

DICHLOBENIL

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Explanation

Dichlobenil is the International Organization for Standardization (ISO)–approved common name for 2,6-dichlorobenzonitrile (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 1194-65-6. It belongs to the group of benzonitrile compounds, which are used as herbicides on cranberry bogs, dichondra, ornamentals, blackberry, raspberry and blueberry fields, apple, pear, filbert and cherry orchards, vineyards, hybrid poplar–cottonwood plantations and rights-of-way to control weeds; and in sewers to remove roots. It inhibits the germination of actively dividing meristems and acts primarily on growing points and root tips.

Dichlobenil has not previously been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

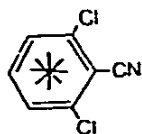
Some of the critical studies do not comply with good laboratory practice (GLP), as the data were generated before the implementation of GLP regulations. Overall, however, the Meeting considered that the database was adequate for the risk assessment.

Evaluation for acceptable daily intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of dichlobenil were studied in rats following a single oral low dose, a single oral high dose, a single oral daily dose repeated for 11 days followed by a radioactive dose and intravenous administration.

Fig. 1 shows the structure of [phenyl-U-¹⁴C]dichlobenil.



* denotes the position of the uniformly labelled phenyl ring

1.1 Absorption, distribution and excretion

In an absorption, distribution, excretion and metabolism study, Sprague-Dawley rats (five of each sex per dose) were administered dichlobenil as a single intravenous dose of 5.6 mg/kg body weight (bw) or a single oral gavage dose of 6.7 mg/kg bw. A separate group of four male bile duct-cannulated rats received dichlobenil as a single oral gavage dose of 6.7 mg/kg bw. In this study, [phenyl-U-¹⁴C]dichlobenil (purity 99.7%) was used. For oral dosing, ¹⁴C-labelled dichlobenil was suspended in 1% tragacanth gum; for intravenous dosing, it was suspended in polyethylene glycol 400/benzylalcohol/isopropylidene glycol (15%/2%/60% weight per volume [w/v]) in water. 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 were collected at 24, 48, 72, 96 and 168 hours after dosing. Bile was collected from bile duct-cannulated animals at 2, 5 and 24 hours after administration, and urine was collected at 5 and 24 hours. At 168 hours, all intact animals were euthanized, and blood and liver were collected. Radioactivity in cage washes, urine, faeces, plasma and liver was measured by combustion followed by liquid scintillation counting.

The total recovery of radioactivity was about 85% and 96% of the administered dose by the oral and intravenous routes of exposure, respectively (Table 1). Seven days after intravenous administration, male rats had excreted about 70% of the dose in the urine and 25% of the dose in the faeces. In females, about 65% and 31% of the dose were excreted in the urine and faeces, respectively. Similar results were obtained 7 days after oral administration. Male and female rats excreted about 65% and 20% of the dose in urine and faeces, respectively. The rate of urinary excretion was rapid; excretion was about 95% complete in 24 hours. Residual radiolabel in the liver, 168 hours after oral or intravenous administration, did not differ significantly between routes or sexes. The bile duct cannula was broken in one rat, so the results of three rats were reported. Approximately 79% of the administered dose was excreted in the bile in 24 hours. Based on a comparison of urinary excretion following oral and intravenous administration, approximately 89% and 97% of the oral dose were absorbed in male and female rats in 7 days, respectively (Koorn, Kaldenberg & De Lance, 1987).

The elimination and time course tissue distribution of [phenyl-U-¹⁴C]dichlobenil (purity 99.6%) were studied following a single gavage dose of 2.5 mg/kg bw to male and female Sprague-Dawley rats. The compound was suspended in 1% tragacanth gum and administered to 14 rats of each sex. Two rats of each sex were terminated at 1, 3, 6, 24, 72, 168 and 336 hours after dosing, and various tissues were collected for the analysis of radioactivity. Daily excretion was also determined in rats killed after 7 and 14 days.

Table 1. Cumulative excretion of radioactivity in rats 24 and 168 hours after oral or intravenous exposure to [phenyl-U-¹⁴C]dichlobenil

Fraction	Excretion (% of administered radiolabel)				
	0–24 h		0–168 h		
	Single oral intact (M/F)	Single oral cannulated (M)	Intravenous intact (M/F)	Single oral intact (M/F)	Intravenous intact (M/F)
Urine	62 ^a /63 ^a	20	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

F: female; M: male; NC: not collected

^a Including final cage wash.

^b Slight differences may occur between the sums of the individual values and the totals listed because of rounding.

Source: Koorn, Kaldenberg & De Lance (1987)

Table 2. Cumulative excretion of radioactivity in rats 168 hours after oral exposure to [phenyl-U-¹⁴C]dichlobenil

Fraction	Excretion (% of administered radiolabel ± SD)	
	Males (<i>n</i> = 4)	Females (<i>n</i> = 4)
Urine ^a	75.1 ± 4.8	55.8 ± 7.1
Faeces	23.8 ± 10.5	20.6 ± 3.1
Total excreted	98.7 ± 7.4	76.4 ± 7.3

SD: standard deviation

^a Excluding cage wash.

Source: Kaldenberg & de Lange (1988)

The elimination of radioactivity in urine and faeces 7 days after dosing is shown in Table 2. Seven days after dosing, male rats eliminated 75.1% and 23.8% of the administered dose in the urine and faeces, respectively. Excretion was slightly lower in female rats. In females, only 55.8% and 20.6% of the administered dose were excreted in the urine and faeces, respectively.

In all tissues and organs, the highest concentrations of radioactivity were found between 1 and 3 hours after administration (Table 3). No large differences in distribution were observed between males and females for most organs and tissues at most time points, except for plasma and brown fat concentrations at 168 and 336 hours: in males, plasma concentrations were approximately 3 times higher than in females, whereas the reverse was encountered for brown fat.

In conclusion, the results show that dichlobenil is rapidly absorbed and distributed. After the highest concentrations have been reached, the concentrations decline rapidly, showing no potential for accumulation (Kaldenberg & de Lange, 1988).

The elimination of radioactivity following oral administration of [phenyl-U-¹⁴C]dichlobenil (purity > 99%) as single or multiple doses (11 days) at 3.75, 30 or 240 mg/kg bw was studied. The radioactive test material was suspended in 1% tragacanth and administered to five Sprague-Dawley rats of each sex per dose. The radiolabelled dichlobenil was given as a single oral dose on day 1 in one study and on days 1 and 11 in a multiple-dose study, with rats receiving unlabelled test material on days 2–10.

Table 3. Levels of radioactivity in selected organs and tissues after oral administration of a single gavage dose of ^{14}C -labelled dichlobenil in male and female rats at various time intervals post-dosing

	Levels of radioactivity (kBq/g)						
	1 h	3 h	6 h	24 h	72 h	168 h	336 h
Males							
Plasma	0.848	0.315	0.197	0.023	0.014	0.016	0.014
Blood	0.571	0.211	0.124	0.016	0.008	0.006	0.005
Liver	2.866	1.332	0.615	0.158	0.074	0.032	0.008
Kidney	3.332	10.005	0.588	0.056	0.019	0.011	0.006
Brown fat	0.782	0.393	0.103	0.017	0.004	0.019	< 0.004
Kidney fat	3.099	4.412	0.200	0.023	< 0.008	0.051	0.013
Females							
Plasma	0.763	0.323	0.283	n.s.	0.006	0.005	< 0.004
Blood	0.500	0.208	0.186	0.020	0.010	0.008	0.005
Liver	1.792	0.098	1.011	0.194	0.078	0.018	0.012
Kidney	3.413	1.302	1.533	0.087	0.027	0.014	0.010
Brown fat	0.956	0.264	0.312	0.016	0.007	0.054	0.015
Kidney fat	3.277	0.609	0.910	0.208	0.012	0.074	0.019

n.s.: not sampled

Source: Kaldenberg & de Lange (1988)

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

Cumulative excretion is shown in Tables 4 and 5. Total recoveries at the two lowest doses were between 89% and 92%. At the high dose, total recoveries accounted for 77–83% of the administered dose. It appears that there is some saturation occurring at the high dose, but it could be due to low total radioactivity recoveries in the high-dose group.

Tissue levels 9 days after the last of 11 oral doses of dichlobenil in selected tissues were dose dependent (Table 6). The highest residue levels were noted in the liver in animals receiving all doses. There were no apparent sex-related differences in tissue distribution.

In conclusion, no consistent differences appear to exist between the different dose groups or between sexes. Some differences are observed between the amounts of radiolabel recovered from urine in the various groups, but they seem to reflect the lower recovery in those groups (Cameron, Mutch & Scott, 1988).

Table 4. Cumulative excretion of radioactivity in rats 24 hours after the first and last doses of dichlobenil in a repeated oral exposure study

Fraction	Excretion (% of administered radioactivity)					
	First dose: 0–24 h			Last dose: 240–264 h		
	3.75 mg/kg bw per day	30 mg/kg bw per day	240 mg/kg bw per day	3.75 mg/kg bw per day	30 mg/kg bw per day	240 mg/kg bw per day
	M/F	M/F	M/F	M/F	M/F	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

bw: body weight; F: female; M: male

^a Slight differences may occur between the sums of the individual values and the totals listed because of rounding.

Source: Cameron, Mutch & Scott (1988)

Table 5. Distribution and cumulative excretion of radioactivity in rats 0–240 and 240–432 hours after repeated oral exposure to dichlobenil

Fraction	Distribution and excretion (% of administered radiolabel, single dose)					
	First dose: 0–240 h			Last dose: 240–432 h		
	3.75 mg/kg bw per day	30 mg/kg bw per day	240 mg/kg bw per day	3.75 mg/kg bw per day	30 mg/kg bw per day	240 mg/kg bw per day
	M/F	M/F	M/F	M/F	M/F	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/ < 0.01	< 0.01/ < 0.01
Recovery ^a	90/92	89/90	83/77	87/87	86/83	78/72

bw: body weight; F: female; M: male; NC: not collected

^a Slight differences may occur between the sums of the individual values and the totals listed because of rounding.

Source: Cameron, Mutch & Scott (1988)

Table 6. Total radioactivity in liver, kidney, whole blood and plasma of rats (n = 5 of each sex) 9 days after the last of 11 doses of dichlobenil

Organ/tissue	Total radioactivity (mg eq/kg (L))		
	3.75 mg/kg bw per day	30 mg/kg bw per day	240 mg/kg bw per day
	M/F	M/F	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	0.01/0.01	0.20/0.28

bw: body weight; eq: equivalent; F: female; M: male

Source: Cameron, Mutch & Scott (1988)

1.2 Biotransformation

The metabolism of [^{14}C]dichlobenil was studied in Sprague-Dawley rats following single oral and repeated oral doses (11 days) of 3.75, 30 and 240 mg/kg bw. In this study (Cameron, Mutch & Scott, 1988), metabolites from urinary and faecal samples were identified. For metabolite fingerprints, the 0- to 48-hour and 240- to 288-hour urinary samples were pooled separately for males and females at the three dose levels. For faeces, the 0- to 24-hour or 0- to 48-hour and 240- to 264-hour or 240- to 288-hour samples were also pooled. For isolation and identification of metabolites, the 0- to 48-hour and 240- to 288-hour urinary and faecal samples of the highest-dose group were pooled. Radioactivity in urinary and faecal samples was measured using (combustion) liquid scintillation counting. Samples of urine were hydrolysed with β -glucuronidase and arylsulfatase. Metabolites were identified by high-performance liquid chromatographic analysis, nuclear magnetic resonance, mass spectrometry, gas chromatography/mass spectrometry and infrared spectroscopy.

This study revealed that the radioactive compound was absorbed to a significant extent following the first of 11 daily oral doses of dichlobenil. An average of more than 60% of the administered radioactivity was recovered in the urine of rats from the three dose groups. Excretion via faeces accounted for less than 25% of the administered radioactivity (at all dose levels) following the first dose of dichlobenil.

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

The metabolite patterns in urine and faeces after the last dose of the repeated-dose study are presented in Table 7. Ten metabolites were identified in the urine, and five metabolites in the faeces. No parent compound was detected in the urine. At the high dose, a significant quantity of parent compound was identified (93% male and female combined) in faeces (Cameron, Mutch & Scott, 1988).

From the chemical structures of the metabolites, two metabolic pathways are involved in the degradation of dichlobenil in rats following oral gavage administration (Fig. 2). One pathway includes hydroxylation at the 3 or 4 position, followed by glucuronidation or sulfation, and the second pathway includes substitution of one chlorine atom by glutathione. The contribution of glutathione-conjugated metabolites decreases with increasing doses, indicating saturation of the metabolic pathway (Vincent et al., 1989).

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies with dichlobenil (including skin and eye irritation and dermal sensitization studies) are summarized in Table 8.

(a) Oral administration

Rats

Five male and five female fasted Wistar albino rats were dosed orally, by gavage, with dichlobenil (purity 99.8%) ground and mixed with corn oil (10 g/40 mL) at a single dose of 2000 mg/kg bw and observed 1, 2 and 4 hours post-dosing and once daily thereafter for mortality and clinical signs. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

Nine of 10 animals survived the 2000 mg/kg bw dose level. One male was terminated on day 2 due to its moribund condition. Clinical signs in the surviving animals included lethargy, chromorhinorrhoea, ataxia, sagging eyelids, soiling of the anogenital area, diarrhoea, red staining of the nose/mouth, wetness of the anogenital area and localized alopecia. Piloerection, flaccidity and

Table 7. Metabolite patterns in rats 192 hours after the last dose following repeated oral exposure to dichlobenil

Metabolite	% of total radioactivity recovered from respective excretion product					
	Urine			Faeces		
	3.75 mg/kg bw per day	30 mg/kg bw per day	240 mg/kg bw per day	3.75 mg/kg bw per day	30 mg/kg bw per day	240 mg/kg bw per day
	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 ^a
L	–	–	–	–	–	< 3 ^a
Dichlobenil (parent compound)	–	–	–	6/4	60/62	93 ^a
Total	93/93	93/96	91/93	67/42	78/89	98 ^a

bw: body weight; F: female; M: male

^a Male and female fractions combined.

Source: Vincent et al. (1989)

coma were the other physical signs noted in the animal that was terminated early. Body weight changes in the surviving animals were normal. Necropsy of the survivors was normal in 2/9 animals. Kidney abnormalities were noted in 7/9 survivors. Necropsy of the early terminated animal revealed abnormalities of the intestines, as well as red staining of the nose/mouth area.

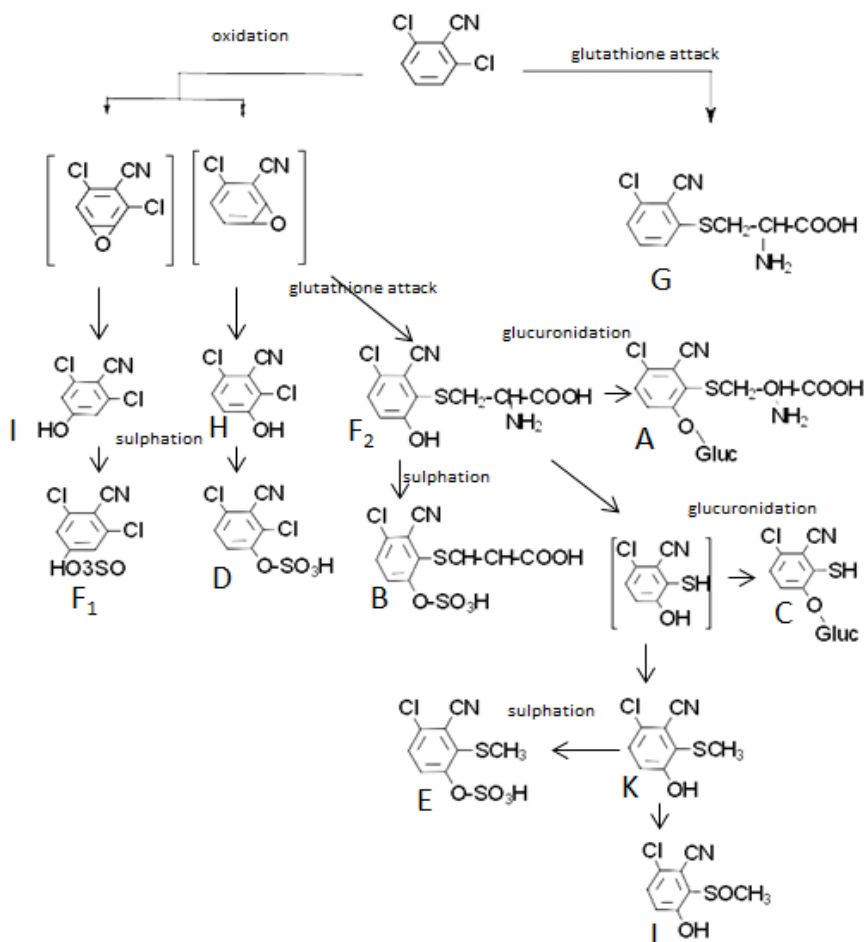
Based on these results, the acute oral median lethal dose (LD₅₀) of dichlobenil was greater than 2000 mg/kg bw (Graver, 1999).

(b) Dermal application

Rabbits

Five female New Zealand White rabbits were dosed dermally with dichlobenil (purity 100%) at a dose of 2000 mg/kg bw. The compound was applied to the prepared site at a dose of 2000 mg/kg bw under a four-layered surgical gauze patch measuring 10 cm × 15 cm. The torso was wrapped with plastic, which was secured with non-irritating tape to keep the test article in contact with the skin for 24 hours, at which time the wrappings were removed and the application sites were cleaned and evaluated according to the Draize method (24 hours, 7 and 14 days). Animals were observed for mortality and clinical signs 1, 2 and 4 hours post-dosing and once daily thereafter for 14 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

Fig. 2. Proposed metabolic pathway in rats



No deaths or clinical signs of an adverse reaction to treatment occurred. Dermal reactions were absent to slight (erythema in one rabbit) at 24 hours and absent on days 7 and 14. Body weight changes were normal. Necropsy results were normal.

Based on these results, the acute dermal LD₅₀ of dichlobenil was greater than 2000 mg/kg bw in female rabbits (Cerven, 2000).

In a separate study, five male and five female New Zealand White rabbits were dosed dermally with dichlobenil (purity 98.8%) at 2000 mg/kg bw based on the results of a preliminary study in two rabbits. The application site was moistened with water, and the dichlobenil was applied to an area of 12 cm × 14 cm. The application site was covered by an occlusive dressing for 24 hours. After a 24-hour exposure period, the application site was cleaned and evaluated according to the Draize method.

Two female rabbits were found dead on day 5. Antemortem signs comprised underactivity, pallor and cold to touch, pigmented staining of the snout and closed eyes. No systemic or local sign of reaction was observed (except one female, which showed pallor on day 4) in the surviving animals. Body weights of all treated rabbits achieved expected gains. Necropsy of the decedents revealed blood staining around the mouth and nares, dark tracheal contents and dark areas on the left lung lobe in one animal, and dark fluid in the thorax and abdomen of the other animal. 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.

