

DICLORAN

*First draft prepared by
C.E. Moase*

Health Evaluation Division, Pest Management Regulatory Agency, Ottawa, Ontario, Canada

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Explanation

Dicloran was evaluated for toxicological effects by the Joint Meetings in 1974 and 1977 (Annex 1, references 22 and 28). A temporary ADI of 0–0.03 mg/kg bw was established in 1974 on the basis of the results of a two-year study in dogs and short- and long-term studies in rats. The 1977 Meeting established an ADI of 0–0.03 mg/kg bw on the basis of these studies, after examination of further data on oculotoxicity in dogs, metabolism and pharmacokinetics in pigs, and the effects of dicloran on liver microsomal enzymes, in compliance with the request of the 1974 Joint Meeting.

Dicloran was reviewed by the present Meeting within the CCPR periodic review programme. In addition to studies previously reviewed, newly available studies, including those on metabolism, short-term toxicity in mice and rats, carcinogenicity in mice, genotoxicity, reproductive toxicity in rats, and developmental toxicity in rabbits, were reviewed. This monograph summarizes the new data and includes relevant data from the previous monograph and monograph addendum (Annex 1, references 23 and 29).

Evaluation for acceptable daily intake

1. Biochemical aspects

(a) *Absorption, distribution, and excretion*

The 1974 Joint Meeting concluded that dicloran is rapidly absorbed, metabolized, and excreted by mammals, including humans, with the formation of chlorinated phenylenediamine and aminophenol, which are conjugated and excreted.

When dicloran was administered orally or intraperitoneally at single doses of 10–40 mg/kg bw to rats, 91% of the oral dose was excreted in the urine within three days (77% in 24 h) and 1% in the faeces, and 83% of the intraperitoneal dose was excreted in the urine within three days (70% in 24 h) and 1.5% in the faeces. A small amount appeared to be excreted in the bile, amounting to 2% of the dose 6 h after intraperitoneal injection and 5% within 12 h after oral delivery (Maté et al., 1967).

¹⁴C-Dicloran administered orally to groups of three male Sprague-Dawley rats at a dose of 1.7 or 8 mg/kg bw was rapidly excreted. Urinary excretion accounted for approximately 90% of the administered dose, which was recovered within 48 h; up to 85% was recovered within 8 h of treatment. The remainder was present in the faeces. Small quantities of radiolabel were detected in the gastrointestinal tract, urinary tract, and liver. ¹⁴C-Dicloran administered to three male volunteers at a dose of 50 mg was also rapidly absorbed and excreted, with 72–79% of the administered dose in urine and 24–36% in faeces. Although recovery was considered to be complete, excretion was somewhat slower in humans than in rats, with 49% of the administered dose recovered in urine and faeces within 24 h and 76% within 48 h; most of the material was excreted within 1.5 days (Eberts, 1965).

[U-¹⁴C]-Dicloran (purity, > 99%) was administered by gavage to groups of five male and five female Sprague-Dawley rats as a single dose of 5 or 500 mg/kg bw. Dicloran appeared to be well absorbed from the intestinal tract and was rapidly excreted in the urine, most of the radiolabel (90–96%) being excreted within 24 h of administration of the low dose and 91% within 48 h of the high dose; 93–98% had been eliminated by day 4 after treatment with both the low and the high dose. The urine was the principle route of elimination (> 77% at the low dose, > 70% at the high dose), with an additional 13% eliminated in the faeces of animals at the low dose and 22% in faeces of those at the high dose. Radiolabelled residues in the tissues and carcass accounted for < 1% of the administered dose, the liver and carcass containing the highest concentrations (O'Boyle & Challis, 1991a,b).

In groups of five hens dosed with ¹⁴C-dicloran in capsules (0.37 or 6 mg/hen per day) for five days, dicloran was rapidly absorbed, metabolized, and eliminated. More than 80% was excreted, and 50–57% of the administered dose was recovered within 24 h of dosing (Dawson, 1988).

In goats intubated with ¹⁴C-dicloran, the administered radiolabel was completely recovered from urine and faeces by 72 h; 38% was recovered within the first 24 h from goats treated with 1.5 mg/kg bw and 96% from those given 8 mg/kg bw. More than 10 times more residues were found in goats than in Sprague-Dawley rats 72 h after treatment, with only trace amounts of residues in other tissues. The liver residues in goats could not be extracted with organic solvents, whereas most of the radiolabelled residues were extractable from rat liver. In contrast, muscle residues from goats treated with a single dose of 8 mg/kg bw were not covalently bound to macromolecules (Jaglan & Arnold, 1985a,b; Jaglan et al., 1985a). In a lactating goat given 613 mg ¹⁴C-dicloran in a capsule daily for five days, 0.5% of the administered radiolabel was detected in milk. The parent compound and 4-amino-2,6-dichlorophenol were the major residues (Cheng, 1996a).

(b) Biotransformation

Preliminary studies suggested that dicloran metabolites in humans are similar to those in rats. 2,6-Dichloro-4-hydroxyaniline sulfate represented about 85% of the total radiolabel excreted in rat urine (Eberts, 1965).

¹⁴C-Dicloran administered to rats intraperitoneally or orally at a dose of 20 mg/kg bw was metabolized to dichloroamino-phenol and dichlorophenylenediamine derivatives. The major metabolite in urine was 2,6-dichloro-4-hydroxyaniline (4-amino-3,5-dichlorophenol), which represented 50% of the dose and 70% of the urinary activity. This was excreted as a conjugate, therefore undergoing rapid deactivation *in vivo*. The only other metabolite detected in the urine was

2,6-dichlorophenylenediamine (4-amino-2,6-dichloroaniline), representing 2.4% of urinary activity, although Cheng (1996b; see below) reported concentrations of < 0.1% of the administered dose. Studies with mouse liver microsomes *in vitro* showed limited conversion of dicloran to the same two metabolites (~20 % each; Maté et al., 1967). These were also reported to be the principal metabolites in dogs and monkeys (Bachmann et al., 1971).

