxicity study in the beagle dog Report [REDACTED] No.56352/41/1995 CA - 3162 GLP, Unpublished	Y	CHEM
5.3.3/01	Newton, P.E.	2002	A 28-day inhalation toxicity study of dichlobenil technical in rats Report [REDACTED] No.399- 218 CA - 4104 GLP, Unpublished	Y	CHEM
5.3.3/02	Allan, S.A.	1995	Dichlobenil technical - twenty-one day dermal toxicity study in the rabbit Report [REDACTED] [REDACTED] No.PDR 621/951245 CA - 3157 GLP, Unpublished	Y	CHEM
5.4.1/01	Seket, J.C.	1983	Ames test to assess the potential mutagenic effect of dichlobenil Report Duphar B.V., The Netherlands No.56645/58/1983 CA - 1901 Non-GLP, Unpublished	Y	CHEM
5.4.1/04	Rundell, J.O., Myhr, B.C.	1984	Evaluation of dichlobenil technical in the in vitro transformation of balb/3t3 cells assay	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
			Report Litton Bionetics, Inc., Maryland, U.S.A. No.56645/62/1983 CA - 2118 GLP, Unpublished		
5.4.1/05	Matthews, E.J., Myhr, B.C.	1984	Evaluation of dichlobenil technical in the in vitro transformation of balb/C-3T3 cells with S9 activation assay Report Litton Bionetics, Inc., Maryland, U.S.A. No.56645/20/1984 CA - 2117 GLP, Unpublished	Y	CHEM
5.4.1/06	Murli, H.	1990	Mutagenicity test on dichlobenil technical in an in vitro cytogenetic assay, measuring chromosomal aberration frequencies in chinese hamster ovary (cho) cells with multiple harvests. Report Hazleton Laboratories America Inc., U.S.A. No. 12176-0-437J (Duphar Int. Doc. No.56645/108/90) CA - 2906 GLP, Unpublished	Y	CHEM
5.4.1/07	Clarke, J.J.	2007	In vitro mammalian cell gene mutation (CHO/HGPRT) test with an independent repeat assay (test article Dichlobenil Technical) Report BioReliance, USA NO. AB37HH.782001.BTL CA - 4238 GLP, Unpublished	Y	CHEM
5.4.2/01	Allen, J.A., Proudlock, R.J.	1983	Autoradiographic assessment of dna repair in mammalian cells after exposure to dichlobenil technical Report [REDACTED] [REDACTED] Reference No. PDR382/831065 (56645/60/1983) CA - 2032 GLP, Unpublished	Y	CHEM
5.4.2/02	Jens, D.H.	1983	Micronucleus test on dichlobenil Report [REDACTED] No.56645/57/1983 CA - 1900 GLP, Unpublished	Y	CHEM
5.5/02a	Inoue, H., Enomoto, M.	1984	Chronic feeding study on dichlobenil in the rat Report No.56645/41/1984 (exp.No.40 (010-002)) CA - 2122 GLP, Unpublished	Y	CHEM
5.5/02b	Inoue, H., Enomoto, M.	1998	Addendum - reply to the comments on conclusion of exp. No. 40 [REDACTED] - chronic feeding study on dichlobenil in the rat Report [REDACTED], Dated 2 September 1998 CA - 2122 GLP, Unpublished	Y	CHEM
5.5/03	Hooks, W.N., Cary, R.J., Colman, K. et al.	1990	Dichlobenil - potential tumorigenic effects in prolonged dietary administration to hamsters Report [REDACTED] [REDACTED] No.PDR 478	Y	CHEM

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			CA - 2942 GLP, Unpublished		
5.5/04	Chambers, P.R., Hornby, S.B., Gibson, W.A., et al	1992a	Dichlobenil potential tumorigenic effects in prolonged dietary administration to hamsters Report [REDACTED] [REDACTED] No.PDR 511/9178 CA - 2985 GLP, Unpublished	Y	CHEM