Table 8. Acute toxicity of dichlobenil

Species	Strain	Sex	Route	Purity; vehicle	Result	Reference
Rat	Wistar	M + F	Oral	99.8%; corn oil	LD ₅₀ > 2 000 mg/kg bw	Graver (1999)
Rabbit	New Zealand White	F	Dermal	100%	LD ₅₀ > 2 000 mg/kg bw	Cerven (2000)
Rabbit	New Zealand White	M + F	Dermal	98.8%	LD ₅₀ > 2 000 mg/kg bw	Johnson (1994)
Rat	CD (Sprague-Dawley derived)	M + F	Inhalation (whole body)	–	LC ₅₀ (4 h) > 3.2 mg/L	Cracknell (1994)
Rat	Wistar	F	Inhalation	–	Not a respiratory irritant at 0.372 mg/L	Janssen (1986)
Rat	Wistar	M + F	Inhalation	99.8%	LC ₅₀ (4 h) > 2.5 mg/L	Janssen & de Rooy (1985)
Rabbit	New Zealand White	M	Skin irritation	98.8%; 1% tragacanth	Not irritating	Koopman (1985)
Rabbit	New Zealand White	M	Eye irritation	98.8%	Not irritating	Koopman (1983)
Guinea-pig	Dunkin Hartley	F	Skin sensitization (Magnusson-Kligman)	99.4%; paraffin oil	Not sensitizing	Cuthbert & Carr (1987)

bw: body weight; F: female; LC₅₀: median lethal concentration; LD₅₀: median lethal dose; M: male

Based on these results, the acute dermal LD₅₀ of dichlobenil was greater than 2000 mg/kg bw in rabbits (Johnson, 1994).

(c) *Exposure by inhalation*

Rats

Five male and five female CD (Sprague-Dawley) rats were exposed in a chamber to an atmosphere containing a maximum technically achievable concentration of 3.21 mg/L of dichlobenil (purity not reported). The mass median aerodynamic diameter (MMAD) of dichlobenil technical was greater than 5.5 µm, with a geometric standard deviation (GSD) of 13.8 ± 2.1. The animals were subjected to a single 4-hour, continuous, snout-only exposure. The animals were observed for 14 days post-treatment. Body weights were recorded on day 0 and weekly thereafter. All animals were subjected to necropsy and postmortem examination.

No deaths occurred during the exposure or observation period. During the exposure, slow and shallow respiration was seen for all animals; wet and soiled fur was recorded for one male and two females. During the 2 hours immediately following the exposure, underactivity, piloerection, hunched posture, staggering gait and wet fur were seen for all animals. Subsequently, all animals were normal in appearance and behaviour. Body weight losses were evident for four males on the day following exposure, but their weight gains were normal thereafter. Body weight gains for the remaining male and for all females were unaffected by treatment. There were no macroscopic findings at necropsy, and lung, liver and kidney weights were unaffected.

Based on these results, the acute median lethal concentration (4-hour; LC₅₀) was estimated to be greater than 3.21 mg/L air (Cracknell, 1994).

In a separate study, the respiratory irritation of dichlobenil (purity not reported) was evaluated in three male Wistar rats. The respiratory movements of the rats were recorded before, during and after a nose-only exposure to an aerosol of dichlobenil. From these recordings, the respiratory frequencies were calculated and expressed as a percentage of the value before exposure. It was concluded that an airborne concentration of 0.372 mg/L (time not given) has no irritating action on the respiratory tract of Wistar rats (Janssen, 1986).

In a third study, a group of Wistar rats (five of each sex) were exposed nose-only (aerosol) to an atmosphere containing dichlobenil (purity 98.8%) for 4 hours. Group I and group II (two of each sex) were exposed to dichlobenil in acetone, and group III (one of each sex) was exposed to acetone only. In the highest-dose group, dichlobenil levels between 2.5 and 3.25 mg/L were measured. The test substance was administered to the animals as an aerosol. The MMAD and GSD were determined twice. The MMAD values for group I were 2.57 and 2.61 μm , and the GSD values were 3.13 and 3.14. In group II, the MMAD values were 2.11 and 2.00 μm , and the GSD values were 2.89 and 2.90. The post-exposure observation period was 14 days.

Decreased respiratory frequency and temporarily decreased reactivity and locomotor activity were observed in the test groups, indicating an irritating action of the test atmosphere on the respiratory system and suggesting a slight action on the nervous system when compared with control rats. No effects on body weight were seen following exposure. No mortalities occurred during the 14-day observation period. There were no macroscopic findings at necropsy.

Based on these results, the acute median lethal concentration (4-hour; LC_{50}) was estimated to be greater than 2.5 mg/L in rats (Janssen & de Rooy, 1985).

(d) *Dermal irritation*

In a study of primary dermal irritation, three male New Zealand White rabbits were dermally exposed to 0.5 g of dichlobenil (purity 98.8%) slightly moistened with 1% tragacanth suspension and placed onto the shorn skin on the back of each rabbit under a 6 cm^2 patch of aluminium foil secured in position with surgical adhesive tape. The test material was in contact with the skin for 4 hours. After removal of the patch, the treated application site was wiped, and the dermal irritation was scored at 30–60 minutes and at 24, 48 and 72 hours after patch removal using the Draize method. The animals were observed for 14 days post-treatment.

There was no erythema or oedema at any time point.

Based on the results of this study, dichlobenil was not irritating to the skin of rabbits (Koopman, 1985).

(e) *Ocular irritation*

Rabbits

In a primary eye irritation study, a sample of 100 mg of dichlobenil (purity 98.8%) was instilled into the left eye of three male New Zealand White rabbits. The eyes were not washed. The ocular reactions were evaluated at 24, 48 and 72 hours and on day 7.

No effects were seen on cornea, iris or conjunctiva during the 7 days of observation.

Based on the results of this study, dichlobenil is not irritating to the eyes of rabbits (Koopman, 1983).

(f) *Dermal sensitization*

Guinea-pigs

The skin sensitization potential of dichlobenil (purity 99.4%) was investigated in 40 female Dunkin Hartley guinea-pigs using the maximization test. The test group and controls consisted of 20 guinea-pigs each. For the intradermal phase of induction, 2% (w/v) dichlobenil in paraffin oil was

selected. The control animals received the same treatment, but with paraffin oil replacing the test article. Twenty-four hours after injection, the dose sites were scored for irritation. Six days after the intradermal injection, all the test and control sites were wetted with 10% aqueous sodium lauryl sulfate to provoke a mild inflammatory response. On day 7, the test group received 10% (w/v) test substance in paraffin oil, and the control group received paraffin oil via topical application during the second phase of induction. Twenty-four hours after patch removal, the treated sites of both test and control groups were assessed for irritancy. Two weeks after the topical induction, animals (both test and control) were challenged with the test article at 25% (w/v) and with the vehicle (paraffin oil) by epidermal application. The application sites were assessed at 24 and 48 hours after removal of the test item and scored for severity of reaction.

The test animals exhibited slight to moderate reactions after the induction phase. The control animals had a slight reaction. None of the animals showed a positive reaction to the treatment after the challenge phase.

Under the conditions of the guinea-pig maximization study, there was no indication of delayed contact hypersensitivity induced by dichlobenil (Cuthbert & Carr, 1987).

2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

In a 90-day oral toxicity study, dichlobenil (purity 99.4%) was administered to Charles River CD-1 mice (10 of each sex per dose group) in the diet at a concentration of 25, 125, 625 or 3125 parts per million (ppm) (equal to 3.8, 19, 91 and 447 mg/kg bw per day for males and 4.8, 24, 114 and 512 mg/kg bw per day for females, respectively). The control group consisted of 20 mice of each sex. Stability, homogeneity and dietary concentrations were measured periodically. Treated animals were inspected twice daily on weekdays and once daily during weekends. Body weight, feed consumption and water consumption were recorded weekly. Ophthalmoscopic examination was conducted at the beginning and end of the study for controls and high-dose animals. Blood was collected at the end of the study from all animals. Some neurological evaluations (e.g. motor activity) were recorded as part of the clinical examinations.

Dietary concentrations were 96–107% of the nominal concentrations. Diets were stable and homogeneously distributed.

No treatment-related effects were observed on mortality, ophthalmoscopic examination, haematology or gross pathology.

During the first 2 weeks of treatment, the following clinical signs (slight) were present at 3125 ppm: reduced locomotor activity (females only), hunched posture, piloerection and ptosis. The onset of clinical signs was not reported. At 3125 ppm, body weight gains were significantly decreased ($P < 0.05$) by 80% in the males compared with controls during week 1 and by 56% during week 4. At termination, the body weight gains in males had returned to normal. In the females at 3125 ppm, body weight gains were decreased by 58% ($P < 0.05$) during week 1 and by 52% in weeks 9–13. There were no treatment-related differences in overall (weeks 1–13) body weight gains. The decreases in the body weight gains at week 1 were not considered adverse, as the changes in body weight gains were minor and occurred together with significant reductions in feed consumption. Feed consumption at 3125 ppm was significantly ($P < 0.01$) decreased by 10–19% in the males and by 14–26% in the females during the first 4 weeks of treatment. Additionally in the females at this dose, cumulative feed consumption was decreased by 14% ($P < 0.01$) compared with controls for weeks 5–8 and weeks 9–13. The decrease in overall feed consumption for weeks 1–4 was coincident with early study decreases in body weight gain. In the 3125 ppm females, water consumption was increased ($P < 0.01$) by 58% during week 1 and by 23% during week 2, resulting in an increase of 21% ($P < 0.01$) over controls for weeks 1–4.

At 625 ppm, feed consumption was decreased ($P < 0.05$) by 8% in the males during week 2 and by 10% in the females during weeks 2 and 4 compared with controls. Cumulative feed consumption in these animals was decreased ($P < 0.05$) by 8% for weeks 1–4 and by 7% for weeks 5–8. Glucose was decreased ($P < 0.05$) in the 625 ppm (16%) and 3125 ppm (10%) males. Treatment-related effects on the liver were observed. At 3125 ppm, increases ($P < 0.01$) were observed in cholesterol in the males (50%) and females (43%) and in phospholipids in the males (46%) and females (40%). Significant ($P < 0.01$) increases in the absolute and relative (to body weight) liver weights were observed in males (17–20%) and females (18–23%) at 3125 ppm. The severity of centrilobular swelling of parenchymal cells was increased at 625 and 3125 ppm in both sexes. Glycogen storage (either diffusely throughout the liver or in isolated single parenchymal cells) was increased in incidence and/or severity in both sexes at 3125 ppm.

In the kidneys, the severity of pyelitis was increased in the 625 and 3125 ppm males; however, the incidence of this finding did not increase in a dose-dependent manner. Additionally in the 3125 ppm males, the incidence of marginal to slight interstitial nephritis was increased over controls.

The only findings at 625 ppm were minor, transient decreases in feed consumption in both sexes, decreased glucose in males and minor increases in severity of centrilobular hepatocyte swelling in both sexes and pyelitis in the kidneys in males. However, the incidences of these microscopic findings were not dose dependent. At this dose, the incidences of centrilobular hypertrophy were not accompanied by increased liver weights or changes in clinical chemistry. Therefore, these changes are likely an adaptive response to the test material and are not considered adverse at this dose level.

The nose (a potential target organ) was not examined histopathologically in this study.

The no-observed-adverse-effect level (NOAEL) was 625 ppm (equal to 91 mg/kg bw per day), based on transient clinical signs of toxicity, decreased body weight gains, decreased feed consumption and liver toxicity (increased liver weights, clinical chemistry, severity of centrilobular hypertrophy and glycogen storage) in both sexes at 3125 ppm (equal to 447 mg/kg bw per day) (Kemp, de Haan & Dawes, 1987).

Hamsters

In a 90-day oral toxicity study, dichlobenil (purity 98.5%) was administered to 10 F1D Alexander Syrian hamsters (supplier Bio Breeders Inc., USA) of each sex per dose in the diet at a concentration of 41, 209, 1289 or 7500/4648 ppm (equivalent to 3, 16, 79 and 395/263 mg/kg bw per day, respectively). Twenty animals of each sex were tested as a control group. Ten hamsters of each sex from the control and high-dose groups (7500/4648 ppm) were kept on study for an additional 4-week recovery period, during which the animals received diet without dichlobenil. As a result of excessive weight loss (13–25%) in both sexes after 2 weeks at 7500 ppm, all high-dose animals received 4648 ppm dichlobenil from week 3 until week 13. Animals were inspected twice daily for signs of toxicity and mortality on weekdays and once on weekends. Animals were weighed weekly. Feed consumption was recorded daily. Ophthalmological examination was performed at study initiation and during week 11. Blood was collected before the treatment and at termination on week 13 or week 17 for haematology and clinical chemistry measurements. All animals were subjected to gross pathological examination. Selected organs were weighed, and tissues were examined histopathologically.

There were no treatment-related effects on survival, and no adverse clinical signs or ophthalmological changes were observed. Body weight gains in high-dose males did not rebound to control levels until week 10; however, mean body weights in 4648 ppm males remained decreased (21%) at the end of treatment. Mean feed consumption was decreased ($\geq 11\%$) in 4648 ppm males after dose lowering. Mean serum cholesterol and phospholipids were increased by 55% each ($P < 0.01$) in females at 4648 ppm. Concentrations of these clinical chemistry parameters, body weight gain and feed consumption returned to control levels during the recovery period. Other changes in haematological or clinical chemistry parameters were slight and not considered toxicologically

significant. Mean absolute liver weights were increased by at least 13% ($P < 0.01$) in males at and above 1289 ppm and by 16% ($P < 0.01$), 34% ($P < 0.01$) and 85% ($P < 0.01$) in females at 209, 1289 and 4648 ppm, respectively. Mean absolute prostate weight was reduced by at least 19% ($P < 0.05$) at and above 209 ppm, whereas mean absolute seminal vesicle and testicular weights were reduced by 16% or greater at 209, 1289 and 4648 ppm. Statistically significant changes in other mean absolute and/or relative organ weights (e.g. kidney, spleen, brain and uterus) were observed at 4648 ppm; however, in the absence of histopathological correlates, these changes were not considered toxicologically significant.

At necropsy, livers of 5/10 ($P < 0.01$) males were enlarged at 4648 ppm, whereas 5/10 or 10/10 ($P < 0.01$) females had enlarged livers at 1289 or 4648 ppm, respectively. Rough surface of the liver was also noted for 3/10 and 8/10 ($P < 0.01$) females at 1289 or 4648 ppm, respectively. Black grit was found in the gallbladders of 2/10 ($P < 0.01$) females at 4648 ppm at the end of the treatment. Control incidences for each of these hepatobiliary observations were 0/20. At 4648 ppm, small testis was observed in 3/9 males ($P < 0.01$), whereas small epididymis was seen in 2/9 males ($P < 0.01$); the control incidence for each of these observations was 0/20. Slight haemorrhagic area in the epididymis and seminal vesicle was also observed in 1/9 males (versus 0/20 controls) at 4648 ppm. Bile granules in the gallbladder were observed in 2/10 males and 3/10 females in the high-dose recovery group (versus 0/10 and 1/10, respectively, in controls).

Histopathological observation revealed a marked decrease in the number of spermatocytes at the end of treatment in 5/9 males at 4648 ppm (versus 1/20 in controls). Marginal to moderate prostatic mineralization was observed in 3/10, 4/10 or 3/9 males at 209, 1289 and 4648 ppm, respectively, after 13 weeks (versus 1/20 in controls). Marginal to marked tubular degeneration in the testes was observed in 7/9 ($P < 0.01$) males at 4648 ppm (versus 2/20 in controls). Calculi in the gallbladder were observed at the end of treatment in 7/10 males ($P < 0.01$) and 10/10 ($P < 0.01$) females at 4648 ppm (versus 0/20 in each control group). Swollen hepatocytes were noted in 2/9 males at 4648 ppm and in 2/10 females at 209 ppm (marginal), 4/10 females at 1289 ppm (marginal to slight; $P < 0.01$) and 4/10 females at 4648 ppm (marginal to moderate; $P < 0.01$). Control incidences for swollen hepatocytes were 1/20 for males and 0/20 for females.

The nose (a potential target organ) was not examined histopathologically in this study.

In conclusion, the NOAEL was 41 ppm (equivalent to 3 mg/kg bw per day), based on decreased weight and mineralization of the prostate and decreased mean absolute seminal vesicle and testicular weights in males at 209 ppm (equivalent to 16 mg/kg bw per day) (Kemp, de Haan & Jager, 1987).

Rats

In a 90-day oral toxicity study, dichlobenil (purity 90.5%) was administered in the diet to albino Rochester Wistar rats (12 of each sex per dose) at a concentration of 0, 100, 1000 or 3000 ppm (equivalent to 0, 10, 100 and 300 mg/kg bw per day, respectively) for 3 months. An additional six males were treated with 10 000 ppm (equivalent to 1000 mg/kg bw per day) for 3 months. Histopathological examination was performed on major organs, and five animals per group were examined.

Five (of six) males treated with 10 000 ppm dichlobenil died by week 7 of the study (causes undetermined). Mortality, due to either respiratory infection or "other causes", was evenly distributed across males in all other groups (2/12, 2/12, 1/12 and 2/12 at 0, 100, 1000 and 3000 ppm, respectively); 2/12 females died on study at 1000 ppm only. Mean body weights were decreased in males ($\geq 14\%$) by the end of the study at and above 3000 ppm. There were no treatment-related effects on urinary protein or glucose up to 3000 ppm. In addition, no adverse effects on haematological parameters were observed. There was an increase in absolute kidney weights at 3000 ppm. Mean absolute liver weights were increased in males (39%) and females (52%) at 3000 ppm. The effect was dose dependent and is considered adverse, as granular swelling and fine foamy vacuolation of the hepatocytes were observed in all rats of each sex at 3000 ppm and in all males at 10 000 ppm. Necrosis was observed in only one 3000 ppm group male. An inflammatory, necrotic

focus was also observed in the liver of one male at 1000 ppm, whereas vacuolar swelling was observed in a second animal at the same dose.

The nose (a potential target organ) was not examined histopathologically in this study. Histopathological results were not reported for all animals, clinical chemistry analysis was not performed, there was a lack of initial haematological measurements, feed consumption was not measured and there was a lack of analysis of test substance concentration, stability and homogeneity. These deficiencies would not be expected to alter the conclusions of this study.

The NOAEL was 100 ppm (equivalent to 10 mg/kg bw per day), based on hepatocytic inflammation and necrosis at 1000 ppm (equivalent to 100 mg/kg bw per day) in males (Hodge, 1961).

Dogs

In a 90-day oral toxicity study, dichlobenil (purity 95.5%) was administered in the diet to two Beagle dogs of each sex per dose group at a concentration of 0, 50, 150 or 450 ppm (equivalent to 0, 1.3, 3.8 and 11 mg/kg bw per day) for at least 90 days; the 450 ppm dose group consisted of three males and one female.

No adverse effects of treatment were observed on mortality, clinical signs, body weights, body weight gains, feed consumption, haematology, urine analysis or gross pathology. Liver effects were observed at 450 ppm. Alkaline phosphatase, alanine aminotransferase (ALT), kidney weights and liver weights were increased at 450 ppm. The study authors reported a finely foamy appearance of the liver cell cytoplasm that was attributed to vacuoles resulting from glycogen storage and concluded that the observed glycogen storage in treated dogs was unrelated to the test substance, because only mild glycogenic infiltration of the hepatic parenchymal cells was observed in dogs in the 450 ppm dose.

The nose (a potential target organ) was not examined histopathologically in this study.

The NOAEL was 150 ppm (equivalent to 3.8 mg/kg bw per day), based on increased alkaline phosphatase, ALT, and liver and kidney weights at 450 ppm (equivalent to 11 mg/kg bw per day) (Til, Feron & de Groot, 1967).