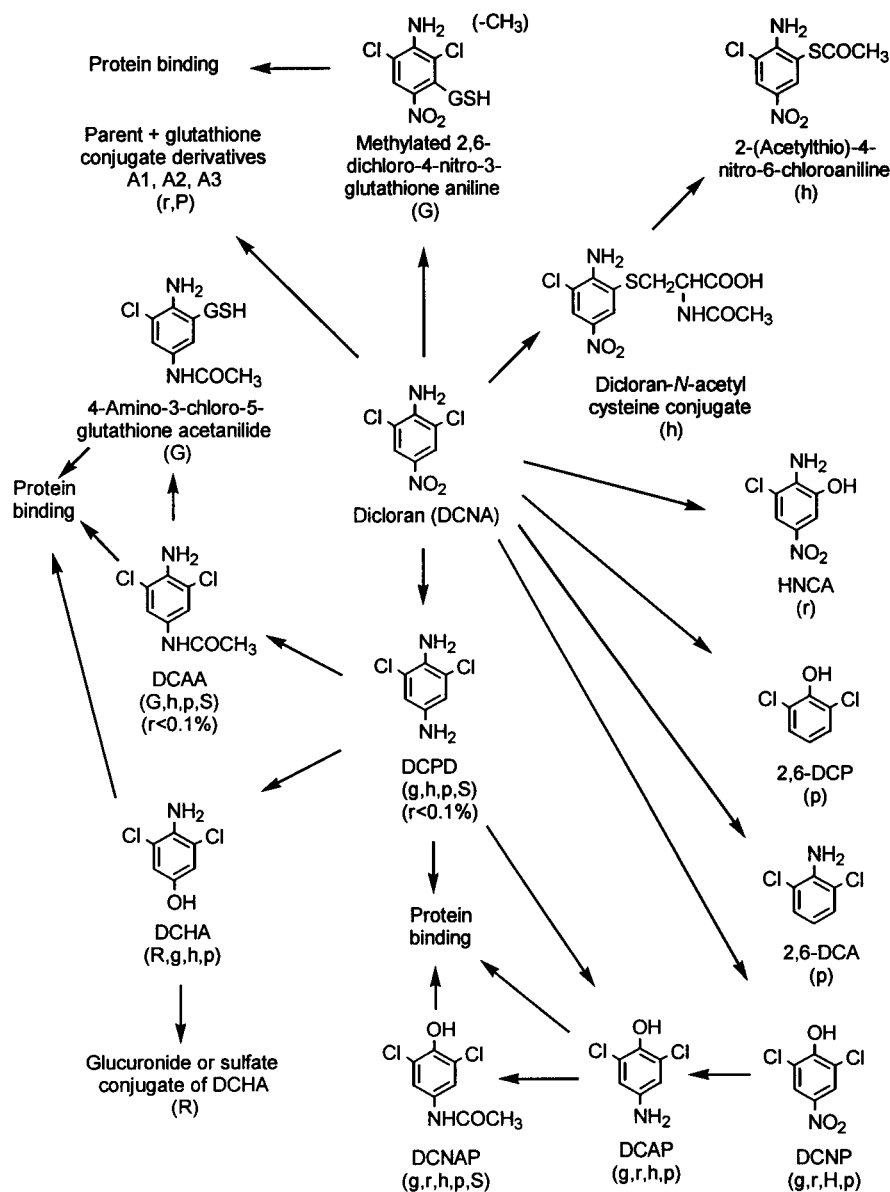
Groups of five Sprague-Dawley rats of each sex received repeated doses of 5 mg/kg bw unlabelled dicloran (purity, > 97%) by gavage for two weeks or a single oral dose of 500 mg/kg bw before treatment with [U-¹⁴C]-dicloran (purity, > 99%). The compound appeared to be well absorbed from the intestinal tract and was rapidly excreted in the urine, most of radiolabel being excreted within 24 h of dosing. There were no apparent sex-related differences in absorption or elimination, and urine was the principal route of excretion (~85% of the low dose, 66% of the high dose) in males and females. Within 48 h, 92–93% of the administered dose had been metabolized and excreted by animals at the low dose and 82–86% by those at the high dose. The concentrations of residues in tissues were low, the highest concentrations being found in liver (0.05–0.06 ppm) and kidney (0.02 ppm) seven days after dosing with 5 mg/kg bw; the concentrations in other tissues were ≤ 0.01 ppm. The concentrations of radiolabelled residues were comparable in males and females, and the total residues accounted for 0.2–0.3% of the administered dose. The major urine metabolites were 2,6-dichloro-4-hydroxyaniline sulfate (22–63% of administered dose) and 2,6-dichloro-4-hydroxyaniline glucuronide (16–29%). Unchanged parent compound was detected in faeces of animals at the high dose only. The major faecal metabolites were derivatives of glutathione conjugates. The excretion and metabolite profiles were essentially independent of dose and pretreatment, although there were some quantitative, sex-dependent differences in the distribution of major urinary metabolite fractions (Cheng, 1996b).

An integrated pathway for the metabolism of dicloran in rats, goats, hens, plants, and soil is indicated in Figure 1. The parent compound and its glutathione conjugates were the major metabolites of plants, comprising 32–51% of residues in peaches, potatoes, and lettuces (Hawkins et al., 1988; Smith, 1989; O'Neal, 1997a,b). None of the metabolites was of toxicological concern.

The highest residues in tissues of hens dosed with capsules containing ¹⁴C-dicloran at 150 g/bird per day for three days were found in egg yolk, fat, and liver (0.74, 0.21, and 0.10 g/g, respectively). Only the parent compound was detected in egg yolk and fat. The residues in liver consisted of 4-amino-3,5-dichloroacetilide (24%), 2,6-dichloro-4-nitrophenol (21%), and parent (54.8%; Dawson, 1988).