5.5/05	Chambers, P.R., Brown, R., Gibson, W.A. et al	1992b	Background tumour incidence in hamsters (background data study) Report [REDACTED] [REDACTED] No.PDR 540/920478 CA - 3019 GLP, Unpublished	Y	CHEM
5.5/06	Oevelen, W.A.M. Van, Leeming, N.	2006	Dichlobenil: rebuttal against the proposed Cat 3 carcinogenicity classification Report Chemtura Netherlands B.V., The Netherlands and TSGE, UK, No. 155802/161/2006 Unpublished	Y	CHEM
5.6.1/01a	Leeming, N.M.	1989	Dichlobenil: 2 generation oral (dietary administration) reproduction study in the rat Report [REDACTED] No.5798-65/18 CA - 2843 GLP, Unpublished	Y	CHEM
5.6.1/01b	Barker, L.	1992a	Dichlobenil: 2 generation oral (dietary administration) reproduction study in the rat. Addendum 1 - pathology report Report [REDACTED] No.5798-65/18 (addendum 1) CA - 2843 GLP, Unpublished	Y	CHEM
5.6.1/01c	Barker, L.	1992b	Dichlobenil: 2 generation oral (dietary administration) reproduction study in the rat. Addendum 2 - revision to pathology report Report [REDACTED] No.5798-65/18 (addendum 2) CA - 2843 GLP, Unpublished	Y	CHEM
5.6.1/01d	Barker, L.	1993	Dichlobenil: 2 generation oral (dietary administration) reproduction study in the rat. Addendum 3 - F1 pathology report Report [REDACTED] No.5798-65/18 (addendum 3) CA - 2843 GLP, Unpublished	Y	CHEM
5.6.2/01	Koeter, H.B.W.M.	1984	Oral embryotoxicity/teratogenicity study with dichlobenil in rats Report [REDACTED] (No.V84.026/231045) No.56645/26/1984 CA - 2055 GLP, Unpublished	Y	CHEM
5.6.2/03a	Barker, L.	1989b	Dichlobenil: oral (gavage) teratology study in the rabbit (experiment II) repeat study Report [REDACTED] No.5942-65/19 (Int.Doc.No.56645/21/1989) CA - 2844 GLP, Unpublished	Y	CHEM
5.6.2/03b	Barker, L.	1991	Dichlobenil: oral (gavage) teratology study in the rabbit (experiment II) repeat study	Y	CHEM

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			amendment to final report Report [REDACTED] No.65/19 Ca - 2844 GLP, Unpublished		
5.8.1/03	Schüngel, M.	2003	AE C653711 (metabolite of ae c638206) acute toxicity in the rat after oral administration Report [REDACTED] No.At00875 CA - 4190 GLP, Unpublished	Y*	BAY
5.8.1/04	Boschman, T.A.C., Kemp, A., Linde, H.M. van der et al .	1967	Dietary administration of 2,6-dichlorobenzamide to male and female rats for 13 weeks Report [REDACTED] No.56645/02/1967 CA - 642 Non-GLP, Unpublished	Y	CHEM
5.8.1/05	Koorn, J.	1993	Evaluation of the possible mutagenic activity of 2,6-dichlorobenzamide in the ames salmonella/microsome test Report Solvay Duphar B.V., The Netherlands No. 56645/69/1992 CA - 3028 GLP, Unpublished	Y	CHEM
5.8.1/06	Herbold, B.	2003a	AE C653711: salmonella/microsome test - plate incorporation and preincubation method Report Bayer Cropscience, Germany No.AT00853 CA - 4189 GLP, Unpublished	Y*	BAY
5.8.1/07	Herbold, B.	2003b	AE C653711 metabolite of AE C638206: V79/HPRT-Test in vitro for the detection of induced forward mutations Report Bayer Cropscience, Germany No.AT00610 CA - 4185 GLP, Unpublished	Y	CHEM