In a 52-week toxicity study, dichlobenil (purity 98.8%) was administered by capsule to five Beagle dogs of each sex per dose at a dose of 0, 1, 6 or 36 mg/kg bw per day. Animals were observed twice daily for morbidity and mortality. Animals were observed for adverse reactions daily. Detailed clinical examinations were conducted on all animals before the initiation of treatment and during weeks 26 and 52. Individual body weights were recorded at least once weekly. Individual feed consumption and water consumption were measured daily up to week 16 of the treatment period, then daily during weeks 20, 24, 28, 32, 36, 40, 44, 48 and 52. Eyes were examined by ophthalmoscopy and fundus photography prior to the initiation of treatment (week -1) and during week 52. Blood was collected from the jugular vein during weeks -1, 26/27 and 52/53. Urine was collected from fasted animals during weeks -1, 26/27 and 52/53. All animals were killed and necropsied after 52 weeks of treatment; selected organs were weighed, and tissue samples were fixed and preserved at necropsy. Selected tissues from all dogs killed post-study were examined histopathologically.

All animals survived to the scheduled termination date. No treatment-related clinical signs of toxicity were observed in any dogs, and no compound-related effects were noted on feed or water consumption, ophthalmoscopic examination, urine analysis or gross pathology. Lower mean body weights (12–16%) and body weight gains (36–44%; $P < 0.05$) were observed throughout the study in males at 36 mg/kg bw per day (relative to controls), whereas no biologically significant changes in body weight or body weight gain were observed in females. Haemoglobin, haematocrit and erythrocyte counts during treatment were slightly decreased in males at and above 6 mg/kg bw per day at weeks 26 and 52. However, as the magnitudes of change (6–12%), although statistically significant, were slight and as haemosiderin was observed in the spleen without dose dependence at

and above 6 mg/kg bw per day, the observed parameters were not considered toxicologically significant. Statistically or biologically significant changes in these same haematological parameters were not observed in females. Increased serum cholesterol, triglyceride, phospholipid and alkaline phosphatase levels were observed in males and females at and above 6 mg/kg bw per day (Table 9). Gamma-glutamyltransferase was also increased in males only. Increased ($\geq 18\%$) absolute and relative liver weights were observed in mid- and high-dose males and females (Table 10). This effect corresponded to periportal hepatocytic hypertrophy in males at and above 6 mg/kg bw per day and at 36 mg/kg bw per day in females. Decreased (78%; $P < 0.05$) uterine weights were also observed in females at 36 mg/kg bw per day. This effect was accompanied by delayed maturation of the uterus at this dose. Other changes in absolute or relative organ weights were not considered treatment related, as the effects were not dose related or were not accompanied by histopathological alterations. No adverse effects were observed in the nasal cavity of treated or control animals at termination.

In conclusion, the NOAEL was 1 mg/kg bw per day, based on increased liver weights and increased serum cholesterol, triglycerides, phospholipids and alkaline phosphatase in males and females and increased serum gamma-glutamyltransferase and periportal hypertrophy of the hepatocytes in males at 6 mg/kg bw per day (Pickersgill, 1995).

In a 2-year toxicity study, dichlobenil (purity 95.5%) was administered in the diet to Beagle dogs (four of each sex per dose) at a concentration of 0, 20, 50 or 350 ppm (equivalent to 0, 0.5, 1.3 and 8.8 mg/kg bw per day, respectively). Body weights were recorded weekly for 16 weeks and then every other week. Feed consumption was measured weekly. Blood was collected before treatment and at 13, 26, 52, 78 and 102 weeks for haematological and clinical chemistry measurements. Urine was collected before treatment and at 12, 26, 78 and 102 weeks. All animals were terminated, and a gross pathological examination was conducted. Selected organs were weighed and examined microscopically.

No animals died during the study, and there were no treatment-related clinical signs of toxicity. No adverse effects were observed on body weight, feed consumption or haematological parameters or at necropsy.

At 350 ppm, mean concentrations of serum alkaline phosphatase were increased 2–3 times over controls in males and females (Table 11). Mean ALT levels in females were 2 times higher than in controls at 350 ppm during the second half of the study, whereas mean ALT concentrations in males were decreased at this same dose and time period. An increase in the number of erythrocytes (moderate to high) in the urine was observed in 3/4 high-dose females at 350 ppm at week 102 only. Mean absolute and relative liver weights were increased by 35% and 28% in males and by 67% and 46% in females at 350 ppm (Table 11). Increases in mean absolute kidney weight (10% in males; 24% in females) and thyroid weight (57% in males; 111% in females) were not considered toxicologically significant at the high dose in the absence of histopathological correlates and also because mean body weight was increased by 45% and 86% in males and females, respectively, at this dose. In addition, no difference in mean relative kidney weight was observed in any treatment group, relative to controls. Mean absolute and/or relative glucose-6-phosphatase and glucose-6-phosphate dehydrogenase activities were increased in the liver at 350 ppm, thereby reflecting the increased metabolic activity of hepatocytes at this dose (Table 11). Moderately severe leukocytic infiltration around the central veins of the liver was observed in 2/4 males and 3/4 females at 350 ppm. “One or a few tiny foci of necrosis” were also observed in 3/4 males at 350 ppm (versus 1/4 controls).

The nose (a potential target organ) was not examined histopathologically in this study.

The NOAEL was 50 ppm (equivalent to 1.3 mg/kg bw per day), based on increased liver weight (both sexes), serum alkaline phosphatase (both sexes) and serum ALT (females only); liver histopathology (leukocytic infiltration around the central veins [both sexes] and necrosis [males only]); and an increase in the number of erythrocytes in the urine (females only) at 350 ppm (equivalent to 8.8 mg/kg bw per day) (Til et al., 1969).

Table 9. Selected clinical chemistry changes in male and female dogs (52-week study)

Parameter	Week	0 mg/kg bw per day	1 mg/kg bw per day	6 mg/kg bw per day	36 mg/kg bw per day
Males					
Cholesterol (g/L)	–1	1.71 ± 0.60	1.72 ± 0.12	1.58 ± 0.19	1.56 ± 0.27
	26	1.37 ± 0.40	1.65 ± 0.15 (20) ^a	2.05 ± 0.37** (50)	3.00 ± 0.76** (119)
	52	1.23 ± 0.36	1.54 ± 0.17	1.90 ± 0.29** (54)	2.56 ± 0.45** (108)
Triglycerides (g/L)	–1	0.29 ± 0.11	0.40 ± 0.10	0.38 ± 0.06	0.34 ± 0.07
	26	0.33 ± 0.11	0.38 ± 0.11 (15)	0.48 ± 0.13* (45)	1.85 ± 1.56** (461)
	52	0.32 ± 0.07	0.43 ± 0.07* (34)	0.46 ± 0.10* (44)	1.55 ± 0.67** (384)
Phospholipids (g/L)	–1	3.22 ± 0.99	3.25 ± 0.20	2.96 ± 0.28	3.05 ± 0.43
	26	3.01 ± 0.59	3.58 ± 0.43 (19)	3.90 ± 0.31** (30)	5.33 ± 1.17** (77)
	52	2.78 ± 0.76	3.45 ± 0.33* (24)	3.86 ± 0.31** (39)	4.94 ± 0.76** (78)
Alkaline phosphatase (IU/L)	–1	278 ± 43	283 ± 71	269 ± 41	268 ± 67
	26	133 ± 42	156 ± 67	191 ± 34* (44)	575 ± 320** (332)
	52	110 ± 54	129 ± 53	191 ± 50* (74)	525 ± 224** (377)
GGT (IU/L)	–1	3 ± 1	4 ± 1	4 ± 1	4 ± 1
	26	3 ± 2	4 ± 1	4 ± 2	5 ± 1* (67)
	52	0 ± 0	2 ± 2	2 ± 1*	3 ± 2*
Females					
Cholesterol (g/L)	–1	1.36 ± 0.22	1.42 ± 0.36	1.27 ± 0.21	1.40 ± 0.19
	26	1.55 ± 0.36	1.85 ± 0.57	2.27 ± 0.31** (46)	3.06 ± 0.41** (97)
	52	1.72 ± 0.53	1.74 ± 0.70	2.14 ± 0.31 (24)	2.99 ± 0.67** (74)
Triglycerides (g/L)	–1	0.33 ± 0.06	0.32 ± 0.08	0.34 ± 0.11	0.34 ± 0.06
	26	0.45 ± 0.11	0.43 ± 0.12	0.64 ± 0.23 (42)	1.13 ± 0.16** (151)
	52	0.51 ± 0.10	0.52 ± 0.10	0.66 ± 0.12* (29)	1.34 ± 0.35** (163)
Phospholipids (g/L)	–1	2.77 ± 0.33	2.85 ± 0.54	2.65 ± 0.41	2.84 ± 0.44
	26	3.43 ± 0.51	3.80 ± 0.97	4.27 ± 0.41* (24)	5.47 ± 0.44** (59)
	52	3.69 ± 1.07	3.53 ± 0.99	4.36 ± 0.38 (18)	5.39 ± 0.83** (46)
Alkaline phosphatase (IU/L)	–1	253 ± 49	281 ± 41	298 ± 54	293 ± 66
	26	135 ± 65	177 ± 49	174 ± 40 (29)	404 ± 118** (199)
	52	142 ± 78	174 ± 72	178 ± 60 (25)	462 ± 65** (225)

bw: body weight; GGT: gamma-glutamyltransferase; IU: International Units; *: $P < 0.05$; **: $P < 0.01$

^a Numbers in parentheses are per cent change, relative to control (reviewer calculated these values only where there are significant results, to examine dose–response relationships).

Source: Data taken from pp. 121–138 of Pickersgill (1995)

Table 10. Selected absolute and relative organ weights in dogs (52-week study)

Organ	Absolute (g) and relative (% of body weight) organ weights			
	0 mg/kg bw per day	1 mg/kg bw per day	6 mg/kg bw per day	36 mg/kg bw per day
Males				
Terminal body weight	10 380 ± 1 275	10 080 ± 1 199	9 700 ± 480 (7) ^a	8 820 ± 853* (15)
Liver	281.1 ± 30.4	287.6 ± 27.6	332.1 ± 22.4* (18)	476.7 ± 95.9** (70)
% body weight	2.73 ± 0.34	2.87 ± 0.28	3.42 ± 0.13** (25)	5.37 ± 0.66** (97)
Kidneys	45.60 ± 3.60	44.80 ± 4.16	45.47 ± 4.87	55.16 ± 5.22** (21)
% body weight	0.444 ± 0.053	0.446 ± 0.028	0.471 ± 0.064	0.627 ± 0.053** (41)
Thyroids	0.691 ± 0.207	0.695 ± 0.309	0.764 ± 0.179	1.080 ± 0.206* (56)
% body weight	0.006 7 ± 0.002 0	0.007 0 ± 0.003 5	0.007 9 ± 0.001 8	0.012 3 ± 0.002 2** (84)
Testes	13.28 ± 1.75	14.99 ± 3.55	14.77 ± 1.07	17.70 ± 4.85 (33)
% body weight	0.129 ± 0.019	0.149 ± 0.035	0.152 ± 0.010* (18)	0.201 ± 0.052** (56)
Prostate	10.168 ± 4.125	8.755 ± 1.863	7.918 ± 2.468 (22)	6.375 ± 1.070* (37)
% body weight	0.095 3 ± 0.034 8	0.087 1 ± 0.018 4	0.081 7 ± 0.025 0 (14)	0.073 1 ± 0.015 5 (23)
Females				
Terminal body weight	8 660 ± 730	8 860 ± 666	9 620 ± 733	8 040 ± 1 088
Liver	252.9 ± 21.1	300.8 ± 38.6 (19)	378.3 ± 56.5** (50)	391.1 ± 60.7** (55)
% body weight	2.92 ± 0.14	3.41 ± 0.51* (17)	3.92 ± 0.43** (34)	4.86 ± 0.18** (66)
Thyroids	0.560 ± 0.065	0.749 ± 0.104** (34)	0.744 ± 0.113** (33)	0.840 ± 0.084** (50)
% body weight	0.006 5 ± 0.001 1	0.008 5 ± 0.001 5	0.007 8 ± 0.001 6	0.010 6 ± 0.001 5** (63)
Thymus	4.477 ± 0.906	2.702 ± 1.003	4.648 ± 0.661	3.086 ± 0.222*
% body weight	0.051 5 ± 0.008 3	0.030 3 ± 0.011 3*	0.048 4 ± 0.006 5*	0.038 7 ± 0.003 3*
Ovaries	1.340 ± 0.289	1.361 ± 0.665	1.828 ± 0.746	0.676 ± 0.145** (50)
% body weight	0.015 7 ± 0.004 2	0.015 5 ± 0.007 9	0.018 7 ± 0.006 7	0.008 5 ± 0.001 9 (46)
Uterus	15.226 ± 6.506	11.522 ± 8.928	17.280 ± 10.842	3.423 ± 0.734* (78)
% body weight	0.179 9 ± 0.085 5	0.132 1 ± 0.105 6	0.175 7 ± 0.106 7	0.042 7 ± 0.009 2* (76)

bw: body weight; *: $P < 0.05$; **: $P < 0.01$ ^a Numbers in parentheses are per cent change, relative to control (reviewer calculated these values only where there are significant results, to examine dose–response relationships).

Source: Data taken from pp. 316–321 of Pickersgill (1995)

*(b) Dermal application**Rabbits*

In a 21-day dermal toxicity study, dichlobenil technical (purity 98.8%) was administered topically (i.e. spread evenly over the intact skin of the clipped dorsal region of animals in treatment groups, then moistened with water) to New Zealand White rabbits (five of each sex per dose) at a dose

Table 11. Clinical chemistry changes in male and female dogs receiving dichlobenil for 2 years

Week	Males				Females			
	0 ppm	20 ppm	50 ppm	350 ppm	0 ppm	20 ppm	50 ppm	350 ppm
Serum alkaline phosphatase (Bessey-Lowry units)								
0	1.0	1.0	1.0	1.1	0.9	0.9	0.9	0.8
12	1.6	1.9	2.0	3.2	2.1	1.6	1.9	4.1
26	1.6	1.7	2.5	2.5	2.2	1.4	2.0	3.9
52	1.0	0.9	1.4	2.6	1.7	1.2	1.5	3.3
78	1.3	1.7	1.6	3.5	1.6	1.1	1.7	4.1
102	1.2	1.5	1.6	3.3	1.5	1.1	1.7	3.5
Serum ALT (rheumatoid factor units)								
0	12.4	11.4	12.4	12.3	12.5	11.0	15.0	12.8
12	9.8	12.4	12.8	11.9	12.8	11.8	11.9	13.1
26	21.8	19.3	26.0	10.5	16.9	14.4	13.1	21.9
52	16.0	25.9	15.0	11.8	13.1	11.9	12.6	18.4
78	22.0	22.3	17.4	9.5	12.6	11.8	7.4	31.4
102	23.9	16.3	25.0	12.5	16.3	15.0	10.5	41.6
Terminal liver weights ^a								
Absolute	452	416	466	610	368	364	408	615
Relative	3.35	3.12	3.07	4.29	2.96	2.90	3.17	4.32
Terminal kidney weights ^a								
Absolute	73	73	81	80	51	56	62	63
Relative	0.54	0.55	0.53	0.56	0.41	0.45	0.48	0.44
Liver enzyme activity								
G6Pase ^b	30.0	34.5	33.6	42.9	27.6	27.6	29.4	37.1
G6PD ^c	5.1	6.8	7.2	15.1	4.7	4.3	5.6	11.8

ALT: alanine aminotransferase; bw: body weight; G6Pase: glucose-6-phosphatase; G6PD: glucose-6-phosphate dehydrogenase; NADPH: nicotinamide adenine dinucleotide phosphate (reduced); ppm: parts per million

^a Absolute organ weight: g; relative organ weight: g/100 g bw.

^b Units: μmol glucose-6-phosphate hydrolysed per minute per 100 g bw at pH 6.6 and 30 °C.

^c Units: μmol NADPH produced per minute per 100 g bw at pH 7.4 and 30 °C.

Source: Til et al. (1969)

of 0, 100, 300 or 1000 mg/kg bw per day, 6 hours/day, for 3 weeks. At the end of the 6-hour exposure period, the skin application was washed with warm water to remove any residues.

Administration of 100, 300 or 1000 mg/kg bw per day produced no adverse clinical signs or skin irritation. No animals died during the study. During the last week of the study, three rabbits (one low-dose male and female and one mid-dose female) were euthanized because they showed hind limb paralysis. All three rabbits showed haemorrhage of the skeletal muscle in the lumbar region around the lower spinal column, and two rabbits showed fractured lower lumbar vertebrae. These findings were considered to be due to trauma, but the cause of the trauma was not established in the report and may have been due to handling. There were no treatment-related effects on body weight, feed consumption, haematology, blood chemistry, organ weights, or gross or microscopic pathology. An increase in mean serum cholesterol (49%, $P < 0.01$) was observed in high-dose females. In the absence of liver histopathology, this effect was not considered toxicologically significant. The nose (a potential target organ) was not examined histopathologically in this study. In addition, an ophthalmological examination was not performed.

The NOAEL for dermal irritation and systemic toxicity in rabbits was 1000 mg/kg bw per day, the highest dose tested; a lowest-observed-adverse-effect level (LOAEL) could not be determined (Allan, 1995).

(c) *Exposure by inhalation*

In a 7-day inhalation toxicity study, Sprague-Dawley [CrI:CD(SD)IGS BR] rats (five of each sex per concentration) were exposed by nose-only inhalation to dichlobenil (purity 98.1%) as a dust aerosol at an achieved concentration of 0, 21, 77 or 200 mg/m³ for 6 hours/day for 7 days. The study served as a dose-range finding study for the 28-day inhalation study.

There were no treatment-related effects on survival. Decreased general activity was observed in 1/5 males at each of 77 and 200 mg/m³. Difficulty breathing was observed in 1/5 and 2/5 males at 77 and 200 mg/m³, respectively. Mean body weight was decreased in females at 200 mg/m³, compared with controls. Mean feed consumption was decreased in both sexes at 77 and 200 mg/m³, compared with controls. No adverse effects were observed at necropsy. Mean absolute and relative liver weights were increased in both sexes at 77 and 200 mg/m³, respectively, compared with controls. Trace to mild nasal degeneration was observed in at least 1/5 animals of each sex at and above 21 mg/m³ (versus 0/5 animals of each sex in controls). This response was expected, as dichlobenil is an olfactory toxicant.

The LOAEL was 21 mg/m³, the lowest dose tested. A NOAEL was not established in this study (Newton, 2002).

In a 28-day inhalation toxicity study, Sprague-Dawley rats (10 of each sex per concentration) were exposed by nose-only inhalation to dichlobenil (purity 98.1%) as a dust aerosol at a concentration of 0, 2.3, 5.1 or 12 mg/m³ (equivalent to 0, 0.0023, 0.0051 and 0.012 mg/L, respectively) for 6 hours/day, 5 days/week, for 4 weeks. These concentrations were selected based on the results of the 7-day inhalation toxicity study described above.

There were no treatment-related effects on survival, clinical observations, functional observational battery, motor activity, body weights, body weight gains, feed consumption, ophthalmoscopy, haematology, clinical chemistry, organ weights, gross pathology or histopathology in either sex at any concentration.