Groups of five hens treated with dicloran at a dose of 0.37 or 6 mg/day for five days excreted more than 80%, 3–11% of which was parent compound. The percentage of the residues that were extractable (i.e. unbound) was 87% (in liver) to 100%, with the highest residue concentrations in liver and egg yolk of hens given either dose and in abdominal fat of those given the high dose. Parent compound was the major component of fat (94%) and egg yolk (> 80%), and 2,6-dichloro-4-nitrophenol was the major metabolite in liver (45–58% of residue). 4-Amino-3,5-dichloroacetilide and 3,5-dichloro-4-hydroxyacetanilide were found at concentrations of 1–12% and 12–33%, respectively, in liver and muscle. The concentrations of other metabolites represented < 10% of radiolabel in tissue. The metabolites underwent subsequent sulfate or glutathione conjugation and excretion (Cheng, 1996c).

The major metabolite in rat urine (2,6-dichloro-4-hydroxyaniline) was not present in goat urine, and rat urine contained more polar metabolites than goat urine, whereas goat urine had more polar conjugates that could not be hydrolysed by glucuronidase or sulfatase. In goats, dicloran is reduced to 2,6-dichlorophenylenediamine and acetylated to 3,5-dichloro-4-aminoacetanilide (4–6% in urine), which is rapidly metabolized and excreted in the urine and faeces. Although the reactive intermediate is formed in goat liver and is bound covalently to macromolecules, goat liver-bound residues had little potential to form reactive intermediates after ingestion by rats (Jaglan & Arnold, 1985a; Jaglan et al., 1985a,b).

Figure 1. Proposed metabolic pathway for dicloran in rats, goats, hens, plants, and soil or sediment

DCNA, 2,6-dichloro-4-nitroaniline; HNCA, 2-hydroxy-4-nitro-6-chloroaniline; DCHA, 2,6-dichloro-4-hydroxyaniline; DCAP, 4-amino-2,6-dichlorophenol; DCNAP, 3,5-dichloro-4-hydroxyacetanilide; DCDP, 2,6-dichlorophenylenediamine; DCAA, 4-amino-3,5-dichloroacetanilide; DCNP, 2,6-dichloro-4-nitrophenol; DCP, 2,6-dichlorophenol; DCA, 2,6 dichloroaniline; A1, A2, A3, glutathione conjugate derivatives. R, G, H, P, S correspond to metabolites in rats, goats, hens, plants, and soil or sediment, respectively. Major metabolites are represented by letters in upper-case, minor metabolites are represented by lower-case letters.

(c) *Effects on enzymes and other biochemical parameters*

Oral administration of dicloran at a dose of 400 or 1000 mg/kg bw per day to rats for three months resulted in increased hepatic demethylase and desulfurase activity, and liver mitochondrial oxygen consumption was increased (Serrone et al., 1967). Dicloran at oral doses of ≥ 10 mg/kg bw stimulated rat liver mixed-function oxidases, and a dose of 500 mg/kg bw decreased mitochondrial

oxidation of succinate without concomitant uncoupling of oxidative phosphorylation. Oxidative phosphorylation was not affected by single doses at any concentration. Daily administration of 1000 mg/kg bw to rats induced uncoupling of oxidative phosphorylation after four days (Bachmann et al., 1971). These liver enzymes were not stimulated in rhesus monkeys treated with 160 mg/kg bw per day dicloran for three months (Serrone et al., 1967), and no effect was seen on brain or liver mitochondrial function in mice (Bachmann et al., 1971).

The 2,6-dichloro-4-hydroxyaniline metabolite was as active as 2,4-dinitrophenol *in vitro* in uncoupling oxidative phosphorylation, whereas dicloran was only one-tenth as effective. 2,6-Dichlorophenylenediamine had no effect on mitochondrial respiration or oxidative phosphorylation *in vitro* (Bachmann et al., 1971).

Dicloran and 2,6-dichloro-4-hydroxyaniline at 10^{-5} mol/L inhibited electron transport and uncoupled oxidative phosphorylation *in vitro*, whereas 2,6-dichlorophenylenediamine at 10^{-4} mol/L induced only slight uncoupling. These inhibitory effects *in vitro* cannot be considered serious adverse effects since they were not confirmed by similar observations *in vivo*. Mitochondria isolated from rats treated with dicloran or its metabolites were functionally normal (Gallo et al., 1976).

In a preliminary study, oral doses of 1000 mg/kg bw dicloran per day for four days to female Sprague-Dawley rats did not significantly induce cytochrome P450 activity (Basting et al., 1984). After oral administration of 100 mg/kg bw twice a day for four days to male Sprague-Dawley rats, however, significant increases were found in the activities of cytochrome P450 (55% increase), cytochrome_{b5} (24% increase), benzphetamine demethylase, and ethoxycoumarin deethylase (51–68% increases) in comparison with controls. The pattern of enzyme induction was reported to indicate that dicloran significantly induces phenobarbital-type enzymes in rat microsomes (Creedy et al., 1985).

2. Toxicological studies

(a) Acute toxicity

The results of studies on the acute toxicity of dicloran are presented in Table 1. Technical-grade dicloran was of low or slight acute toxicity by all routes of administration in all species examined. Oral administration resulted in nasal haemorrhage, paralysis, depression, and excessive yellow urine and faeces. Intraperitoneal administration resulted in sleep, depression, and excessive yellow urine and faeces. The effects of subcutaneous injection were confined to local damage in which the test substance became enclosed in a fibrous sac, sometimes followed by abscess formation and eventual ulceration. Dermal administration resulted in no systemic clinical effects.

Technical-grade dicloran (purity, 96%) was not irritating to the intact or abraded skin of male or female New Zealand white rabbits at a concentration of 500 mg per site (Racznik & Wood, 1980b) or after repeated daily applications of 10 mg dry material or 0.1 ml of 10% aqueous suspension to the abraded or unabraded skin of albino rabbits over five days (Boots Pure Drug Co., 1962).