5.8.1/08	Waart, E.J. van de	1993a	Evaluation of DNA repair inducing ability of 2,6-dichlorobenzamide (BAM) in a primary culture of rat hepatocytes (with independent repeat) Report [REDACTED] No.56345/23/1993 CA - 3032 GLP, Unpublished	Y	CHEM
5.8.1/09	Waart, E.J. van de	1993b	Micronucleus test in bone marrow cells of the mouse with 2,6-dichlorobenzamide (BAM) Report [REDACTED] No.56345/13/1993 CA - 3024 GLP, Unpublished	Y	CHEM
5.8.1/10a	Wheldon, G.H., Street, A.E., Heywood, R. et al	1971	Effects of BAM in dietary administration to rats for two years Report [REDACTED] [REDACTED] No.56645/16/1971 CA - 515 Non-GLP, Unpublished	Y	CHEM
5.8.1/10b	Johnson, S.F.	1996	Bam - validation of an analytical method and	Y	CHEM

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5.8.1/10c	Connick, H., Crome, S.J., Gopinath, C.	1996	Re-assessment of liver lesions/tumours from study PDR/49; BAM: dietary administration to rats for 2 years Report [REDACTED] URL97/961569 CA - 515 GLP, Unpublished	Y	CHEM
5.8.1/11	Story, D.L.	2006	The effect of dichlobenil on the olfactory mucosa of rodents Report Crompton Corporation, USA No.AM950/23/2002 Unpublished	Y	CHEM
5.8.1/12	Payraudeau, V, Freeman, E.	2006	Regulatory toxicology position paper: 2,6-dichlorobenzamide (BAM) – Toxicity profile and lack of carcinogenicity potential Report Bayer CropScience, France and Chemtura Corporation, USA No. M-274220-02-1 Unpublished	Y	CHEM
5.8.1/13	Pallen, C.	2006	Regulatory toxicology position paper: Re-assessment of liver lesions/tumors from study PDR/49; BAM: dietary administration to rats for 2 years – complementary statistical analysis of hepatocellular tumors in female rats Report Bayer CropScience, France No. M-273467-01-1 Unpublished	Y	CHEM
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7.1.1/02	Berryman, L.J., Daamen, P.A. M.	1989	Assessment of acute oral toxicity with Casoron G in the mouse Report [REDACTED] No. 019912 CA – 2883 Yes, Unpublished	Y	CHEM
7.1.2/01	Koopman, T.S. M.	1985a	Acute dermal toxicity study with Casoron G-6.75 in rats Report [REDACTED] No.56645/86/1985 CA – 2458 Yes, Unpublished	Y	CHEM
7.1.4/01	Koopman, T.S. M.	1985b	Primary irritation of Casoron G-6.75 to the skin of the male rabbit Report [REDACTED] No.56645/87/1985 CA – 2459 Yes, Unpublished	Y	CHEM
7.1.5/01	Daamen, P.A. M.	1989	Assessment of acute eye irritation/corrosion of Casoron G in the rabbit	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
			Report [REDACTED] No.019923 CA – 2875 Yes, Unpublished		
7.1.6/01	Huygevoort, A.H.B.M. Van	2003	Assessment of contact hypersensitivity to Casoron G in the albino guinea pig (Buehler test) Report [REDACTED] No.387541 CA – 4153 Yes, Unpublished	Y	CHEM
7.2/01	Oevelen, W.A.M. Van	2006	Supplementary information to the proposed GAP – dichlobenil/Casoron G® Report Chemtura Netherlands B.V., The Netherlands No.155802/155/2006 Unpublished	Y	CHEM
7.3/01	Kane, T.J.	2006	Casoron G (ready-to-use granule containing dichlobenil) in vitro dermal penetration study at one dose level using human skin Report Huntingdon Life Science Ltd., England No.DNL0004/062792 CA – 4220 Yes, Unpublished	Y	CHEM