In conclusion, the NOAEL was 12 mg/m³ (equivalent to 0.012 mg/L), the highest dose tested. A LOAEL was not observed in this study (Newton, 2002).

2.3 *Long-term studies of toxicity and carcinogenicity*

Hamsters

In a carcinogenicity study, dichlobenil (purity 99.4%) was administered in the diet for 91 weeks (males) or 78 weeks (females) to F1D Alexander Syrian hamsters (50 of each sex per dose in the treatment groups and 100 of each sex in the control group) at a concentration of 0, 675, 1500 or

3375 ppm (equal to 0, 51, 117 and 277 mg/kg bw per day for males and 0, 55, 121 and 277 mg/kg bw per day for females, respectively).

No treatment-related mortality or clinical signs were observed. Overall body weight gain was decreased for all treated groups compared with controls. In the 3375 ppm dose group, a large drop in body weight (16% decrease) was seen as early as 2 weeks after the initiation of the study and was sustained throughout the study. No treatment-related effects were noted in eyes during ophthalmoscopic examination. A statistically significant increase in body weight-adjusted liver weights was observed in females in all treatment groups and in mid- and high-dose males at termination. Macroscopic examination revealed enlargement of the liver in both sexes receiving 3375 ppm diet, together with a slightly increased incidence of liver masses in males receiving 3375 ppm. A slight increase in the incidence of pale areas on the liver together with a reduction in adipose tissue were also noted in males in all treated groups. In the high-dose males, an increased incidence of benign liver tumours (6/50 versus 0/100 controls) and one malignant liver tumour were observed. One benign tumour was observed in males at 1500 ppm (Table 12). Eosinophilic hepatocytes were observed in males receiving 1500 ppm diet (not statistically significant) or 3375 ppm diet; minimal centrilobular hepatocyte enlargement was seen in all treated male groups and in females receiving 3375 ppm diet. Hepatitis was observed in both sexes receiving 3375 ppm diet and in females receiving 1500 ppm diet, as were pigmented giant cells in males and females receiving 3375 ppm diet, pigmented sinusoidal cells in males and females receiving 3375 ppm diet and brown pigments in hepatocytes in males receiving 1500 or 3375 ppm diet. The nose (a potential target organ) was not examined histopathologically in this study.

Table 12. Selected liver lesions in carcinogenicity study in hamsters

Observation	Incidence of finding							
	Males				Females			
	0 ppm	675 ppm	1 500 ppm	3 375 ppm	0 ppm	675 ppm	1 500 ppm	3 375 ppm
Microscopic non-neoplastic lesions in liver								
Centrilobular hepatocyte enlargement	7/100	15/50*	10/50*	14/50*	7/100	5/49	5/50	11/50*
Finely vacuolated hepatocytes	1/100	3/50	6/50*	8/50*	0/100	0/49	0/50	0/50
Hepatitis	1/100	1/50	2/50	5/50*	0/100	0/49	3/50*	4/50*
Pigmented giant cells	10/100	4/50	5/50	20/50*	17/100	5/49	11/50	17/50*
Pigmented sinusoidal cells	5/100	1/50	3/50	9/50*	7/100	4/49	2/50	9/50*
Brown pigment in hepatocytes	9/100	7/50	16/50*	19/50*	0/100	0/49	0/50	0/50
Eosinophilic hepatocytes	0/100	0/50	1/50	5/50*	0/100	0/49	0/50	0/50
Liver tumours								
Hepatocellular adenoma	0/100	0/50	1/50	6/50*	0/100	0/49	0/50	0/50
Hepatocellular carcinoma	0/100	0/50	0/50	1/50	0/100	0/49	0/50	0/50

ppm: parts per million; *: $P < 0.05$ (Fisher's exact test)

Source: Chambers et al. (1992b)

In conclusion, a NOAEL could not be identified in this study, based on decreased body weight gain in males and females and increased relative liver weight in females at 675 ppm (equal to 51 mg/kg bw per day), the lowest dose tested. The NOAEL for carcinogenicity in male hamsters was 1500 ppm (equal to 117 mg/kg bw per day), based on an increased incidence of hepatocellular adenoma and carcinoma at 3375 ppm (equal to 277 mg/kg bw per day). No treatment-related tumours were observed in female hamsters (Chambers et al., 1992b).

In a separate carcinogenicity study, dichlobenil (purity 99.4%) was administered in the diet for 88 weeks (males) or 80 weeks (females) to F1D Alexander Syrian hamsters (50 of each sex per dose) at a concentration of 5, 26, 132 or 675 ppm (equal to 0.34, 1.69, 9.39 and 45.6 mg/kg bw per day for males and 0.35, 1.78, 9.20 and 48.9 mg/kg bw per day for females, respectively). Control groups consisted of 100 animals of each sex. The criterion for study termination in males was 50% mortality in controls.

Diets were prepared weekly. The test compound was stable and homogeneously distributed in the diet, and measured concentrations were within an acceptable range.

Mortality in control females at termination was 42%. There were no dose-dependent differences in survival of males or females at termination. Mean body weight was decreased (10–11%) in males at 675 ppm from weeks 80 to 88 only of the study. Mean overall body weight gain was decreased at 675 ppm by 96% ($P < 0.01$) in males from weeks 0 to 88 and by 14% in females from weeks 0 to 80. No differences in feed consumption or feed efficiency were observed across the doses. No adverse effects were observed on differential leukocyte counts or ophthalmological end-points or at necropsy.

Mean relative liver weights were increased in females by 15% ($P < 0.01$) at 675 ppm; however, the effect showed no relationship with dosing. Non-neoplastic histopathological observations are summarized in Table 13. Increased incidences ($P < 0.05$) of reduced secretion of the prostate and seminal vesicles were observed in males at and above 132 ppm, at which the dose–response curve plateaued for both end-points. The dose–response curve for the increased incidence ($P < 0.05$) of hyperplasia of the non-glandular region of the stomach in males also plateaued at and above 26 ppm. The toxicological relevance of this end-point is questionable, however, as humans lack a homologue for the rodent non-glandular stomach. Increased incidences ($P < 0.05$) of hyperplasia of the non-glandular region of the stomach were also observed in females; however, the effect was not dose dependent. Increased incidences of hyperplasia ($P < 0.05$) were also observed at several other sites at 675 ppm, including pancreatic islet cells and skin (acanthosis) in males and adrenal cortex, small intestine and bone marrow (sternum) in females. Hypertrophy of the skin (hyperkeratosis) and hepatocytes were also observed at the high dose in females. The nose (a potential target organ) was not examined histopathologically in this study.

The NOAEL was 26 ppm (equal to 1.69 mg/kg bw per day), based on reduced secretion of the prostate and seminal vesicles in males at 132 ppm (equal to 9.39 mg/kg bw per day) (Hooks et al., 1991a,b).

An additional study was conducted to collect background data on incidences of spontaneously occurring tumours in hamsters of the F1D Alexander Syrian strain, supplied by Bio Breeders Inc., USA. This strain was used in the carcinogenicity studies with dichlobenil by Chambers et al. (1992b) and Hooks et al. (1991a,b). The conditions and procedures used in this study were designed to duplicate those of the two studies. This study included one untreated control group consisting of 50 male and 50 female hamsters monitored from about 6 weeks of age until approximately 50% survival was reached in each group, at which time all surviving animals were euthanized. This occurred after 77 weeks for females and 90 weeks for males. Additional data were also generated on survival, body weight change and feed consumption, with limited haematological and organ weight parameters assessed.

Table 13. Non-neoplastic histopathological observations in hamsters

	Incidence of finding				
	0 ppm	5 ppm	26 ppm	132 ppm	675 ppm
Males					
Reduced secretion of the prostate	5/100	1/21	2/27	5/21*	9/48*
Reduced secretion of the seminal vesicles	9/100	5/34	5/32	7/27*	10/48*
Islet cell hyperplasia of the pancreas	1/99	0/21	1/26	0/20	4/50*
Epithelial hyperplasia of the stomach (non-glandular region)	18/100	6/50	18/50*	22/50*	20/50*
Acanthosis of the skin	1/100	0/21	0/27	0/25	7/50*
Females					
Centrilobular hepatocytes	1/100	1/50	1/50	1/50	6/50*
Cortical hyperplasia of the adrenals	49/99	25/50	23/49	29/49	36/49*
Epithelial hyperplasia of the stomach (non-glandular region)	29/100	30/50*	21/50	19/50	27/50*
Epithelial hyperplasia of the stomach (limiting ridge)	20/100	18/50*	18/50*	13/50	21/50*
Submucosal inflammatory cells in the stomach (non-glandular region)	12/100	12/50	5/50	11/50	17/50*
Peritonitis	1/100	2/50	2/50	1/50	5/50*
Epithelial hyperplasia of the duodenum	13/100	2/30	3/37	2/37	13/49*
Epithelial hyperplasia of the ileum	9/100	0/27	1/31	2/36	11/49*
Epithelial hyperplasia of the caecum	3/100	3/33	1/32	1/37	9/50*
Prominent mucous cells in the caecum	9/100	4/33	2/32	3/37	11/50*
Limiting dilation of the rectum	3/98	0/28	0/32	0/36	8/50*
Hyperkeratosis of the skin	1/100	0/29	0/35	1/36	6/50*
Hyperplasia of the bone marrow in the sternum	33/100	11/28	11/32	11/36	25/50*

ppm: parts per million; *: $P < 0.05$ (Fisher's exact test)

Source: Data adapted from Table 9, pp. 83–148, of Hooks et al. (1991a)

Based on the results of this study, the study authors (Chambers et al., 1992a) concluded that values for all parameters investigated in this study were essentially similar to those of the respective control groups of the previously mentioned carcinogenicity studies in hamsters (Hooks et al., 1991a,b; Chambers et al., 1992b).

Rats

In a combined chronic toxicity and carcinogenicity study, dichlobenil (purity 96.7%) was administered in the diet to Fischer 344 rats (50 of each sex per dose) for 110 weeks at a concentration of 0, 50, 400 or 3200 ppm (equal to 0, 3.2, 29 and 241 mg/kg bw per day for males and 0, 3.2, 26 and 248 mg/kg bw per day for females, respectively). Interim terminations of 10 additional animals of each sex per dose took place at each of 52 and 78 weeks. Diets were prepared weekly, and dietary concentration, stability and homogeneity were evaluated periodically. Animals were inspected daily for clinical signs and mortality. Body weights were measured weekly for 26 weeks and then biweekly. Feed consumption and water consumption were measured weekly. Ophthalmoscopic examination of

eyes was not performed. Blood was collected from 10 rats of each sex per dose at 52, 78 and 110 weeks. Urine was collected at 52, 73 and 110 weeks from 10 animals of each sex per dose. Animals that died and at termination were subjected to gross pathological examination, and selected tissues were collected for histopathological examination. Selected organs were removed and weighed.

Diets were homogeneous and stable for 7 days, and the concentrations were within an acceptable range.

Mortality was 100% in males by week 98 at 3200 ppm. Survival of males at the middle and low doses was similar to that of controls and of females at all doses tested. No treatment-related clinical signs of toxicity were observed. At 3200 ppm, body weight was decreased by 33% ($P < 0.001$) by week 88 in males and by 34% ($P < 0.001$) by week 104 in females. Body weight decrements were proportional to time on study in both sexes. Similarly, mean body weight gain in males at 3200 ppm was decreased by 19% from weeks 1 to 13 and by 160% from weeks 52 to 66. In females, mean body weight gain at 3200 ppm was decreased by 24% from weeks 1 to 13 and by 40% from weeks 52 to 66. No differences were observed in feed consumption when corrected for body weight, whereas feed efficiency was decreased by 35% in males and by 29% in females at 3200 ppm. Water consumption (corrected for body weight) in high-dose animals was increased by 30% and 55% in males and by 22% and 39% in females at weeks 5 and 15, respectively. Urinary volume was also over 2-fold higher in high-dose males at week 78 ($P < 0.01$). No toxicologically significant effects on haematological parameters were observed. Mean blood urea nitrogen (BUN) was 3-fold higher ($P < 0.01$) at week 78 in males treated with 3200 ppm; in males treated with 400 ppm, mean BUN was increased by 75% ($P < 0.05$) at termination (Table 14). Mean serum uric acid levels were also increased by 35% ($P < 0.01$) at 3200 ppm in males. Mean cholesterol in males treated with 3200 ppm was increased by 91% ($P < 0.05$) at week 52 and by 176% ($P < 0.01$) at week 78; in males treated with 400 ppm, mean cholesterol was increased by 136% ($P < 0.01$) at termination. In females treated with 3200 ppm, mean BUN was over 3-fold higher ($P < 0.05$) at week 110. Mean cholesterol in females treated with 3200 ppm was increased by 168% ($P < 0.01$), 157% ($P < 0.01$) and 83% ($P < 0.01$) at weeks 52, 78 and 110, respectively. No treatment-related changes in mean serum uric acid levels were observed in females.

Mean absolute and relative liver weights were increased in males by 48% ($P < 0.01$) and 118% ($P < 0.01$), respectively, after 78 weeks at 3200 ppm (changes at 78 weeks were similar to those at 52 weeks and at termination); however, the increased relative liver weight was likely due to a combination of increased absolute liver weight and decreased body weight (Table 15). In females of the 400 ppm group, mean absolute liver weights were increased by 16% ($P < 0.01$) at 52 and 78 weeks, and relative liver weights were increased by 23% ($P < 0.01$) at 78 and 110 weeks. At 3200 ppm, mean absolute liver weights of females were increased by 50% and above at 52, 78 and 110 weeks, and relative liver weights were increased by 113% and above at 52, 78 and 110 weeks. Absolute kidney weights were increased by 16% ($P < 0.05$) at 78 weeks in both males and females at 3200 ppm, as was the absolute weight of the adrenals in males (22%, $P < 0.01$) at 78 weeks at the same dose. At terminal necropsy, dose-dependent increases in the incidences of enlarged liver and kidneys were observed in males and females. The incidences of enlarged liver in males were 3/50, 9/50, 15/50 and 45/50 at 0, 50, 400 and 3200 ppm, respectively, whereas enlarged liver was observed in 4/50, 3/50, 11/50 and 44/50 females, respectively. The incidence of enlarged kidney was 2/50, 5/50, 8/50 and 40/50 in males and 0/50, 0/50, 2/50 and 13/50 in females at 0, 50, 400 and 3200 ppm, respectively.

Non-neoplastic histopathological observations are summarized in Table 16. Incidences of fatty metamorphosis (the presence of microscopically visible droplets of fat in the cytoplasm of cells) of the liver were increased above controls at and above 50 ppm at week 52 in males and females; however, dose dependence was lacking for the effect in both sexes at week 78. At study termination, increased incidences of fatty metamorphosis (with increased severity) of the liver were clearly observed in males (45/50) and females (39/40) at 3200 ppm. Liver necrosis was increased in males at 3200 ppm at week 52 only and in females at weeks 52 and 78, but without a dose-response relationship. Cytological alterations (polyploidy with hepatocytic swelling) were increased in females at and above 400 ppm at study termination, whereas increased incidences of "hepatic nodules"

(hyperplasia) were observed in males and females at 3200 ppm at study termination. Clear increases in calcification of the kidney were observed at 3200 ppm in both males (24/50) and females (46/47) at study termination, whereas nephrosis (kidney damage) was observed in 100% of males at and above 400 ppm (increased severity at high dose) and in 98% of females at 3200 ppm at study termination. Nephrosis was also increased in both sexes at both interim termination times at and above 400 ppm. Last, parathyroid hyperplasia was increased at study termination only at and above 50 ppm in males and at 3200 ppm in females. Parathyroid hyperplasia was likely a response to the reported kidney damage (nephrosis), to maintain normal serum calcium concentrations. The nose (a potential target organ) was not examined histopathologically in this study.

There was an increased incidence of hepatocellular tumours at the high dose in both sexes (Table 17). In female rats, there were statistically significant increases in adenomas and combined adenomas/carcinomas by pairwise comparison. There were statistically significant positive dose-related trends for adenomas and carcinomas, alone and combined in both sexes. In male rats, there were no statistically significant increases in hepatocellular tumours at any dose by pairwise comparison.

The NOAEL for systemic toxicity was 50 ppm (equal to 3.2 mg/kg bw per day), based on changes in clinical chemistry (increased BUN, cholesterol), gross pathology (enlarged liver, enlarged kidney) and histopathology (nephrosis, parathyroid hyperplasia) in males; and increased liver weight, enlarged liver and cytological alterations (polyploidy with hepatocytic swelling) in the liver in females at 400 ppm (equivalent to 26 mg/kg bw per day). The NOAEL for carcinogenicity was 400 ppm (equivalent to 26 mg/kg bw per day), based on an increased incidence of hepatocellular tumours at 3200 ppm in both sexes, reaching statistical significance only in females (Inoue & Enomoto, 1984).

Table 14. Selected clinical chemistry values in a chronic toxicity and carcinogenicity study in rats

Week	Males				Females			
	0 ppm	50 ppm	400 ppm	3 200 ppm	0 ppm	50 ppm	400 ppm	3 200 ppm
Phosphorus (mg/dL)								
52	4.7	4.5	4.5	5.3*	3.9	3.5	4.2	4.3
78	5.2	5.0	5.3	7.9*	4.2	4.5	4.7	5.2*
110	5.5	5.0	5.8	—	5.1	6.0	4.8	8.6
BUN (mg/dL)								
52	19	17	18	25	20	20	18	18
78	19	20	22	59**	20	19	18	26*
110	20	30	35*	—	20	21	18	64*
Creatine (mg/dL)								
52	0.60	0.58	0.54*	0.52**	0.60	0.58	0.53	0.58
78	0.68	0.69	0.71	0.84	0.62	0.60	0.57**	0.63
110	0.60	0.58	0.85*	—	0.61	0.61	0.52**	0.83
Cholesterol (mg/dL)								
52	101	108	143*	193*	120	118	147	321**
78	129	141	177	357**	141	147	185	362**
110	151	187	357**	—	241	205	224	442**

BUN: blood urea nitrogen; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

Source: Inoue & Enomoto (1984)

Table 15. Selected organ weight changes in a chronic toxicity and carcinogenicity study in rats

Week	Absolute (g) and relative (g/100 g bw) organ weights							
	Males				Females			
	0 ppm	50 ppm	400 ppm	3 200 ppm	0 ppm	50 ppm	400 ppm	3 200 ppm
Liver								
Absolute								
52	9.90	10.47	11.54**	13.23**	5.27	5.32	6.16**	8.72**
78	10.40	11.03	11.92	15.41**	6.28	6.42	7.30**	11.01**
110	9.87	10.78	12.19**	–	7.76	7.79	8.65*	11.64**
Relative								
52	2.53	2.60	3.05**	4.34**	2.28	2.91	2.72**	4.86**
78	2.40	2.59	2.99*	5.25**	2.35	2.49	2.90**	5.17**
110	2.63	2.79	3.66**	–	2.85	2.70	3.29**	6.62**
Kidney								
Absolute								
52	2.38	2.66*	2.66*	2.99**	1.60	1.62	1.76	2.00**
78	2.80	2.88	2.90	3.24*	1.92	1.92	2.01	2.23**
110	2.84	2.95	3.25**	–	2.23	2.28	2.34	2.43
Relative								
52	0.61	0.66	0.70**	0.96**	0.69	0.70	0.78	1.11**
78	0.65	0.69	0.73	1.10**	0.72	0.75	0.79	1.14**
110	0.76	0.77	0.98**	–	0.83	0.80	0.89	1.41**

bw: body weight; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

Source: Inoue & Enomoto (1984)

2.4 Genotoxicity

The results of studies of genotoxicity with dichlobenil are summarized in Table 18. All the studies, either in vitro or in vivo, were negative. Dichlobenil is not considered to possess any mutagenic or genotoxic potential.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In a two-generation reproductive toxicity study, dichlobenil technical (purity 99.4%) was administered in the diet to Crl:CD(SD)BR rats (30 of each sex per dose in the P generation; 25 of each sex per dose in the F₁ generation) at a concentration of 0, 60, 350 or 2000 ppm (equivalent to 0, 4, 23 and 130 mg/kg bw per day, respectively) for two consecutive generations. Stability and homogeneity of the prepared diet were measured periodically. Animals were observed once per day for overt signs of toxicity. Body weights and feed consumption were measured weekly and on gestation days (GDs) 0, 6, 12, 15 and 20. Pups were weighed on days 1, 4, 7, 14 and 21 of lactation. Adults were necropsied and examined macroscopically, and histopathological examination was conducted on selected tissues.