Technical-grade dicloran (purity, 96%) caused a minimal conjunctival response in the eyes of New Zealand white rabbits, consisting of redness and chemosis 24 and 48 h after treatment. Corneal opacity or injury to superficial layers of the corneal or conjunctival epithelium was not observed (Racznik & Wood, 1980c).

Four successive daily applications of 2–3 mg dicloran to the conjunctival sac of four rabbits resulted in a slight inflammatory reaction of the cornea, which was of short duration (Boots Pure Drug Co., 1962).

Table 1. Acute toxicity of dicloran

Species	Strain	Sex	Route	LD ₅₀ (mg/kg bw)	Reference
Rat	Sherman SPF	M/F	Oral	> 4000	Gaines & Linder (1986)
Rat	NR	NR	Oral	4040	Boots Pure Drug Co. (1962)
Rat	NR	NR	Oral	~ 8000	Serrone et al. (1967)
Rat	NR	NR	Oral	> 10 000	Johnston & Ceru (1961)
Mouse	NR	NR	Oral	1500–2500	Boots Pure Drug Co. (1962)
Guinea-pig	NR	NR	Oral	1400	Boots Pure Drug Co. (1962)
Rabbit	New Zealand white	M/F	Dermal (abraded)	> 2000	Raczniak & Wood (1980a)
Mouse	NR	NR	Intraperitoneal	5500	Johnston & Ceru (1961)
Mouse	NR	NR	Intraperitoneal	2500	Boots Pure Drug Co. (1962)
Rat	NR	NR	Intraperitoneal	1500	Boots Pure Drug Co. (1962)
Rat	NR	NR	Subcutaneous	> 5000	Boots Pure Drug Co. (1962)
Mouse	NR	NR	Subcutaneous	> 6000	Boots Pure Drug Co. (1962)

SPF, specific pathogen-free; M/F, male and female; NR, not reported

Dicloran was inactive in two tests for skin sensitization in guinea-pigs (Boots Pure Drug Co., 1962; Johnston & Schwikert, 1963).

(b) *Short-term studies of toxicity*

Mice

Groups of Charles River COBS mice were fed diets containing 0, 1250, 2500, 5000, or 10 000 ppm technical-grade dicloran for two weeks, equivalent to 0, 190, 380, 750, and 1500 mg/kg bw per day. At 10 000 ppm, 20% of animals of each sex died; the only clinical sign observed was hunched posture. Food consumption and body weight were significantly reduced. Female mice fed 5000 or 10 000 ppm had significantly increased absolute liver weights, and animals of each sex fed doses of 2500 ppm or more had an increased liver:body-weight ratio. Food consumption was slightly reduced in animals of each sex at 2500 and 5000 ppm, and a slight reduction in food consumption was noted at 1250 ppm only in females (Mallyon & Rawcliffe, 1985).

Groups of 10 male and 10 female CD-1 (ICR) mice received dicloran (purity, 95.8%) in the diet at 0, 300, 600, or 1200 ppm, equal to 0, 49, 100, and 200 mg/kg bw per day in males and 0, 53, 100, and 220 mg/kg bw per day in females, for 60 days. There were no treatment-related effects on survival, clinical signs, body weight, food or water consumption, or food conversion. A significant increase in methaemoglobin concentrations in the blood of both males and females was noted at 600 and 1200 ppm (males: 1, 1.3, 1.4, and 1.6%; females: 0.6, 0.7, 0.9, and 1.2%, control to high dose, respectively). Cholesterol and bilirubin concentrations were significantly increased in high-dose females (cholesterol: 2.4 ± 0.58 , 2.2 ± 0.50 , 2.5 ± 0.2 , 3.0 ± 0.65 mmol/L; bilirubin: 4.3 ± 0.6 , 5.4 ± 1.4 , 5.5 ± 2.4 , 5.8 ± 1.7 mol/L, control to high dose, respectively). These changes were dose-related but were not observed in males, which, unlike females, had specific liver lesions. Minimal to slight centrilobular hepatocytic enlargement (2–5/10 versus 0/10 controls) and fatty liver degeneration (2–4/10 versus 0/10 controls) with an increased severity of haemosiderosis in the spleen was noted in males at 600 and 1200 ppm, and a slight increase in the degree of haematopoietic activity was noted in the spleen of males and females combined at the intermediate and high doses. The NOAEL was 300 ppm, equal to 49 mg/kg bw per day in males (Mallyon et al., 1986).

Dicloran (purity, 97%) was administered to groups of 15 B6C3HF₁ mice of each sex (five of each sex per dose for interim sacrifice) by gavage at doses of 0, 15, 45, 135, 400, or 600 mg/kg bw per day for 90 days, with an interim sacrifice at 49 days. There were no treatment-related effects on survival or body weight; data on food consumption were not provided. Significant polycythaemia was seen in males, and hypercholesterolaemia at 1.2–2.1 times the control level and a significant increase in the incidence of splenic extramedullary haematopoiesis were seen in females at