Table 16. Selected non-neoplastic lesions in rats

Week	Incidence of lesions							
	Males				Females			
	0 ppm	50 ppm	400 ppm	3 200 ppm	0 ppm	50 ppm	400 ppm	3 200 ppm
Liver								
Fatty metamorphosis								
52	1/10 ^a	5/10	8/10	9/10+	0/10	2/10	6/10	10/10+
78	9/10	6/10	7/10	9/10+	5/10	10/10	6/10	10/10+
All others ^b	15/47	17/47	13/47	45/50+	42/47	34/47	36/47	39/40+
Necrosis								
52	0/10	0/10	0/10	5/10	0/10	4/10	1/10	7/10
78	0/10	0/10	0/10	1/10	0/10	0/10	1/10	2/10
All others	2/47	4/47	0/47	1/50	1/47	1/47	1/47	0/47
Cytological alterations								
52	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
78	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
All others	0/47	3/47	0/47	1/50	3/47	3/47	14/47	25/47
Nodules								
52	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
78	0/10	0/10	1/10	0/10	1/10	0/10	0/10	0/10
All others	1/47	0/47	1/47	6/50	1/47	2/47	0/47	5/47
Kidney								
Calcification								
52	3/10	0/10	3/10	1/10	10/10	10/10	10/10	10/10
78	0/10	3/10	1/10	5/10	10/10	9/10	10/10	9/10
All others	3/47	2/47	5/47	24/50	22/47	21/47	25/47	46/47
Nephrosis								
52	0/10	0/10	5/10	10/10+	0/10	2/10	7/10	10/10+
78	6/10	8/10	10/10	10/10+	1/10	0/10	2/10	10/10+
All others	36/47	38/47	47/47	50/50+	22/47	21/47	25/47	46/47+
Parathyroid								
Hyperplasia								
52	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
78	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
All others	3/44	9/45	16/46	37/48	0/47	4/47	1/47	17/46

+: increased severity; ppm: parts per million

^a Tissues affected/tissues examined.^b All others = all animals examined except those terminated at 52 and 78 weeks.

Source: Inoue & Enomoto (1984)

Table 17. Liver tumours in rats at 104 weeks

Liver tumour	Males				Females				Historical controls (104 weeks)
	0 ppm	50 ppm	400 ppm	3 200 ppm	0 ppm	50 ppm	400 ppm	3 200 ppm	
Adenoma	0/47	0/47	0/47	4/50	0/47	0/47	2/47	6/47*	5/474 (0.0–0.2%)
Carcinoma	0/47	0/47	0/47	1/50	0/47	0/47	0/47	1/47	3/474 (0.0–2.2%)
Total	1/67 ^a	0/67	0/67	5/70	0/67	0/67	2/67	8/67** ^b	

ppm: parts per million; *: $P = 0.014$; **: $P < 0.01$ (Fisher's exact test)

^a One adenoma (1/10) was observed in controls terminated at 52 weeks.

^b One carcinoma (1/10) was observed in the 3200 ppm dose group terminated at 78 weeks.

Source: Inoue & Enomoto (1984)

Table 18. Results of studies of genotoxicity with dichlobenil

Type of study	Organism/cell line	Dose range tested	Purity (%)	Result	Reference
In vitro					
Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537 and TA1538	40–5 000 µg/plate (in DMSO)	Not reported	Negative ±S9 mix	Sekèt (1983)
Chromosomal aberration	Human lymphocytes	0–1 µg/mL (in DMSO)	Not reported	Negative ±S9 mix	Allen et al. (1984)
Gene mutation (CHO/HGPRT)	Chinese hamster ovary cells	0.15–670 µg/mL (in DMSO)	99.3	Negative ±S9 mix	Clarke (2007)
Chromosomal aberration (CHO-WBL)	Chinese hamster ovary cells	10–100 µg/mL (in DMSO)	≥ 99	Negative ±S9 mix	Murli (1990)
DNA repair (UDS)	HeLa S3 cells	0.05–102.4 µg/mL (in DMSO)	Not reported	Negative ±S9 mix	Allen & Proudlock (1984)
Gene mutation	BALB/3T3 cells	62.5–8 000 µg/mL (in DMSO)	Not reported	Negative ±S9 mix	Matthews (1984)
Gene mutation in mammalian cells	Mouse lymphoma L5178Y cells	5–280 µg/mL in DMSO	Not reported	Negative ±S9 mix	Richold et al. (1983)
In vivo					
Mouse micronucleus	Male and female Swiss mice	0, 300, 600 and 1 000 mg/kg bw (gavage in 1% tragacanth)	Not reported	Negative	Jens (1983)

bw: body weight; CHO: Chinese hamster ovary; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; HGPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × g supernatant fraction from rat liver homogenate; UDS: unscheduled DNA synthesis

Dietary concentrations were within the acceptable range (85–103% of the nominal), and the test material was homogeneously distributed in the diets.

One (of 30) high-dose P male was found dead during week 14 of the study. The cause of death was liver necrosis and haemorrhage. There were no treatment-related clinical signs of toxicity in the study. During the 10-week premating period for P animals, mean cumulative body weight gains were decreased by 25–26% ($P < 0.05$) at 2000 ppm in both males and females. Similarly, mean overall body weight gains for F₁ males were decreased by 25% during the 10-week premating period and by 18% for F₁ females at 2000 ppm. During gestation, mean body weight gains were decreased by 13–14% in both P and F₁ females at 2000 ppm. Decrements in body weight gain were not observed in adult females of either generation during lactation. Mean feed consumption at 2000 ppm was decreased by 11–33% ($P < 0.001$) in P males and by 17–28% ($P < 0.001$) in P females during premating. Mean feed consumption during premating was decreased by 16–21% ($P < 0.001$) in F₁ males and by 16–20% ($P < 0.001$) in F₁ females at 2000 ppm. Mean feed consumption for P and F₁ females during gestation and lactation was not reported. No treatment-related effects on fertility index, fecundity index, gestation index or mean gestation length were observed in either P or F₁ females. The mean number of implantations per dam was unreported for P females and decreased at 2000 ppm in F₁ females (12.3 versus 14.6 in controls).

No treatment-related effects on live birth index, viability index, lactation index or sex ratio were observed in either F₁ or F₂ offspring (Table 19). In addition, the mean number of pups born live per litter was unaffected in F₁ offspring but decreased ($P < 0.001$) at 2000 ppm in F₂ offspring (11.0 versus 13.7 in controls). This was likely due to the decreased number of implantations per F₁ dam at 2000 ppm. At and above 350 ppm, the mean pup body weight of F₁ offspring was decreased by 16–23% ($P < 0.05$) from postnatal day (PND) 4 (precll) to PND 21, compared with controls. The effect was dose dependent. In F₂ offspring, mean pup body weight was also dose-dependently decreased by 19–22% ($P < 0.05$) from PNDs 14 to 21. An increased incidence of pelvic cavitation of the kidney at 2000 ppm (3% versus 0%) was observed during necropsy in weanling F₂ offspring; however, the incidence was similar to the sporadic incidence observed in P and F₁ animals and was not considered toxicologically significant.

The NOAEL for parental systemic toxicity was 350 ppm (equivalent to 23 mg/kg bw per day), based on decreased body weight gains during premating (males and females) and gestation (females) in both generations, decreased feed consumption during premating in both generations (males and females) and a decreased number of implantations per dam in F₁ females at 2000 ppm (equivalent to 130 mg/kg bw per day). The NOAEL for reproductive toxicity was 350 ppm (equivalent to 23 mg/kg bw per day), based on a decreased number of implantations per dam in F₁ females at 2000 ppm (equivalent to 130 mg/kg bw per day). The NOAEL for offspring toxicity was 60 ppm (equivalent to 4 mg/kg bw per day), based on decreased body weight during weaning in both generations at 350 ppm (equivalent to 23 mg/kg bw per day) (Leeming, 1989; Barker, 1992).

(b) *Developmental toxicity*

Rats

In a prenatal developmental toxicity study, dichlobenil (purity not reported) was administered by gavage in 1% gum tragacanth to 25 pregnant Wistar Cpb:WU rats per dose from GD 6 to GD 15 inclusive at a dose level of 0, 20, 60 or 180 mg/kg bw per day. Animals were terminated on GD 21, and uteri were examined for live fetuses and intrauterine deaths. Fetuses were weighed and examined for external, visceral and skeletal alterations.

No treatment-related mortality or clinical signs of toxicity were observed in pregnant does in the study. Twenty-nine per cent ($P < 0.01$) and 36% ($P < 0.01$) decreases in mean body weight gain were observed during the dosing period in does treated at 60 and 180 mg/kg bw per day, respectively. This effect was accompanied by a 15% ($P < 0.01$) and 21% ($P < 0.01$) decrease in feed consumption at 60 and 180 mg/kg bw per day, respectively, as well as a respective 19% ($P < 0.05$) and 24% ($P < 0.01$) decrease in feed efficiency. During the post-dosing period (GDs 16–21), body weight gain, feed consumption and feed efficiency values in mid- and high-dose animals rebounded to levels that were similar to or greater than those of controls.

Table 19. Summary of effects of dietary administration of dichlobenil on F_1 reproductive parameters, offspring survival and pup body weights in rats

Parameter	0 ppm	60 ppm	350 ppm	2000 ppm
Number of matings (P parents)	25	25	25	25
Number of pregnancies	25	20	24	25
Fertility index: female (%)	100	80	96	100
Gestation index	100	100	100	100
Mean gestation length (days)	21.5	21.6	21.7	21.7
Mean number of implantations/dam	14.6	14.4	14.2	12.3
Total number of live pups				
Day 0	342	245	306	274
Day 4	237	180	232	238
Day 21	152	116	153	178
Mean number of live pups/litter				
Day 0	12.0	13.7	13.8	11.2
Day 4 precull	9.3	10.2	11.0	8.9
Day 21	6.6	6.9	6.9	6.0
Live birth index	82.7	92.7	31.2	92.9
Viability index	100	100	100	99.4
Lactation index	71.0	67.7	62.8	67.0
Mean pup body weight (g)				
Day 1	6.1	5.7	5.5**	5.6*
Day 4 precull	8.6	7.9	7.2***	6.9***
Day 14	29.8	29.1	27.4**	21.9***
Day 21	49.8	49.5	46.7*	38.2***
Sex ratio (% males)	48	50	51	48

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Source: Leeming (1989); Barker (1992)

No treatment-related changes were observed in caesarean section parameters (death, altered growth) for either embryos or fetuses. It is unclear from the study report whether statistical analysis was performed for litter incidences of external, skeletal or visceral observations. With regard to external anomalies, a shallow, dose-dependent increase in the incidence of small subcutaneous haemorrhage or petechia was observed (0/23, 1/22, 2/22 and 3/21 litters at 0, 20, 60 and 180 mg/kg bw per day); however, the increase in the number of fetuses affected (0–3) was only slightly increased across doses and was not statistically significant. An increased litter (13.6–14.3%) and fetal (3.1–3.5%) incidence of unilateral supernumerary rib (14th) was observed at and above 60 mg/kg bw per day. The fetal incidences were not statistically significantly different from those of concurrent controls. The litter incidence (19%) of bilateral supernumerary rib (14th) was also increased at 180 mg/kg bw per day, as was the fetal incidence (4.7%; $P < 0.05$). However, both the litter and fetal incidences of supernumerary (14th) rib were below the historical control incidences (32.26% and 10.86%, respectively). An increase ($P < 0.01$) in the “degree” of absence of ossification of the sternbrae (expressed as transformed ossification values per litter) was also observed at 180 mg/kg bw per day (7.03 versus 0.59 in controls). The increase was outside the historical control range (0–0.31).

However, given that the concurrent control value (0.59) was above the upper limit of the historical control range and a dose–response relationship for the effect was lacking, the calculated value is not considered toxicologically significant. With respect to visceral anomalies, a very slight increase in the malformations unilateral microphthalmia and intestinal alteration of the situs viscerum (combined with focal fibrosis of the peritoneum and mesentery) was observed at the high dose in one animal in 1/21 litters only. The malformation soft consistency of the lens/unilateral folded retina was observed in a different animal in 1/21 litters only. The incidence of each effect was above that of the historical controls (1/2691 fetuses; 1/401 litters); however, fetal incidences were not statistically significant, and a dose–response relationship was lacking at the doses tested.

The NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased body weight gain, feed consumption and feed efficiency during the dosing period at 60 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 180 mg/kg bw per day, the highest dose tested (Koëter, 1984).

Rabbits

In a prenatal developmental toxicity study, dichlobenil technical (purity 98.5%) was administered by gavage in 1% gum tragacanth to 16–17 pregnant New Zealand White rabbits per dose from GD 7 to GD 19 inclusive at a dose level of 0, 15, 45 or 135 mg/kg bw per day. A total of three, four, four and eight animals died or were killed following abortions at 0, 15, 45 and 135 mg/kg bw per day, respectively. Owing to an inadequate number of litters for developmental evaluations, the study was repeated (Barker, 1989a).

In a repeat prenatal developmental toxicity study, dichlobenil technical (purity 98.5%) was administered by gavage in 1% gum tragacanth to 18 pregnant New Zealand White rabbits per dose from GD 7 to GD 19 inclusive at a dose level of 0, 15, 45 or 135 mg/kg bw per day.

No treatment-related mortality, clinical signs of toxicity (including abortions) or gross pathology was observed in the study. A decrease (129%; $P < 0.05$) in body weight gain was observed during the dosing period only (GDs 7–19) in does treated at 135 mg/kg bw per day. This effect was accompanied by a 30% ($P < 0.01$) decrease in feed consumption at the high dose during the dosing period only. Body weight gain and feed consumption in high-dose animals rebounded during the post-dosing period (GDs 19–29).

Increases in total resorptions per dam (1.3) and post-implantation loss (17.9%) were observed at 135 mg/kg bw per day. Although the effects were not dose dependent, the incidences were outside the historical control range and considered treatment related. Although generally occurring at very low incidences (1–3 fetuses), several external, visceral and skeletal defects or anomalies were reported at 135 mg/kg bw per day (Table 20). These effects were not observed in either concurrent or historical controls or were observed at incidences outside historical control ranges and were therefore considered toxicologically significant. External anomalies included bilateral open eye (3/115 fetuses; 3/14 litters), cleft palate and adactyly. High-dose visceral anomalies included abnormal cystic gallbladder and distended ureter with bilateral severe hydronephrosis. Skeletal defects at 135 mg/kg bw per day consisted of malformed and malpositioned right scapula, right radius absent with malpositioned ulna and humerus, fused cervical vertebral arches, asymmetrically ossified and fused cervical vertebra centra, abnormally shaped cranium with enlarged and misshapen fontanelle, enlarged fontanelle (19/115 fetuses; 13/14 litters), misshapen frontals (2/115 fetuses; 2/14 litters), skull and frontals foreshortened and nasal malpositioned, and major fusion of sternebrae (3/115 fetuses; 3/14 litters).

The NOAEL for maternal toxicity was 45 mg/kg bw per day, based on decreased body weight gain and feed consumption during the dosing period at 135 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 45 mg/kg bw per day, based on an increased number of total resorptions per dam, increased post-implantation loss and increased incidences of external, visceral and skeletal malformations at 135 mg/kg bw per day (Barker, 1989b).

Table 20. Selected skeletal, visceral and external anomalies in rabbits

	0 mg/kg bw per day	15 mg/kg bw per day	45 mg/kg bw per day	135 mg/kg bw per day
No. of fetuses (litters) examined	142 (16)	154 (16)	146 (16)	135 (14)
Skeletal findings (no. of fetuses showing major effects/no. examined)	1/142	0/154	0/146	24/115*
Fontanelle enlarged	0	0	0	19 (13)
Frontals misshapen	0	0	0	2 (2)
Skull and frontals foreshortened	0	0	0	1
Major fusion of sternebrae	0	0	0	3 (3)
No. of fetuses showing minor skeletal defects	33	45	36	15
External and visceral observations (no. of fetuses showing major effects/no. examined)	0/142	1/154	1/164	11/115*
Open eye(s) bilateral	0	0	0	3 (3)
Open eye(s) unilateral	0	0	0	1
Arthrogryposis; adactyly	0	0	0	1
Cleft palate	0	0	0	1
External hydrocephaly	0	0	0	1
Gallbladder cystic	0	0	0	1
Distended ureter with bilateral hydronephrosis	0	0	0	1
Aortic distended	0	1	1	1
Aortic arch	0	0	0	1
No. of fetuses showing minor external/visceral defects	5	14	2	12

bw: body weight; *: $P < 0.01$

Source: Barker (1989b)

2.6 Special studies

(a) Immunotoxicity

Rats

In an immunotoxicity study, dichlobenil technical (purity 99.0%) was administered daily to groups of 10 male Sprague-Dawley rats by oral gavage at a dose level of 0, 20, 60 or 180 mg/kg bw per day for 28 days. A concurrent positive control group of 10 male Sprague-Dawley rats received the positive control cyclophosphamide monohydrate at a concentration of 15 mg/kg bw per day by intraperitoneal injection on days 23–28. All rats received a single intravenous injection of 1×10^8

sheep red blood cells (sRBCs) on day 23. Body weight, feed intake and water intake were monitored weekly or biweekly during the study. At the end of the study, blood was collected to determine anti-sRBC immunoglobulin M (IgM) concentration, and absolute and relative (to body weight) liver, spleen and thymus weights were measured.

There was no mortality in the study, nor were there any adverse clinical signs. Feed consumption was decreased by 17% at 180 mg/kg bw per day during week 1. Terminal body weights were decreased by 8% in the 180 mg/kg bw per day group. No treatment-related effects on absolute or relative spleen or thymus weights were found in dichlobenil-treated rats. Absolute and relative liver weights were increased in a dose-related manner. Relative liver weights were increased by 14–62% in the treated groups.

Treatment with dichlobenil doses up to 180 mg/kg bw per day for 28 days did not suppress humoral response, as indicated by the anti-sRBC IgM response. The cyclophosphamide monohydrate positive control responded appropriately, by decreasing the absolute and relative (to body weight) spleen and thymus weights and decreasing the anti-sRBC IgM concentrations.

The LOAEL for systemic toxicity in male Sprague-Dawley rats treated with dichlobenil was 20 mg/kg bw per day, the lowest dose tested, based on increased liver weights at all doses. The NOAEL for immunotoxicity was 180 mg/kg bw per day, the highest dose tested (Johnson, 2014).

(b) Olfactory response evaluations

Oral exposure to dichlobenil

No effects were observed upon histopathological evaluation of nasal tissue in a 1-year dog oral (capsule) toxicity study up to a dose level of 36 mg/kg bw per day (Pickersgill, 1995).

Dermal exposure to dichlobenil

A single dermal application of dichlobenil in acetone caused olfactory damage in mice at doses of 50 mg/kg bw and higher; however, five sequential applications of 25 mg/kg bw had no effect on the olfactory mucosa (Deamer, O'Callaghan & Genter, 1994).

Inhalation exposure to dichlobenil

There appear to be no published studies evaluating the effects of inhalation exposure to dichlobenil.

A 1-week range-finding study in rats was conducted at concentrations 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 (Newton, 2002).

Crompton Corporation completed a 28-day inhalation study in rats at concentrations of 2.3, 5.1 and 12 mg/m³. Histopathology on the nasal tissue showed that there was no effect of dichlobenil at doses up to 12 mg/m³ (Newton, 2002).

The results of the above two studies show that 12 mg/m³ is a no-observed-effect concentration for dichlobenil-induced nasal toxicity for an exposure period up to 28 days. However, an exposure to 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 are similar to those seen with dermal exposure to dichlobenil (see above).

Intraperitoneal exposure to dichlobenil

In a published study, single intraperitoneal injections of dichlobenil were administered to C57Bl mice and Sprague-Dawley rats at 12, 25 or 50 mg/kg bw. In mice, necrosis of Bowman's glands was evident 8 hours after the lowest dose of 12 mg/kg bw. The olfactory region was covered by an attenuated surface epithelium or by a respiratory-like epithelium 3–7 days after dosing. Seven to 20 days after dosing, there was fibrosis of the olfactory region. Partial regeneration of the olfactory

epithelium and scattered intact Bowman's glands were observed after 20 days. The study authors proposed that dichlobenil induces a primary lesion in Bowman's glands resulting from the pronounced binding of a metabolite in these glands (Brandt, Eriksson & Brittebo, 1991).

A single intraperitoneal dichlobenil dose of 50 mg/kg bw was found to cause extensive damage to the olfactory epithelium and Bowman's glands of rats. Histological evaluations showed that Bowman's glands in rats were reduced or eliminated 4 days after the intraperitoneal administration, but began to reappear at day 11 and were fairly normal 25 days after treatment (Hastings, Andringa & Miller, 1993).

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 intraperitoneal dichlobenil dose of 50 mg/kg bw. Two weeks later, the rats were given 100 mg/kg bw, and again there were no effects on sensory function. After a single dichlobenil injection of 200 mg/kg bw, it was found that performance on the odour trials dropped to 50%; however, performance on the odour task was nearly normal 5 days after exposure (Hastings, Andringa & Miller, 1993).

In another study, dichlobenil (100 mg/kg bw) given intraperitoneally to rats previously trained in an olfactory task of finding feed buried in bedding caused a transient increase in the mean latency time to find the feed pellet. Treated animals were normal by day 28 (Genter et al., 1996). This type of olfactory sensory recovery has also been seen after methyl bromide exposure at 200 ppm (Hurt et al., 1988). 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.

Covalent binding to the olfactory mucosa after intraperitoneal administration of [¹⁴C]dichlobenil to mice at 12 mg/kg bw was 6 times greater than the binding seen after administration of 6 mg/kg bw (Brittebo, Eriksson & Brandt, 1992), establishing a threshold for the covalent binding that corresponds with the no-observed-effect level of 6 mg/kg bw determined in previous studies (Brandt et al., 1990; Brandt, Eriksson & Brittebo, 1991; Brittebo et al., 1991). However, depletion of glutathione with phorone prior to intraperitoneal treatment with dichlobenil at 6 mg/kg bw resulted in increased covalent binding and extensive toxicity in the olfactory mucosa (Brittebo, Eriksson & Brandt, 1992). 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 intraperitoneal dose of up to 6 mg/kg bw.

Intraperitoneal exposure to chlorthiamid and BAM

The toxic effects of the herbicide 2,6-dichlorothiobenzamide (chlorthiamid), a dichlobenil analogue, and its major environmental metabolite 2,6-dichlorobenzamide (BAM), which is also an environmental metabolite of dichlobenil, were examined in the nasal passages of C57Bl mice following single intraperitoneal injections. The doses administered to mice were 25, 50 and 100 mg/kg bw for BAM and 6, 12, 25 and 50 mg/kg bw for chlorthiamid. BAM (100 mg/kg bw) and chlorthiamid (12 mg/kg bw and above) induced an extensive destruction of the olfactory region, similar to that observed with the analogue dichlobenil at 12 mg/kg bw and above. Necrosis of Bowman's glands was evident first, whereas degeneration and necrosis of the olfactory neuroepithelium developed less rapidly (Brittebo et al., 1991). The toxicity caused by dichlobenil and chlorthiamid to the olfactory neuroepithelium may be secondary to the destruction of Bowman's glands (Brandt et al., 1990).

In vitro studies with dichlobenil

In vitro studies with subcellular homogenates of the olfactory mucosa from C57Bl mice and Sprague-Dawley rats showed a highly efficient cytochrome P450-dependent metabolic activation of dichlobenil in the olfactory mucosa. Based on similar toxicity and the ability of chlorthiamid to inhibit the covalent binding of dichlobenil in vitro, the authors proposed that dichlobenil and chlorthiamid are metabolized to a common cytotoxic product or to closely related cytotoxic products (Brandt, Eriksson & Brittebo, 1991).

Mechanistic studies on the olfactory response produced by dichlobenil

It has also been reported that dichlobenil is metabolized in mouse olfactory mucosa homogenates to a greater degree than in liver homogenates (Eriksson & Brittebo, 1991). This is considered to be a cytochrome P450-dependent mechanism (Brandt et al., 1990; Brandt, Eriksson & Brittebo, 1991; Eriksson & Brittebo, 1991, 1993; Walters, Buchheit & Maruniak, 1993a,b) that results in the formation of an electrophilic intermediate in the olfactory mucosa, which covalently binds to tissue proteins (Brandt et al., 1990; Eriksson & Brittebo, 1993, 1995; Ding, Sheng & Bhama, 1994). 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 (Eriksson, Busk & Brittebo, 1996). [¹⁴C]Dichlobenil binds irreversibly to Bowman's glands to an even greater extent than in the olfactory epithelium (Brandt et al., 1990).

Conclusion

The results of these studies demonstrate that dichlobenil and/or BAM, the major soil metabolite of dichlobenil (see section 2.7(a) below), produce harmful effects on nasal mucosa (olfactory response) via the intraperitoneal route. Dichlobenil is also shown to produce an olfactory response via dermal, inhalation and oral routes of exposure. If the level is exceeded, it has been shown that animals can recover from the harmful effects of dichlobenil on the nasal mucosa, and also there is a threshold for dichlobenil and BAM below which the effects are not observed.

2.7 Studies on metabolites

(a) 2,6-Dichlorobenzamide (BAM)

The substance 2,6-dichlorobenzamide (BAM; CAS No. 2008-58-4) is the major soil metabolite of dichlobenil and the major residue observed in plants and livestock matrices, such as milk, kidney, liver, fat and muscle. It was not detected in a rat metabolism study.

Biochemical aspects

No absorption, distribution or metabolism studies for BAM were submitted. However, in a published study (Bakke et al., 1988), oral absorption, distribution and metabolism of BAM were studied following a single gavage dose of radioactive BAM at 5 mg/kg bw administered to male Sprague-Dawley rats, and excretion was measured. Absorption and excretion of BAM were also studied in male bile duct-cannulated Sprague-Dawley rats. The distribution of radioactive BAM at 48 hours in rats was approximately 62%, 14.6%, 1.8% and 14.9% of the administered dose in urine, faeces, gastrointestinal tract and carcass, respectively. The distribution of radioactive BAM in male bile duct-cannulated rats at 48 hours was approximately 27.6%, 33.5%, 2.2%, 2.4% and 24.6% of the administered dose in urine, bile, faeces, gastrointestinal tract and carcass, respectively. In a metabolism study in rats, the major component in the urine and bile was the unchanged parent compound BAM, and BAM was the only compound detected in tissues. The other metabolites were 2-monohydroxy BAM, 2-chloro-5-hydroxy-6-(methylthio)benzamide and 2-chloro-5-hydroxy-6-[S-(N-acetyl)cysteinyl]benzamide.

Whole-body radioautography and microautography showed the accumulation of non-extractable residues from BAM in nasal mucosa in mice after a single intravenous administration of BAM at 7 mg/kg bw (Bakke et al., 1988).

BAM is a metabolite of fluopicolide in rats and plants. BAM was evaluated as part of the 2009 JMPR assessment of fluopicolide (Annex 1, reference 118). Some of these studies were not made available to the present Meeting. As stated in the 2009 monograph, absorption, distribution and excretion were similar following a single oral dose of 10 or 150 mg/kg bw or following repeated dosing of 10 mg/kg bw per day for 14 days in Sprague-Dawley rats. The radiolabel was eliminated mostly in the urine (approximately 82% of the administered dose), with low levels eliminated in the faeces (approximately 13% of the administered dose). The highest concentrations in tissues were seen in the kidney and liver after the 10 mg/kg bw dose and in the skin and fur, kidneys and liver after the

150 mg/kg bw dose. Tissue concentrations increased by approximately 5-fold for a 15-fold increase in dose. The rat metabolism study suggested that biotransformation of BAM consisted of 1) hydrolysis of the amide group, 2) hydroxylation and subsequent conjugation with either glucuronic acid or sulfate and 3) the loss of a chlorine atom, followed by glutathione conjugation and further metabolism of the glutathione group to the mercapturic acid or *S*-methyl metabolites (Annex 1, reference 118).

Acute toxicity

In an acute oral toxicity study, groups of five male and five female fasted Swiss mice were given a single oral dose of BAM (batch no. FUN81D15A; purity not reported) in a 1% tragacanth suspension at a dose of 0, 156, 312, 625, 1250, 2500 or 5000 mg/kg bw and observed for up to 13 days. Animals were observed periodically for clinical signs. Body weights were recorded 2 days prior to treatment and at 2, 7 and 13 days post-treatment. All animals were examined macroscopically at the end of the study.

All mice in the 5000 mg/kg bw group, 4/5 males and 4/5 females in the 2500 mg/kg bw group and 2/5 males and 4/5 females in the 1250 mg/kg bw group died between 30 minutes and 24 hours following dosing. Clinical signs at lethal or sublethal doses (625–5000 mg/kg bw) included locomotor impairment and dyspnoea. These signs appeared within 5 minutes of dosing and lasted between 1 and 4 days post-dosing. The mice at 156 and 312 mg/kg bw were subdued, with slight locomotor impairment, which lasted for 24 hours post-dosing. No individual animal observations or onset of clinical signs was reported. Males at 2500 and 1250 mg/kg bw and females at 1250, 625 and 312 mg/kg bw lost weight in the first few days after dosing and recovered thereafter. No macroscopic findings were noted at necropsy.

The acute oral LD₅₀ of BAM in mice was 1538 mg/kg bw (95% confidence interval 992–2385 mg/kg bw) and 1144 mg/kg bw (95% confidence interval 722–1813 mg/kg bw) for males and females, respectively (Koopman, 1981).

Short-term studies of toxicity

In a 13-week oral toxicity study, BAM technical (batch no. 133/2/4/104; purity not reported) was administered to 10 Wistar rats of each sex per dose in the diet at a concentration of 0, 50, 180, 600 or 2300 ppm (equal to 0, 4, 14, 49 and 172 mg/kg bw per day, respectively). Animals were observed daily for general condition. Body weight and feed consumption were measured weekly. The effect of the compound on skeletal muscle tone was measured at four intervals during the experiment. Haematology, blood chemistry and urine analysis were performed at intervals during the experiment. No ophthalmological examinations were performed. At the end of the experiment, the clearance of bromosulphthalein by the liver and blood clotting time were measured. Postmortem analysis included recording the weight of the principal organs and their histopathology together with that of other tissues and an estimation of liver glycogen content.

There were no treatment-related effects on survival. There was significant hair loss in the female rats in the 600 and 2300 ppm dose groups. Mean terminal body weights were decreased in both males (18%) and females (11%) at 2300 ppm, relative to controls. Mean body weight gain in males treated at 2300 ppm was decreased by 30% ($P < 0.01$) during weeks 2–11, whereas feed consumption in the same group was decreased by 16% ($P < 0.01$). In females, mean body weight gain was decreased by 18% ($P < 0.01$) after 11 weeks at 600 ppm and by 30% ($P < 0.01$) at 2300 ppm. Feed consumption in females was decreased by 17% ($P < 0.01$) at 2300 ppm only. Feed efficiency in the 2300 ppm dose group was reduced in females, but not in males. Increased relaxation scores (muscle hypotonus; $P < 0.05$) were observed at 600 ppm on day 4 only in males and on days 91 and 92 only in females (Table 21). At 2300 ppm, increases in relaxation scores ($P < 0.05$) were observed at each measurement (days 4, 21, 91 and 92) in both sexes. No treatment-related changes were observed in haematological or urine analysis parameters. A 19% ($P < 0.02$) decrease in mean blood coagulation time in males at 2300 ppm was not considered clinically significant and was also not dose dependent. Mean serum urea concentration in males was increased by 55% ($P < 0.001$) after 6 weeks at 2300 ppm. After 12 weeks in males, mean serum urea concentration was increased by 53% ($P <$

0.04) at 600 ppm and by 69% ($P < 0.04$) at 2300 ppm. In the absence of histopathological correlates in the kidney or liver, increased urea concentrations were not considered toxicologically significant. No biologically significant changes in clinical chemistry were observed in females. There was also no difference in bromosulfthalein serum retention (measure of liver function) between control and high-dose animals. Mean absolute thymus, heart and brain weights were decreased in males by 28% ($P < 0.01$), 21% ($P < 0.01$) and 7% ($P < 0.01$), respectively, whereas mean absolute spleen, thymus and heart weights were decreased in females by 13% ($P < 0.05$), 23% ($P < 0.01$) and 9% ($P < 0.01$), respectively. However, there was no difference in mean relative weights for these organs in either sex, and the differences from control values are likely related to the observed decreases in body weight. For these reasons and because histopathological correlates were lacking in these organs, the changes in organ weights were not considered toxicologically significant. The nose (a potential target organ) was not examined histopathologically in this study.

Table 21. Muscle relaxation scores in rats

Group	Total score							
	Day 4		Day 21		Day 91		Day 92	
	Males	Females	Males	Females	Males	Females	Males	Females
Control	4	10	5	10	6	7	7	6
50 ppm	7	7	6	7	11	2	11	6
180 ppm	6	19	13	8	11	9	11	10
600 ppm	17*	17	16	13	10	21*	16	23*
2 300 ppm	27*	30*	34*	33*	34*	25*	36*	27*

ppm: parts per million; *: $P < 0.05$

Source: Boschman et al. (1967)

In conclusion, the NOAEL was 180 ppm (equal to 14 mg/kg bw per day), based on reduced skeletal muscle tone (males and females) and decreased body weight gain (females) at 600 ppm (equal to 49 mg/kg bw per day) (Boschman et al., 1967).

In a 2-year toxicity study, BAM (batch no. 133-2-4-104; purity 97%) was administered in the diet to Beagle dogs (four of each sex per dose) at a concentration of 0, 60, 100, 180 or 500 ppm (equivalent to 0, 1.5, 2.5, 4.5 and 12.5 mg/kg bw per day, respectively). Stability and homogeneity analyses of the active ingredient in the feed were not performed. Dogs were observed daily for general health and behaviour. Body weights were recorded weekly. Selected haematological and clinical chemistry parameters were measured prior to study initiation and every 3 months during the study. Urine analysis and ophthalmological examinations were not performed. Necropsies were performed on animals, selected organs were removed and weighed and histopathological examination was carried out.

No animals died during the study, and it was reported that there were no treatment-related clinical signs of toxicity. Mean body weight in males treated at 500 ppm was decreased (non-statistically significantly) at weeks 54 (14%) and 104 (12%), whereas in females, mean body weight was decreased ($P < 0.01$) at weeks 15 (12%), 54 (21%) and 104 (23%) at 500 ppm, relative to controls. Mean cumulative body weight gains (weeks 0–104) in males were 19% and 38% lower than control values at 180 and 500 ppm, respectively. However, neither was statistically significantly different from controls. Similarly, in females, mean cumulative body weight gains at 100, 180 and 500 ppm were 27%, 32% and 69% lower than control values, respectively, without statistical significance. Changes in body weight or body weight gain at or below 180 ppm were not considered toxicologically significant based on the wide variation in these measured parameters. No adverse

effects were observed on haematological or clinical chemistry parameters, at necropsy or on organ weights. Microscopic pathology was not observed as a function of dose.

In conclusion, the NOAEL was 180 ppm (equivalent to 4.5 mg/kg bw per day), based on decreased body weight and body weight gain at 500 ppm (equivalent to 12.5 mg/kg bw per day) (Wilson & Thorpe, 1971).

Long-term studies of toxicity and carcinogenicity

In a carcinogenicity study, BAM (batch no. 195; purity 9.5%) was administered in the diet for 106 weeks to CrI:CD[®]BR rats (35 of each sex per dose) at a concentration of 0, 60, 100, 180 or 500 ppm (equal to 0, 2.2, 3.6, 6.5 and 19 mg/kg bw per day for males and 0, 2.8, 4.7, 8.5 and 24 mg/kg bw per day for females, respectively). Stability and homogeneity of prepared diets were determined periodically. The frequency of clinical observations was not stated. It was stated that all signs of ill-health or reaction to treatment were recorded. Animals were weighed at initiation and weekly throughout the study. The quantity of feed consumed by each cage of rats ($n = 5$) was recorded daily. Eyes of all rats in the control and high-dose groups were examined using a Keeler indirect ophthalmoscope at 0, 13, 26, 52 and 104 weeks. Blood was collected by orbital sinus puncture from 10 animals of each sex in the control and high-dose groups at 0, 13, 26, 39, 52 and 103 weeks, and selected haematological and clinical chemistry parameters were evaluated. Urine was collected from five fasted animals of each sex in the control and high-dose groups at 13, 26, 52 and 103 weeks. All animals that died or were terminated on schedule were subjected to gross pathological examination, and the selected tissues were collected from all rats for histological examination. Selected organs were weighed.

Homogeneity of the diet at 60 and 500 ppm was within 3% and 4% of the nominal concentrations, respectively. Measured dietary concentrations were 99.7% and 94.7% at 60 and 500 ppm. The stability of the 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 (Johnson, 1996).