≥ 45 mg/kg bw per day. Dose-related increases in cholesterol concentrations (1.3–2.3 times the control concentration) were also noted in males, which were statistically significant at ≥ 135 mg/kg bw per day. A deep-yellow colouration of the urine was noted in all treated groups and was attributed to the yellow colour of the test material. In the absence of any associated lesion in the group at the lowest dose, this effect was considered not to be adverse. Other effects at higher doses included kidney nephrosis in males at 400 and 600 mg/kg bw per day (5, 4, 6, 5, 15, 14/15, control to high dose, respectively) and females (3, 5, 2, 4, 8, 12/15), with kidney tubular regeneration in males (0, 0, 3, 1, 8, 11/15), increased liver weights in animals of each sex at ≥ 135 mg/kg bw per day (7–64% increase), hepatocyte hypertrophy in males (0, 0, 0, 4, 15, 15/15) and females (0, 0, 0, 0, 5, 12/15) at ≥ 400 mg/kg bw per day, liver necrosis in males (0, 0, 0, 0, 3, 3/15) and females (0, 0, 0, 0, 1, 1/15) at 400 and 600 mg/kg bw per day, a significantly higher incidence of splenic extramedullary haematopoiesis in males at ≥ 135 mg/kg bw per day (0, 1, 2, 13, 15, 15/15) and in females at ≥ 45 mg/kg bw per day (2, 5, 9, 10, 14, 15/15), increased spleen weight at the two higher doses (11–29% increase), and epithelial hyperplasia of the urinary bladder in males (0, 1, 2, 10, 12, 15/15) and females (0, 0, 0, 1, 7, 13/15) at doses ≥ 135 mg/kg bw per day. The NOAEL was 15 mg/kg bw per day (Kakuk, 1986).

Rats

Dicloran (unspecified purity) was administered by gavage to rats (strain unspecified) at 400 or 1000 mg/kg bw per day for three months. Deaths occurred among rats at the high dose. Significantly enlarged livers and unspecified renal changes were observed by light and electron microscopy, with increases in liver mitochondrial enzyme activity and mitochondrial oxygen use (Serrone et al., 1967).

Groups of five newly weaned rats (strain unspecified) of each sex were given dicloran (purity unspecified) by gavage at doses of 0, 140, or 350 mg/kg bw per day, six days/week for four weeks. The growth of males at 350 mg/kg bw per day was reduced to 60% of the control value. At both doses, increased liver weight (120–155% of control) with hepatic vacuolation were noted. There was no apparent effect on blood parameters or on the kidneys at the end of the study (Boots Pure Drug Co., 1962).

Groups of 10 rats (strain unspecified) were given dicloran (purity unspecified) by gavage at doses of 35, 140, or 350 mg/kg bw per day, six days/week, for four weeks. Groups of 20 controls of each sex were available. Growth depression was observed in animals of each sex at 350 mg/kg bw per day and in males at 140 mg/kg bw per day. Increased liver weights and microscopic findings were also seen at these doses. The liver lesions included a dose-related increase in hepatocytic hypertrophy with increased vacuolization, especially in the outer lobes. The liver had returned to normal size in half of the animals which were maintained for two weeks after the end of the treatment, except for males at the highest dose. The histopathological changes in the liver caused by repeated short-term dosing was apparently reversible within two weeks of the end of treatment. The NOAEL was 35 mg/kg bw per day on the basis of effects on body weight and liver at 140 mg/kg bw per day (Boots Pure Drug Co., 1962).

Technical-grade dicloran was fed to rats (strain unspecified) for six months at doses of 0 (25 rats), 30 (15 rats), 300 (15 rats), or 3000 (10 rats) ppm, equal to 0, 2.2, 22, and 230 mg/kg bw per day in males and 0, 2.5, 25, and 270 mg/kg bw per day in females. Another 10 animals of each sex per group received recrystallized dicloran at 3000 ppm, equivalent to 230 mg/kg bw per day for males and 260 mg/kg bw per day for females. At 3000 ppm, growth of both males and females was impaired (70–83% of control), whereas males fed purified material had only a slight reduction in growth (89% of control). Significantly increased liver weights (120–144% of control) were noted in both groups at the high dose, with a significant increase in spleen weight (129% of control) in females. Haematological parameters were not affected at 12 weeks or at termination, and no microscopic lesions were noted. The NOAEL was 300 ppm, equal to 22 mg/kg bw per day, on the basis of changes in body weight and increased liver and spleen weights at 3000 ppm (Boots Pure Drug Co., 1962).

Dicloran (purity, 97.4%) was administered to groups of 10 male and 10 female Sprague-Dawley rats in the diet at doses of 0, 1000, 3000, or 5000 ppm, equal to 0, 75, 230, and 370 mg/kg bw per day in males and 0, 80, 230, and 420 mg/kg bw per day in females, for 90 days. There were no treatment-related effects on survival or ophthalmological parameters. Significantly lower body-weight gain was seen in all treated groups (17–32% less than controls for males and 18–22% less for females). Although palatability may have accounted for the lower body-weight gains during the first week of the study, food consumption was comparable in all groups at week 2; however, it was consistently lower than control values throughout the remainder of the study (males, 10–14% less than controls; females, 12–21%; weeks 2–13). Many of the haematological and clinical chemical findings were considered to be associated with reductions in food consumption. The treatment-related hepatic effects included increased cholesterol concentrations, which were dose-related and statistically significant in males at all doses (1.3–1.5 times the control concentration) and in females at the intermediate and high doses (1.4–2.0 times control), minimal to moderate centrilobular hypertrophy in males in all treated groups (0, 3, 9, 9/10) and in females at the two higher doses (0, 0, 9, 9/10), and significant increases in liver weight in males and females at the two higher doses (29–66%). Minimal thyroid follicular-cell hypertrophy was noted in males in all treated groups (0, 5, 6, 6/10) and in females at the intermediate and high doses (0, 0, 4, 7/10). The dose of 1000 ppm, equal to a daily intake of 75 and 80 mg/kg bw per day for males and females, respectively, was considered to be at the upper limit of a suitable high-level dose for a long-term study of toxicity and carcinogenicity in rats. An NOAEL was not identified because significantly lower body-weight gains were seen at all doses (Peters et al., 1990).