No treatment-related clinical signs of toxicity, mortality or effects on feed consumption, feed efficiency, ophthalmoscopic examinations, clinical chemistry parameters, urine analysis or macroscopic findings were observed in this study. Body weight, relative to controls, at 500 ppm was decreased by 13% ($P < 0.05$) in males at termination and by 10–21% in females from week 13 until the end of the study. At 180 ppm, body weight in females was decreased by 13% at termination only. At 500 ppm, mean body weight gain was decreased by 10–16% in males and by 16–26% in females from week 26. Body weight gain in females treated at 180 ppm was also decreased by 10–17% during the second half of the study. In the 500 ppm dose group, there were statistically significant decreases in mean haematocrit levels, mean haemoglobin concentration, mean erythrocyte counts and mean corpuscular volume in male and female rats and decreased haemoglobin concentration in female rats. Although statistically significant, the magnitude of change in the haematological parameters (3–9% less than controls) was not biologically significant. A 25% increase in mean relative liver weight was observed at 500 ppm in females, as was a 48% increase in mean relative adrenal weight. The increase in liver weight was considered toxicologically significant due to the presence of liver histopathology at 500 ppm. Non-neoplastic histopathological observations in the liver are summarized in Table 22. Dose dependence was lacking for several parameters measured in the liver (Table 22; Wheldon et al., 1971).

The microscopic examination of tissues in the study by Wheldon et al. (1971) was incomplete, but the tissues had been fixed and stored. In 1996, a new study was undertaken to re-evaluate the tissues. On reanalysis of the non-neoplastic liver findings (Connick, Crome & Gopinath, 1996), increased incidences of eosinophilic (focal and areas) and basophilic (focal and areas) hepatocytes were detected in treated rats from the 100, 180 and 500 ppm BAM dose groups. The effects were generally more pronounced in female rats. No treatment-related histopathological effects were observed in any other examined organ.

Table 22. Non-neoplastic and neoplastic lesions in rats

	Incidence of finding									
	Males					Females				
	0 ppm	60 ppm	100 ppm	180 ppm	500 ppm	0 ppm	60 ppm	100 ppm	180 ppm	500 ppm
<i>No. of animals examined</i>	27	29	32	26	35	26	28	28	32	35
Fat deposition										
None	0	7	2	3	5	4	3	1	4	1
Occasional/minimal	13	7	10	7	12	8	11	12	11	7
Moderate	7	6	11	6	9	6	2	7	5	14
Marked	3	5	6	3	3	4	9	4	4	9
Fat degeneration										
Marked	0	2	0	0	0	4 ^a	3	1	3 ^a	3 ^a
Necrosis	0	0	0	1	0	1	1	0	0	0
Non-neoplastic lesions										
<i>Total no. of livers examined</i>	26	28	32	25	34	25	28	28	32	35
Eosinophilic hepatocytes: focal	5	12	17**	11	21**	5	4	7	16*	23**
Eosinophilic hepatocytes: areas	1	3	0	2	4	2	2	1	5	18**
Basophilic hepatocytes: focal	7	11	5	6	9	9	10	6	14	23**
Basophilic hepatocytes: areas	1	0	1	0	1	3	3	0	2	5
Hepatocyte vacuolation: centrilobular	5	7	10	5	16*	5	7	5	8	11
Neoplastic lesions										
<i>Total no. of livers examined</i>	26	28	32	25	34	24	28	27	32	35
Hepatocellular adenoma	1	0	1	0	1	0	1	0	0	5
Hepatocellular carcinoma	2	1	2	1	0	0	0	0	0	0
Pairwise comparison <i>P</i> -value							0.25	0.50	0.50	0.049
<i>P</i> -value for trend test									0.65	0.003

ppm: parts per million; *, $P < 0.05$; **, $P < 0.01$ (Fisher's exact test)^a Similar findings with Oil Red O and haematoxylin/eosin staining, recorded for one animal in each group, were tallied only once.

Source: Connick, Crome & Gonipath (1996)

With respect to neoplastic observations, upon reanalysis of the tumour data (Connick, Crome & Gopinath, 1996), a marginally significant increase ($P = 0.049$ for the pairwise comparison) in the incidence of hepatocellular adenomas was observed in females at 500 ppm (Table 22). Statistical analysis using the time to tumour method demonstrated evidence ($P = 0.003$ for the trend test) of an

increase in hepatocellular tumours in the 500 ppm females. No effect was seen at the 180 ppm dose level. No hepatocellular carcinomas were observed in female rats, and neither adenomas nor carcinomas were observed in males.

Previously, JMPR (Annex 1, reference 118) established a NOAEL of 60 ppm (equal to 2.0 mg/kg bw per day) from the above study for the systemic toxicity of BAM based on body weight reductions, increased incidences of eosinophilic and basophilic foci and fat deposition and cellular degeneration in the livers at 100 ppm (equal to 3.5 mg/kg bw per day).¹ However, the current Meeting re-evaluated the data and concluded that the LOAEL was 500 ppm (equal to 19 mg/kg bw per day) in males, based on decreased body weight and body weight gain and an increased incidence of hepatocellular alteration (eosinophilic foci); and 180 ppm (equal to 8.5 mg/kg bw per day) in females, based on decreased body weight and body weight gain and an increased incidence of hepatocellular alteration (eosinophilic foci). Body weight changes at 100 ppm were minor and were not considered adverse by the Meeting, and the hepatocellular degeneration (eosinophilic foci) in males has no clear dose–response relationship. Therefore, the Meeting concluded that the NOAEL for systemic toxicity in this 2-year toxicity and carcinogenicity study in rats was 100 ppm (equal to 4.7 mg/kg bw per day).

The NOAEL for carcinogenicity was 180 ppm (equal to 8.5 mg/kg bw per day), based on a marginally significant increase in the incidence of hepatocellular adenomas seen in females at 500 ppm (equal to 25 mg/kg bw per day) (Weldon et al., 1971; Connick, Crome & Gopinath, 1996; Johnson, 1996).

Genotoxicity

Results of studies of genotoxicity with BAM are summarized in Table 23.

Table 23. Results of studies of genotoxicity with BAM

Type of study	Organism/cell line	Dose range tested	Purity (%)	Result	Reference
In vitro					
Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	625–5 000 µg/plate (+S9 mix) 625–5 000 µg/plate (–S9 mix) (in DMSO)	100	Negative ±S9 mix	Koorn (1993)
Unscheduled DNA synthesis assay	Primary rat hepatocytes	3–1 000 µg/mL (+S9 mix) 3–1 000 µg/mL (–S9 mix) (in 1% DMSO)	100	Negative ±S9 mix	Van de Waart (1993a)
In vivo					
Mouse micronucleus	Male and female Swiss OF-1 mice (5/sex/dose)	250 mg/kg bw (gavage in corn oil)	100	Negative	Van de Waart (1993b)
Mouse micronucleus	Male and female Swiss OF-1 mice (3/sex/dose)	1 000, 2 000 and 4 000 mg/kg bw (M) ^a 100, 250 and 500 mg/kg bw, M/F (gavage in corn oil)	100	Negative	Van de Waart (1995)

bw: body weight; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; F: female; M: male; S9: 9000 × g supernatant fraction from rat liver homogenate

^a Treated animals in the 2000 and 4000 mg/kg bw dose groups died immediately after dosing, and animals in the 1000 mg/kg bw dose group died in 24 hours. No information on onset of clinical signs or individual animal data were presented.

¹ Note that the current reviewer calculated doses of 2.2 and 3.6 mg/kg bw per day for the dietary concentrations of 60 and 100 ppm.

Multigeneration studies

In a three-generation reproductive toxicity study, BAM (batch no. 195; purity 99.5%) was administered in the diet to Long-Evans rats (10 males per dose and 20 females per dose in the P, F₁ and F₂ generations) at a concentration of 0, 60, 100 or 180 ppm (equivalent to 0, 4.0, 6.7 and 12 mg/kg bw per day, respectively). Two litters were produced in each generation. The number of pups per litter was counted, and litters were weighed on PND 21. Litters were culled to 10 animals each on PND 5. P, F₁ and F₂ generation parental animals were weighed and examined for gross pathology on the day of termination (unspecified). Organ weights were measured, and histopathology was performed on select F_{3b} weanlings only.

No treatment-related mortality was observed in the P, F_{1b} or F_{2b} parental generations. A 6% ($P < 0.05$) decrease in mean parental terminal body weight was observed at 180 ppm in the F_{2b} generation; however, the decrease was not biologically significant and was not observed in P or F_{1b} parental animals. No treatment-related gross pathology was observed in P, F_{1b} or F_{2b} parental animals. Fertility and gestation indices were similar across treatment groups in P, F₁ and F₂ generation dams.

No treatment-related differences were observed in the mean number of pups per litter on PNDs 1, 5 (precull) or 21 in the F₁, F₂ or F₃ generations. Viability and lactation indices were similar across the treatment groups in F₁, F₂ and F₃ generation pups. Mean survival from birth to PND 5 (viability index) was slightly reduced (86.2%, $P < 0.01$; versus 95.7% in controls) at 180 ppm in the F_{3b} generation; however, this decrease was not observed in any other generation. In addition, mean survival from birth to weaning was similar across doses for each of the three generations of offspring. Hyperexcitability was observed in pups (number not reported) from four litters in the F_{1b} generation only.

Mean weanling weights (calculated as entire litter weight divided by number of pups per litter) at 180 ppm were decreased by 15% ($P < 0.05$), 12% ($P < 0.05$) and 14% ($P < 0.01$) in the F_{1b}, F_{3a} and F_{3b} generations, respectively; however, the decreases were not dose dependent, not observed in the F₂ generation and not calculated from individual pup body weights. No treatment-related differences were observed in mean absolute brain weights or mean brain weight to body weight ratios in F_{3b} generation weanling rats of either sex. A 12% increase in both mean absolute kidney weights and mean kidney weight to body weight ratios ($P < 0.01$) was observed in F_{3b} generation female weanlings at 180 ppm. A 10% increase in both mean absolute liver weights and mean liver weight to body weight ratios ($P < 0.05$) in males at 180 ppm and in females at and above 100 ppm was observed in this same generation. Changes in mean organ weights in F_{3b} generation weanlings were not considered toxicologically significant, however, because of a lack of histopathological correlates in the liver and kidney.

The NOAEL for parental systemic toxicity, reproductive toxicity and offspring toxicity was 180 ppm (equivalent to 12 mg/kg bw per day), the highest dose tested.

No individual animal data were provided. The study was completed in 1971, and the report was reissued in 1993 (Hine, Eisenlord & Loquvam, 1993).

Developmental toxicity

In a prenatal developmental toxicity study, BAM (batch no. FUX001000; purity 99.4%) was administered by gavage in 1% gum tragacanth to 16 pregnant New Zealand White rabbits per dose from GD 7 to GD 19 inclusive at a dose level of 0, 10, 30 or 90 mg/kg bw per day. Animals were checked for mortality and moribundity twice daily. Observations for clinical signs of toxicity were made once daily. Body weight and feed consumption data were recorded on GDs 0, 7, 13, 19, 23 and 28. On GD 28, all surviving animals were terminated, and litters were delivered by caesarean section. A gross pathological examination was conducted. Various fetal and litter parameters were evaluated.

Five (of 16) females treated at 90 mg/kg bw per day were terminated in extremis. Three of the five terminated high-dose dams had late abortions (GDs 19, 21 and 22, respectively). The incidence of abortion followed by termination at 0, 10 and 30 mg/kg bw per day was 1/16, 1/16 and 0/16, respectively. The other 2/5 high-dose animals were terminated moribund. Moribund condition

followed by termination was observed in 1/16, 0/16 and 2/16 animals at 0, 10 and 30 mg/kg bw per day, respectively. The incidence of thin appearance was increased at 90 mg/kg bw per day (10/16), whereas the incidences at 0, 10 and 30 mg/kg bw per day were 1/16, 0/16 and 2/16, respectively. A decrease (129%) in mean body weight gain, relative to controls, was observed during the dosing period (GDs 7–19) in does treated at 90 mg/kg bw per day. Similarly, feed consumption at 90 mg/kg bw per day was decreased during the dosing period by 49%, relative to controls. Body weight gain and feed consumption in high-dose animals rebounded above control levels during the post-dosing period (GDs 20–28). No treatment-related gross pathology was observed.

No treatment-related effects were observed on several developmental end-points, including the number of resorptions, post-implantation loss, litter size and sex ratio. Mean fetal body weight at 90 mg/kg bw per day (33.9 g) was decreased by 6%, relative to controls; however, the change was not statistically significant, and the mean value fell within the historical control range (27.7–39.4 g). No treatment-related changes in the incidences of external defects were observed.

An increase in the incidence of bipartite interparietal bone was observed at 90 mg/kg bw per day. Litter incidences were 1/14, 1/15, 2/14 and 3/11 at 0, 10, 30 and 90 mg/kg bw per day. Bipartite interparietal bone is considered a malformation in rats. Historical control data were not provided for the litter incidences of bipartite interparietal bone. However, the historical control data for fetal incidences were not statistically significantly different from those of concurrent controls, and the concurrent control fetal incidence (0.8%) exceeded that of the historical controls (0.3%). An increase in the incidence of postcaval lung lobe agenesis was also observed at 90 mg/kg bw per day. Litter incidences were 0/14, 0/15, 1/14 and 3/11 at 0, 10, 30 and 90 mg/kg bw per day. The fetal incidence of postcaval lung lobe agenesis at 90 mg/kg bw per day (3.2%) exceeded that of the historical controls (1.2%), whereas that at 30 mg/kg bw per day (0.9%) did not.

The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on increased incidences of clinical signs (late abortion, thin appearance) and decreased (severe) body weight gain and feed consumption during the dosing period at 90 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 30 mg/kg bw per day, based on increased incidences of late abortion and skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) malformations at 90 mg/kg bw per day (McIntyre, 1987).

Olfactory response evaluations

BAM was evaluated for its olfactory toxicity. The nasal passages of C57Bl mice were examined following single intraperitoneal injections of BAM at doses of 25, 50 and 100 mg/kg bw. No morphological changes were observed in the nasal tissue following dosing at 25 and 50 mg/kg bw. At 100 mg/kg bw, BAM induced an extensive destruction of the olfactory region, similar to that observed with dichlobenil and chlorthiamid at 12 mg/kg bw and above. Necrosis of Bowman's glands was evident first, whereas degeneration and necrosis of the olfactory neuroepithelium developed less rapidly (Brittebo et al., 1991). This study suggest that BAM is less potent than dichlobenil and chlorthiamid in producing a nasal olfactory response.

In conclusion, both BAM and dichlobenil (see section 2.6(b)) produce harmful effects on nasal mucosa (olfactory response) via the intraperitoneal route. BAM is less potent than dichlobenil in eliciting an olfactory response. Dichlobenil is also shown to produce an olfactory response via dermal, inhalation and oral routes of exposure. If the level is exceeded, it has been shown that animals can recover from the harmful effects of dichlobenil on the nasal mucosa, and also there is a threshold for dichlobenil and BAM below which the effects are not observed.

Toxicity of metabolites

The current Meeting evaluated the following BAM metabolites found in plants or animal tissues: 2,6-dichloro-4-hydroxybenzonitrile, 6-chloro-3-hydroxy-2-thiobenzamide, 6-chloro-3-hydroxy-2-methylsulfinylbenzamide and 6-chloro-3-hydroxy-2-methylthiobenzamide. These chemicals were evaluated using the DEREK knowledge-based program to determine whether there are any structural alerts for toxicity. The DEREK analysis did not give any alerts for long-term

toxicity, similar to the findings for the parent chemical BAM. Based on a lack of any structural alerts and the fact that these compounds are the result of either hydroxylation or sulfate conjugation, the Meeting concluded that the above-mentioned BAM metabolites are likely to be of lower toxicity than the parent compound BAM.

3. Observations in humans

No information was provided.

Comments

Biochemical aspects

Following gavage dosing of rats at 5.6 mg/kg bw, dichlobenil was extensively absorbed; about 89% and 97% of the administered dose were absorbed in males and females, respectively, in 7 days, based on a comparison of urinary excretion following oral and intravenous administration. Oral absorption after repeated exposure was at least 60%, irrespective of dose or sex. Liver, kidney and kidney fat reached the highest concentrations of radioactivity; concentrations peaked 1–3 hours after administration in these organs and in plasma. The elimination of the radioactivity associated with [¹⁴C]dichlobenil following oral administration was rapid, with most (about 80%) being eliminated within 24 hours at the low dose. About 60% was excreted in the urine and about 20% in the faeces, irrespective of sex or dose regimen, within 7–10 days after initiation of exposure. Approximately 79% of the administered dose was excreted in the bile within 24 hours in bile duct–cannulated rats, suggesting enterohepatic recirculation.

Dichlobenil was extensively metabolized, and no parent compound was detected in the urine. Most of the urinary metabolites were conjugates, such as sulfate, glutathione (and derivatives) and glucuronic acid conjugates. In faeces, three major metabolites (each representing at least 5% of the administered dose) were observed, as well as parent compound (dichlobenil). The proportion of conjugated metabolites decreased with increasing dose, whereas the amount of parent compound in faeces increased. Dichlobenil was metabolized in rats via two metabolic pathways: the first pathway involves hydroxylation at the 3 or 4 position, followed by glucuronidation or sulfation, and the second pathway involves substitution of one chlorine atom by glutathione.

Toxicological data

The acute oral and dermal LD₅₀s were greater than 2000 mg/kg bw in rats and rabbits, respectively. The acute inhalation LC₅₀ in rats was greater than 3.2 mg/L. Dichlobenil was non-irritating to rabbit skin and eyes. It was not a skin sensitizer in guinea-pigs, as determined by the Magnusson and Kligman maximization test.

The liver was the primary target organ in mice, rats, hamsters and dogs in repeated-dose toxicity studies.

In a 90-day toxicity study in mice using dietary dichlobenil concentrations of 0, 25, 125, 625 and 3125 ppm (equal to 0, 3.8, 19, 91 and 447 mg/kg bw per day for males and 0, 4.8, 24, 114 and 512 mg/kg bw per day for females, respectively), the NOAEL was 625 ppm (equal to 91 mg/kg bw per day), based on transient clinical signs of toxicity, decreased body weight gains, decreased feed consumption and liver toxicity (increased liver weights, clinical chemistry, severity of centrilobular hypertrophy and glycogen storage) at 3125 ppm (equal to 447 mg/kg bw per day).

In a 90-day toxicity study in hamsters using dietary dichlobenil concentrations of 0, 41, 209, 1289 and 7500/4648 ppm (equivalent to 0, 3, 16, 79 and 395/263 mg/kg bw per day, respectively), the NOAEL was 41 ppm (equivalent to 3 mg/kg bw per day), based on decreased weight and mineralization of the prostate and decreased absolute seminal vesicle and testicular weights at 209 ppm (equivalent to 16 mg/kg bw per day).

In a 90-day toxicity study in rats, dichlobenil was administered in the diet at a concentration of 0, 100, 1000 or 3000 ppm (equivalent to 0, 10, 100 and 300 mg/kg bw per day, respectively); an additional group of males was fed 10 000 ppm (equivalent to 1000 mg/kg bw per day). The NOAEL was 100 ppm (equivalent to 10 mg/kg bw per day), based on hepatocellular necrosis and inflammation in males seen at 1000 ppm (equivalent to 100 mg/kg bw per day).

In a 90-day toxicity study in dogs using dietary dichlobenil concentrations of 0, 50, 150 and 450 ppm (equivalent to 0, 1.3, 3.8 and 11 mg/kg bw per day), the NOAEL was 150 ppm (equivalent to 3.8 mg/kg bw per day), based on increased alkaline phosphatase, ALT and liver and kidney weights at 450 ppm (equivalent to 11 mg/kg bw per day).