A second short-term study was conducted to investigate the effects of dicloran at doses lower than those administered in the 90-day study. Dicloran (purity, 96.4%) was given in the diet to groups of 10 Sprague-Dawley rats of each sex at doses of 0, 500, or 750 ppm, equal to 0, 44, and 71 mg/kg bw per day for males, and 0, 48, and 71 mg/kg bw per day for females, for eight weeks. There were no treatment-related effects on survival. The overall body-weight gain of females at 750 ppm was significantly less than that of controls (12% less overall), the greatest difference occurring during the final two weeks of treatment (41–58% less than control). This decrease correlated with a slight decrease in food consumption in this group during treatment, which was 10–11% less than that of controls during the last two weeks of treatment. Increased liver weight relative to body weight (13–21%) in animals of each sex at 750 ppm was associated in 10/10 males with minimal centrilobular hepatocyte enlargement. The NOAEL was 500 ppm, equal to 44 mg/kg bw per day, on the basis of lower body-weight gain and decreased food consumption in females at 750 ppm and increased liver weights in males and females, with associated centrilobular hepatocyte enlargement in males at 750 ppm; however, a full histological examination was not carried out (only of the liver and thyroid), and although alterations were found in the livers of males at the high dose, the livers of males at the low dose were not examined histologically (Waterson et al., 1992).

Rabbits

Technical-grade dicloran (purity, 96.2%) was moistened with distilled water and applied to the intact skin of groups of five male and female New Zealand white rabbits under an occluded patch for 6 h per day for 21 consecutive days at doses of 12, 120, or 1200 mg/kg bw per day. Animals at the two higher doses showed yellow staining of untreated fur and extremities from day 4 onwards, but there were no adverse clinical findings. One male and one female rabbit at 12 mg/kg bw per day had slight, transient erythema lasting for two to four days, and most rabbits at 120 and 1200 mg/kg bw per day had slight erythema beginning in the second week of the study, which tended to persist until study termination. The reaction did not progress beyond slight erythema, and there did not appear to be a dose-related difference above 120 mg/kg bw per day. At termination, male rabbits at 1200 mg/kg bw per day had significantly greater (36%) adrenal weights than controls, but the adrenals were not examined histologically. The liver weights were 18% greater in females at 1200 mg/kg bw per day than in controls. No other effects were seen on body weight, food consumption, or haematological, biochemical, or histological parameters. The NOAEL was 120 mg/kg bw per day (Elliot et al., 1988).

Rats, rabbits, and dogs

Groups of 10 male and 10 female TUC/SPD rats, one male and one female New Zealand white rabbit, and two male beagle dogs were exposed to a dust aerosol of technical-grade dicloran by whole-body exposure for 6 h per day, five days per week for three weeks. The nominal concentration of the aerosol was 2 mg/L; the actual concentration and particle size were not provided. Two rats died on the third day of exposure, and one rabbit died on day 13. The food consumption and body-weight gains of the treated animals were depressed. There were no consistent haematological changes attributable to treatment. The blood cholesterol concentration was significantly elevated in exposed dogs and rabbits. The liver weights of exposed animals were increased, although the histological appearance was unremarkable, with no evidence of hepatocellular effects in any species (Seaman et al., 1980).

Monkeys

Oral doses of 160 mg/kg bw per day were lethal to rhesus monkeys within three months, with a greater effect on females than males. The colouration of the urine differed from that of rat urine, suggesting a difference in metabolism in the two species. Centrilobular fatty infiltration was observed in the liver, and liver and kidney changes were observed on electron microscopic examination, which included swelling of mitochondria with distortion of the cristae. Hepatic microsomal enzyme activity was not enhanced (Serrone et al., 1967).

*(c) Long-term studies of toxicity and carcinogenicity**Mice*

In a screening study, groups of 18 mice of each sex of each of two susceptible hybrid strains (unspecified) were given dicloran at 215 mg/kg bw per day for three weeks from day 7 after birth. Thereafter, for 18 months, the mice were fed 600 ppm (equivalent to 100 mg/kg bw per day) in the diet, sacrificed, and examined for tumours. Dicloran did not cause a significant increase in the incidence of tumours (Innes et al., 1969).

Dicloran (purity, 96.2–97.2%) was administered to groups of 50 CD-1 mice of each sex at dietary concentrations of 0, 50, 175, or 600 ppm, equal to 0, 7.4, 24, and 86 mg/kg bw per day in males and 0, 10, 36, and 120 mg/kg bw per day in females, for 80 weeks. The doses were based on the results of a 60-day dietary study in mice in which a concentration of 600 ppm was associated with increased methaemoglobin concentration, splenic pigment change, increased haematopoiesis, centrilobular hepatocyte enlargement, and fatty degeneration of hepatocytes. There was no adverse effect on mortality (percent survival at termination: males, 50, 50, 64, and 56%; females, 84, 76, 76, and 78%, control to high dose, respectively). There were no treatment-related effects on clinical signs, body weight, palpable masses, food consumption, food conversion, or differential leukocyte counts. Clinical chemistry and urinalysis were not performed. There was no increase in tumour incidence. The liver was the principal target organ, with increased absolute and relative weights (10–12%) in males and females at the high dose, which were statistically significant in females, and histopathological changes. These included centrilobular hepatocyte enlargement (males: 8, 8, 7, and 26/50; females: 1, 0, 2, and 5/50), centrilobular haemosiderosis (males: 1, 2, 4, and 12/50; females: 7, 5, 3, and 13/50), focal (4, 2, 5, and 10/50) and single-cell (1, 1, 0, and 6/50) liver necrosis in males, and vacuolation of centrilobular hepatocytes (4, 3, 3, and 12/50) in females. Acute inflammatory cell infiltration was seen in males at the intermediate and high doses (2, 4, 9, and 9/50), but no other hepatic changes were seen in males at this dose. Other changes at 600 ppm included a higher incidence of erythropoiesis in the spleens of males (7, 7, 8, and 15/50), an increased number of females with an enlarged/distended uterus (5, 9, 9, and 15/50), associated with a significant increase in the incidence and severity of cystic uterine endometrial hyperplasia (17, 21, 24, and 31/50), and an increased number of females at the high dose with distended mammary gland ducts (9/50 versus 3/50 in control; animals at the low and intermediate doses were not assessed). An 11% increase in kidney weight in males was not associated with

histopathological lesions. On the basis of the above findings, the NOAEL was 175 ppm, equal to 24 mg/kg bw per day (Mallyon & Markham, 1989).

Rats

Groups of 35 rats (strain unspecified) of each sex were fed dicloran (purity unspecified) in the diet at concentrations of 0, 20, 100, or 3000 ppm for two years, equal to 0, 1.6, 8, and 240 mg/kg bw per day, with an interim sacrifice of five animals of each sex per dose at 13 weeks. The dose of 100 ppm had no effect on behaviour, mortality, or growth, and all values were comparable to those of controls. At 3000 ppm, growth and food consumption of males and females were depressed, and haematological parameters (haemoglobin and packed cell volume) were reduced but only after the first year of treatment. Gross and microscopic examination at 13 weeks and at the conclusion of the study showed slightly heavier livers, kidneys, and testes in males at 3000 ppm. The incidence and location of neoplasms were similar in treated and control rats. Histological examination revealed changes in the liver at 3000 ppm, characterized by hepatic-cell enlargement, increased severity of glycogen depletion, increased basophilia of the cytoplasm, and the presence of dead cells. Other observations were increased thyroid hypertrophy and increased pigmentation of the spleen in males and females at 3000 ppm. Hepatic-cell changes and slight adrenal cortical atrophy were seen in several animals at this dose at 13 weeks, but the adrenal changes were not seen at 104 weeks. The NOAEL was 100 ppm, equal to 8 mg/kg bw per day (Woodard et al., 1964).

Groups of 25 Boots-Wistar strain rats of each sex were fed dicloran (purity unspecified) in the diet at a concentration of 0 or 1000 ppm for two years, equal to 59 mg/kg bw per day in males and 71 mg/kg bw per day in females. There was no effect on survival, food consumption, growth, haematological parameters, or gross or histological appearance of tissues and organs at the conclusion of the study. There were no differences in the size or cellular constitution of the liver, kidney, or spleen. The incidence of tumours in control and treated groups was similar. The NOAEL was 1000 ppm, equal to 59 mg/kg bw per day, the highest dose tested (Lessel, 1974).

Dogs

Groups of four male and four female beagle dogs were fed dry diets containing dicloran (purity unspecified) at concentrations of 0, 20, 100, or 3000 ppm for two years, equal to 0, 0.33, 1.7, and 44 mg/kg bw per day for males and 0, 0.36, 1.8, and 62 mg/kg bw per day for females, with an interim sacrifice of one animal of each sex per dose at 14 weeks. No compound-related changes in behaviour, food consumption, or growth were observed. One female at 3000 ppm died at 74 weeks, with treatment-related signs of haemolytic anaemia (reduced haemoglobin, immature erythrocytes, polychromophilic macrocytes, increased leukocyte count, and increased myeloid: erythroid ratio in the bone marrow) before death. One control male lost considerable weight but survived to the end of the study. All dogs at 3000 ppm had watery lachrymation, which persisted throughout treatment; the examination of the eyes was not adequate to determine any ocular toxicity of dicloran. Yellowing of the sclera, mucous membranes, and abdominal skin were noted at the high dose. Changes in haematological and clinical chemical parameters in males and females at the high dose included significant reductions in haemoglobin from week 26 to termination and reduced serum protein at termination; increased activity of alanine and aspartate aminotransferases and increased prothrombin time, blood urea nitrogen, and bromosulphthalein retention were observed in one male and the one female that died at the high dose, both of which had received 20–94% more test material than the other dogs in this group owing to gradual body-weight loss relative to the constant concentration in the diet. Gross and microscopic examination revealed significantly increased liver, spleen, and kidney weights accompanied by histological changes at 3000 ppm, which included irregular hepatic-cell size (3/5 versus 0/6 in other groups), moderate hepatic-cell hypertrophy (3/5 versus 0/6 in controls) and increased pigmentation of the liver (4/5 versus 0/6 in control), and spleen (5/5 versus 0/6 in controls). One male and two females at 3000 ppm (including the female that died) and one male at 20 ppm also had enlarged gall-bladders containing tar-like,

viscous bile, although this was stated to be a spontaneous lesion in dogs (cystic mucinous hypertrophy). Changes at 100 ppm which included irregular hepatic cells, slight focal hepatocytic hypertrophy, slight vacuolation, moderate liver pigmentation, and/or marked spleen pigmentation were noted in two female dogs. A company re-evaluation attributed the findings at 100 ppm to enzyme induction, although the histopathological findings in this group were complicated by the presence of parasitic hepatitis. No degenerative lesions were noted at study termination, and there was no progression in the severity of hepatocellular effects between interim and terminal sacrifice. The NOAEL was 100 ppm, equal to 1.7 mg/kg bw per day, on the basis of significant reductions in haemoglobin, reduced serum protein, and histopathological changes in the liver and spleen at 3000 ppm (Woodard et al., 1964; Kakuk et al., 1979).

(d) *Genotoxicity*

The results of studies on the mutagenicity and genotoxicity of dicloran are presented in Table 2. Dicloran gave occasional positive responses in the most recent tests for reverse mutation and in a test for mitotic recombination, with a 2–2.5-fold increase in mitotic recombination. The results of the test for sex-linked recessive lethal mutation in *Drosophila* were equivocal, even after re-analysis in conjunction with historical control data.

(e) *Reproductive toxicity*

(i) *Single-generation reproductive toxicity*

Male rats (strain unspecified) were treated with dicloran (purity unspecified) in the diet at concentrations of 0, 1000, or 2000 ppm, equivalent to 0, 50, and 100 mg/kg bw per day, for 90 days. The dose of 1000 ppm increased liver and kidney weights. When the males were mated with untreated females, no difference was observed in the number of litters or in the number of animals born or weaned (US Environmental Protection Agency, 1974).