In a 52-week toxicity study in dogs administered dichlobenil by capsule at a dose of 0, 1, 6 or 36 mg/kg bw per day, the NOAEL was 1 mg/kg bw per day, based on increases in liver weights, serum cholesterol, triglycerides, phospholipids and alkaline phosphatase in both sexes and in gamma-glutamyltransferase and periportal hypertrophy of the hepatocytes in males at 6 mg/kg bw per day.

In a 2-year toxicity study in dogs using dietary dichlobenil concentrations of 0, 20, 50 and 350 ppm (equivalent to 0, 0.5, 1.3 and 8.8 mg/kg bw per day), the NOAEL was 50 ppm (equivalent to 1.3 mg/kg bw per day), based on increases in liver weight and serum alkaline phosphatase (in both sexes) and in serum ALT (females only) and liver histopathology (leukocytic infiltration around the central veins in both sexes and necrosis in males) seen at 350 ppm (equivalent to 8.8 mg/kg bw per day).

The overall NOAEL for the 1- and 2-year dog studies was 50 ppm (equivalent to 1.3 mg/kg bw per day), and the overall LOAEL was 6 mg/kg bw per day.

In a first toxicity and carcinogenicity study in hamsters (91 weeks for males and 78 weeks for females) using dietary dichlobenil concentrations of 0, 675, 1500 and 3375 ppm (equal to 0, 51, 117 and 277 mg/kg bw per day for males and 0, 55, 121 and 277 mg/kg bw per day for females, respectively), decreased body weight gain in males and females and increased relative liver weight in females were observed at all doses. No NOAEL could be identified. The NOAEL for carcinogenicity in male hamsters was 1500 ppm (equal to 117 mg/kg bw per day), based on an increased incidence of hepatocellular adenoma and carcinoma at 3375 ppm (equal to 277 mg/kg bw per day). No treatment-related tumours were observed in female hamsters.

In the second toxicity and carcinogenicity study in hamsters, dichlobenil was administered at a dietary concentration of 0, 5, 26, 132 or 675 ppm (equal to 0, 0.34, 1.69, 9.39 and 45.6 mg/kg bw per day for males and 0, 0.35, 1.78, 9.20 and 48.9 mg/kg bw per day for females, respectively) for 88 weeks (males) or 80 weeks (females). The NOAEL was 26 ppm (equal to 1.69 mg/kg bw per day), based on reduced secretion of the prostate and seminal vesicles at 132 ppm (equal to 9.39 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study in rats using dietary dichlobenil concentrations of 0, 50, 400 and 3200 ppm (equal to 0, 3.2, 29 and 241 mg/kg bw per day for males and 0, 3.2, 26 and 248 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 3.2 mg/kg bw per day), based on changes in clinical chemistry (increased BUN, cholesterol), gross pathology (enlarged liver and kidney) and histopathology (nephrosis, parathyroid hyperplasia) in males and enlarged liver with increased liver weight and polyploidy with hepatocytic swelling in the liver in females at 400 ppm (equal to 26 mg/kg bw per day). There was an increased incidence of hepatocellular tumours at 3200 ppm in both sexes, reaching statistical significance only in females. The NOAEL for carcinogenicity was 400 ppm (equal to 26 mg/kg bw per day).

The Meeting concluded that dichlobenil is carcinogenic in rats and hamsters.

Dichlobenil was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that dichlobenil is unlikely to be genotoxic.

In view of the lack of genotoxicity and on the basis of other available toxicological information, the Meeting concluded that the mode of action for the increased incidences of hepatocellular adenomas and carcinomas in male and female rats and male hamsters, although not completely understood, is likely to involve a threshold. Therefore, the Meeting concluded that dichlobenil is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats given diets containing dichlobenil at a concentration of 0, 60, 350 or 2000 ppm (equivalent to 0, 4, 23 and 130 mg/kg bw per day, respectively), the NOAEL for parental toxicity was 350 ppm (equivalent to 23 mg/kg bw per day), based on decreased body weight gains during premating (males and females) and gestation (females) in both generations, decreased feed consumption during premating in both generations (males and females) and a decreased number of implantations per dam in F₁ females at 2000 ppm (equivalent to 130 mg/kg bw per day). The NOAEL for offspring toxicity was 60 ppm (equivalent to 4 mg/kg bw per day), based on decreased body weight during weaning in both generations seen at 350 ppm (equivalent to 23 mg/kg bw per day). The NOAEL for reproductive toxicity was 350 ppm (equivalent to 23 mg/kg bw per day), based on a decreased number of implantations per dam in F₁ females seen at 2000 ppm (equivalent to 130 mg/kg bw per day).

In a prenatal developmental toxicity study in rats that used dichlobenil doses of 0, 20, 60 and 180 mg/kg bw per day, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased body weight gain, feed consumption and feed efficiency during the dosing period seen at 60 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 180 mg/kg bw per day, the highest dose tested.

In a prenatal developmental toxicity study in rabbits that tested at dichlobenil doses of 0, 15, 45 and 135 mg/kg bw per day, the NOAEL for maternal toxicity was 45 mg/kg bw per day, based on decreased body weight gain and feed consumption during the dosing period at 135 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 45 mg/kg bw per day, based on an increased number of total resorptions per dam, increased post-implantation loss and increased incidences of external, visceral and skeletal malformations (e.g. open eyes, major fusion of sternebrae, cleft palate) seen at 135 mg/kg bw per day.

The Meeting concluded that dichlobenil is teratogenic in rabbits, but not in rats.

No evidence of immunotoxicity was observed in an immunotoxicity study in rats administered dichlobenil by gavage at a dose level of 0, 20, 60 or 180 mg/kg bw per day for 28 days.

The Meeting concluded that dichlobenil is not immunotoxic.

Dichlobenil produces harmful effects on nasal mucosa via dermal, inhalation and oral routes of exposure. These effects on nasal mucosa are reversible, and there is a threshold below which the effect was not observed.

Toxicological data on metabolites and/or degradates

The acute oral LD₅₀s for 2,6-dichlorobenzamide (BAM; a plant metabolite and soil degradate) were 1538 and 1144 mg/kg bw for male and female mice, respectively.

In a 13-week toxicity study in rats using dietary BAM concentrations of 0, 50, 180, 600 and 2300 ppm (equal to 0, 4, 14, 49 and 172 mg/kg bw per day, respectively), the NOAEL was 180 ppm (equal to 14 mg/kg bw per day), based on reduced skeletal muscle tone (males and females) and decreased body weight gain (females) seen at 600 ppm (equal to 49 mg/kg bw per day).

In a 2-year study of toxicity in dogs using dietary BAM concentrations of 0, 60, 100, 180 and 500 ppm (equivalent to 0, 1.5, 2.5, 4.5 and 12.5 mg/kg bw per day, respectively), the NOAEL was 180 ppm (equivalent to 4.5 mg/kg bw per day), based on decreased body weight and body weight gain seen at 500 ppm (equivalent to 12.5 mg/kg bw per day).

In a 2-year study of carcinogenicity in rats using dietary BAM concentrations of 0, 60, 100, 180 and 500 ppm (equal to 0, 2.2, 3.6, 6.5 and 19 mg/kg bw per day for males and 0, 2.8, 4.7, 8.5 and 25 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 4.7 mg/kg bw per

day), based on decreased body weight and body weight gain and an increased incidence of hepatocellular alteration (eosinophilic foci) seen in females at 180 ppm (equal to 8.5 mg/kg bw per day). The NOAEL for carcinogenicity was 180 ppm (equal to 8.5 mg/kg bw per day), based on a marginally significant increase in the incidence of hepatocellular adenomas seen in females at 500 ppm (equal to 25 mg/kg bw per day).

The Meeting concluded that BAM is carcinogenic in rats.

BAM was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that BAM is unlikely to be genotoxic.

In view of the lack of genotoxicity and on the basis of other available toxicological information, the Meeting concluded that the mode of action for the increased incidence of hepatocellular adenomas in female rats, although not completely understood, is likely to involve a threshold. Therefore, the Meeting concluded that BAM is unlikely to pose a carcinogenic risk to humans from the diet.

In a three-generation reproductive toxicity study in rats given diets providing a BAM dose of 0, 60, 100 or 180 ppm (equivalent to 0, 4.0, 6.7 and 12 mg/kg bw per day, respectively), the NOAEL for parental toxicity, offspring toxicity and reproductive toxicity was 180 ppm (equivalent to 12 mg/kg bw per day), the highest dose tested.

In a prenatal developmental toxicity study in rabbits that administered BAM by gavage at a dose of 0, 10, 30 or 90 mg/kg bw per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day, based on late abortion, thin appearance and severely decreased body weight gain and feed consumption during the dosing period at 90 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 30 mg/kg bw per day, based on increased incidences of late abortion and skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) malformations observed at 90 mg/kg bw per day.

The Meeting concluded that BAM is teratogenic in rabbits.

BAM produces harmful effects on nasal mucosa via the intraperitoneal route of exposure. The reversibility of these effects was not investigated. There is a threshold below which the effects were not observed.

Human data

No information on employees working in dichlobenil manufacturing plants was provided.

The Meeting concluded that the existing database on dichlobenil was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

Dichlobenil

The Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw on the basis of an overall NOAEL of 1.3 mg/kg bw per day in 1- and 2-year dietary studies of toxicity in dogs, on the basis of liver toxicity (increased liver weight, liver enzymes, cholesterol and triglycerides) at 6 mg/kg bw per day. A safety factor of 100 was applied. This ADI is supported by an overall NOAEL of 1.69 mg/kg bw per day in a dietary carcinogenicity study in hamsters, based on reduced secretion of the prostate and seminal vesicles, decreased body weight gain and hyperplasia of the adrenal cortex, small intestine and bone marrow observed at 9.39 mg/kg bw per day. The upper bound of the ADI provides a margin of exposure of at least 24 000 relative to the LOAEL for liver tumours in hamsters and rats.

An acute reference dose (ARfD) of 0.5 mg/kg bw was established on the basis of a NOAEL of 45 mg/kg bw per day in a study of developmental toxicity in rabbits, based on increased incidences of external, visceral and skeletal malformations (e.g. open eyes, major fusion of sternebrae, cleft palate) seen at 135 mg/kg bw per day. A safety factor of 100 was applied. This ARfD applies to women of childbearing age only. The Meeting concluded that it is not necessary to establish an ARfD for the remainder of the population in view of the low acute oral toxicity of dichlobenil and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

2,6-Dichlorobenzamide (BAM)

Based on a re-evaluation of the data, the Meeting withdrew the ADI and ARfD for 2,6-dichlorobenzamide (BAM) established by the 2009 JMPR as part of the evaluation of fluopicolide.

The Meeting established an ADI of 0–0.05 mg/kg bw for 2,6-dichlorobenzamide (BAM) on the basis of a NOAEL of 4.5 mg/kg bw per day in a 2-year dietary study of toxicity in dogs, based on decreased body weight and body weight gain at 12.5 mg/kg bw per day. A safety factor of 100 was applied. This ADI is supported by a NOAEL of 4.7 mg/kg bw per day in a 2-year dietary carcinogenicity study in rats, based on decreased body weight and body weight gain and an increased incidence of hepatocellular alteration (eosinophilic foci) in females observed at 8.5 mg/kg bw per day. The upper bound of the ADI provides a margin of exposure of at least 500 relative to the LOAEL for liver tumours in rats and also a 240-fold margin of exposure relative to the highest dose tested in a three-generation reproductive toxicity study in rats, at which no effects were observed.

The Meeting established an ARfD of 0.3 mg/kg bw for 2,6-dichlorobenzamide (BAM) on the basis of a NOAEL of 30 mg/kg bw per day in a developmental toxicity study in rabbits, based on increased incidences of skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) malformations seen at 90 mg/kg bw per day. A safety factor of 100 was applied. This ARfD applies to women of childbearing age only. The Meeting concluded that it is not necessary to establish an ARfD for the remainder of the population in view of the low acute oral toxicity of BAM and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment of dichlobenil

Species	Study	Effect	NOAEL	LOAEL
Hamster	Eighteen-month studies of toxicity and carcinogenicity ^{a,b}	Toxicity	26 ppm, equal to 1.69 mg/kg bw per day	132 ppm, equal to 9.39 mg/kg bw per day
		Carcinogenicity	1 500 ppm, equal to 117 mg/kg bw per day	3 375 ppm, equal to 277 mg/kg bw per day
Rat	Two-year study of toxicity and carcinogenicity ^a	Toxicity	50 ppm, equal to 3.2 mg/kg bw per day	400 ppm, equal to 26 mg/kg bw per day
		Carcinogenicity	400 ppm, equal to 26 mg/kg bw per day	3 200 ppm, equal to 241 mg/kg bw per day
	Two-generation study of reproductive toxicity ^a	Reproductive toxicity	350 ppm, equivalent to 23 mg/kg bw per day	2 000 ppm, equivalent to 130 mg/kg bw per day
		Parental toxicity	350 ppm, equivalent to 23 mg/kg bw per day	2 000 ppm, equivalent to 130 mg/kg bw per day
		Offspring toxicity	60 ppm, equivalent to 4 mg/kg bw per day	350 ppm, equivalent to 23 mg/kg bw per day
	Developmental toxicity study ^c	Maternal toxicity	20 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	180 mg/kg bw per day ^d	–

Species	Study	Effect	NOAEL	LOAEL
Rabbit	Developmental toxicity study ^c	Maternal toxicity	45 mg/kg bw per day	135 mg/kg bw per day
		Embryo and fetal toxicity	45 mg/kg bw per day	135 mg/kg bw per day
Dog	One- ^c and 2-year studies of toxicity ^{a,b}	Toxicity	50 ppm, equivalent to 1.3 mg/kg bw per day	6 mg/kg bw per day

^a Dietary administration.

^b Two or more studies combined.

^c Gavage administration, including capsules.

^d Highest dose tested.

Levels relevant to risk assessment of BAM

Species	Study	Effect	NOAEL	LOAEL
Rat	Two-year study of toxicity and carcinogenicity ^a	Toxicity	100 ppm, equal to 4.7 mg/kg bw per day	180 ppm, equal to 8.5 mg/kg bw per day
		Carcinogenicity	180 ppm, equal to 8.5 mg/kg bw per day	500 ppm, equal to 25 mg/kg bw per day
	Three-generation study of reproductive toxicity ^a	Reproductive toxicity	180 ppm, equivalent to 12 mg/kg bw per day ^b	–
		Parental toxicity	180 ppm, equivalent to 12 mg/kg bw per day ^b	–
		Offspring toxicity	180 ppm, equivalent to 12 mg/kg bw per day ^b	–
	Developmental toxicity study ^c	Maternal toxicity	30 mg/kg bw per day	90 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	90 mg/kg bw per day
Dog	Two-year study of toxicity ^a	Toxicity	180 ppm, equivalent to 4.5 mg/kg bw per day	500 ppm, equivalent to 12.5 mg/kg bw per day

^a Dietary administration.

^b Highest dose tested.

^c Gavage administration.

Estimate of acceptable daily intake (ADI)

0–0.01 mg/kg bw for dichlobenil

0–0.05 mg/kg bw for 2,6-dichlorobenzamide (BAM)

Estimate of acute reference dose (ARfD)

0.5 mg/kg bw for dichlobenil (applies to women of childbearing age only)

0.3 mg/kg bw for 2,6-dichlorobenzamide (BAM) (applies to women of childbearing age only)

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to dichlobenil*Absorption, distribution, excretion and metabolism in mammals*

Rate and extent of oral absorption	Rapidly and completely absorbed from gastrointestinal tract (at least 89% in 7 days)
Dermal absorption	No data
Distribution	Rapidly distributed, highest concentrations in liver, kidney, kidney fat and brown fat; concentrations peaked in 1–3 hours
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid; about 80% excreted within first 24 hours following single low dose
Metabolism in animals	Extensive, 10 metabolites in urine (no parent) and four metabolites and parent compound in faeces
Toxicologically significant compounds in animals and plants	Dichlobenil and 2,6-dichlorobenzamide (BAM; plant metabolite)

Acute toxicity

Rat, LD ₅₀ , oral	> 2 000 mg/kg bw
Rabbit, LD ₅₀ , dermal	> 2 000 mg/kg bw
Rat, LC ₅₀ , inhalation	> 3.2 mg/L (4 hours)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)

Short-term studies of toxicity

Target/critical effect	Liver
Lowest relevant oral NOAEL	1.3 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, highest dose tested (rabbit)
Lowest relevant inhalation NOAEC	12 mg/m ³ , highest concentration tested (rat)

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Liver and kidney (rat) and liver (hamster)
Lowest relevant oral NOAEL	1.69 mg/kg bw per day (hamster)
Carcinogenicity	Liver tumours (male hamster, male and female rats); unlikely to pose a carcinogenic risk to humans from the diet

Genotoxicity

Unlikely to be genotoxic

Reproductive toxicity

Target/critical effect	Decreased body weights in adults and pups, decreased number of implantations per dam in F ₁ females
Lowest relevant parental NOAEL	23 mg/kg bw per day
Lowest relevant offspring NOAEL	4 mg/kg bw per day
Lowest relevant reproductive NOAEL	23 mg/kg bw per day

Developmental toxicity

Target/critical effect	Increased total resorptions, post-implantation loss and
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	malformations (rabbit)
Lowest relevant maternal NOAEL	20 mg/kg bw per day (rat)
Lowest relevant embryo/fetal NOAEL	45 mg/kg bw per day (rabbit)
<i>Neurotoxicity</i>	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data
<i>Other toxicological studies</i>	
Immunotoxicity NOAEL	180 mg/kg bw per day, highest dose tested
<i>Medical data</i>	
	No data provided
<i>Critical end-points for setting guidance values for exposure to BAM</i>	
<i>Acute toxicity</i>	
Mouse, LD ₅₀ , oral	1 140 mg/kg bw
<i>Short-term studies of toxicity</i>	
Target/critical effect	Body weight and body weight gain
Lowest relevant oral NOAEL	4.5 mg/kg bw per day (dog)
<i>Long-term studies of toxicity and carcinogenicity</i>	
Target/critical effect	Liver and body weights
Lowest relevant oral NOAEL	4.7 mg/kg bw per day (rat)
Carcinogenicity	Hepatocellular adenomas in female rats; unlikely to pose a carcinogenic risk to humans from the diet
<i>Genotoxicity</i>	
	Unlikely to be genotoxic
<i>Reproductive toxicity</i>	
Target/critical effect	None
Lowest relevant parental NOAEL	12 mg/kg bw per day, highest dose tested
Lowest relevant offspring NOAEL	12 mg/kg bw per day, highest dose tested
Lowest relevant reproductive NOAEL	12 mg/kg bw per day, highest dose tested
<i>Developmental toxicity</i>	
Target/critical effect	Increased incidences of skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) anomalies
Lowest relevant maternal NOAEL	30 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	30 mg/kg bw per day (rabbit)
<i>Neurotoxicity</i>	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data

Summary

	Value	Study	Safety factor
Dichlobenil			
ADI	0–0.01 mg/kg bw	One-year and 2-year studies of toxicity (dog)	100
ARfD ^a	0.5 mg/kg bw	Developmental toxicity study (rabbit)	100
2,6-Dichlorobenzamide (BAM)			
ADI	0–0.05 mg/kg bw	Two-year study of toxicity (dog)	100
ARfD ^a	0.3 mg/kg bw	Developmental toxicity study (rabbit)	100

^a Applies to women of childbearing age only.

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