Dicloran (purity unspecified) was fed to groups of 10 female rats at concentrations of 0, 500, or 1000 ppm, equivalent to 0, 25, and 50 mg/kg bw per day, for 188 days before mating and then through gestation and lactation. A reduced number of pups was reported when females were treated with 1000 ppm. There was no apparent effect on the survival of pups, although the mean body weight of those at 1000 ppm was slightly reduced (US Environmental Protection Agency, 1974).

(ii) *Multigeneration reproductive toxicity*

In a two-generation study of reproductive toxicity, with one litter per generation, dicloran (purity, 99.2%) was administered to groups of 28 male and 28 (F_0) and 24 male and 24 female (F_1) female Sprague-Dawley rats in the diet at doses of 0, 50, 250, or 1250 ppm, equal to 0, 4, 21, and 110 mg/kg bw per day. The doses were based on the results of a range-finding study (Wilcox & Barton, 1996) in which concentrations up to 1000 ppm did not produce any obvious alterations in body weight, food consumption, duration of gestation, or litter parameters. The animals were fed the test diet during the 10- (F_0) or 11-week (F_1) pre-mating period and then randomly allocated to mating pairs (1:1) for a maximum of seven nights. Treatment was continued for animals of each sex throughout the mating, gestation, and lactation periods until termination of the F_2 litters.

The treatment-related clinical signs included yellow staining of the tray paper and, in some cases, yellow staining of the fur of all animals at the high dose in both generations, due to the yellow colour of the test material. There were no significant differences in mean body weights or body-weight gain between control and treated males. The mean body weights of F_0 females at the high dose throughout the pre-mating period was 2–5% lower than that of controls, resulting in a 12% lower mean overall gain at the end of the pre-mating period. This was considered to be treatment-related. The mean body weights of F_1 females at the high dose were, on average, 6% lower than those of controls during pre-mating, but the overall mean body-weight gain was only 5% lower. F_0 and F_1 females at the high dose had slightly lower overall body-weight gain during gestation,

Table 2. Results of assays for the genotoxicity of dicloran

End-point	Test object	Concentration	Purity (%)	Result	Reference
<i>In vitro</i> Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	16, 31, 62, 125, 250, 500, 1000 µg/plate in DMSO	99.9	Negative ^b	Everest & Tuplin (1977)
Reverse mutation ^a	<i>S. typhimurium</i> TA1535, TA1536, TA1537, TA1538	100 µg/plate in DMSO	NR	Negative	Shirasu et al. (1976)
Reverse mutation ^c	<i>S. typhimurium</i> TA98, TA100	20–200 µg/plate in DMSO	99.5	Negative ^d	Jeang & Li (1978)
Reverse mutation ^c	<i>S. typhimurium</i> TA98, TA100	2, 20, 200 µg/plate in DMSO	99.5	Negative ^e	Jeang & Li (1980)
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	6 concentrations up to 10 000 µg/plate	NR	Negative	Waters et al. (1982); Garrett et al. (1986)
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA98 NRF, TA100, TA100 NRF	62, 100, 155, 200, 310, 415 µg/plate in ethyl cellosolve/ethanol or DMSO	NR	Positive ^f	Basting et al. (1983); Myers-Basting (1986)
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	50, 150, 500, 1500, 5000 µg/plate in DMSO	97.5	Positive ^h	Jones & Fenner (1987)
Reverse mutation ^a	<i>S. typhimurium</i> TA98, TA100	10, 33, 100, 333, 1000, 2000 µg/plate in DMSO	98	Positive ⁱ	Zeiger et al. (1992)
Reverse mutation ^c	<i>E. coli</i> WP2, WP2uvrA ⁻	62, 125, 250, 500, 1000 µg/plate in DMSO	99.9	Negative ^j	Everest & Tuplin (1977)
Reverse mutation ^c	<i>E. coli</i> B/r try WP2, WP2 try hcr	20 µg/plate in DMSO	NR	Negative	Shirasu et al. (1976)
Reverse mutation ^a	<i>E. coli</i> WP2uvrA ⁻	6 concentrations up to 10 000 µg/plate	NR	Negative ^k	Waters et al. (1982); Garrett et al. (1986)
Mitotic recombination ^a	<i>S. cerevisiae</i> D3	5 concentrations (unspecified)	NR	Negative	Waters et al. (1982)
Mitotic aneuploidy	<i>Neurospora crassa</i> I-41-5, I-34-8	5 or 10 µg/ml in DMSO	NR	Negative ^l	Griffiths et al. (1986); Moustacchi (1986)
DNA repair ^c	<i>B. subtilis</i> H17 rec ⁺ , M45 rec ⁻	20 g/disc in DMSO	NR	Negative	Shirasu et al. (1976)
DNA repair ^a	<i>B. subtilis</i> H17 rec ⁺ , M45 rec ⁻	20 g/disc in DMSO	99.5	Negative ^m	Jeang & Li (1978)
DNA repair ^c	<i>B. subtilis</i> H17 rec ⁺ , M45 rec ⁻	2 concentrations (not specified)	NR	Negative ^m	Waters et al. (1982); Garrett et al. (1986)
DNA repair ^c	<i>E. coli</i> P3478 (polA ⁻), W3110 (polA ⁺)	2 concentrations (not specified)	NR	Negative ^m	Waters et al. (1982); Garrett et al. (1986)
Unscheduled DNA synthesis	Rat hepatocytes	3, 4, 5, 6, 7, 8, 9, 10 µg/ml in DMSO	97.5	Negative ⁿ	McBride & McGregor (1987)
Mitotic recombination and aneuploidy	<i>Aspergillus nidulans</i> (heterozygous diploid strain)	2070, 4140, 6210 µg/plate in ethanol	NR	Positive ^o	Kappas (1978); Moustacchi (1986)
Cytogenetic alterations ^a	Human lymphocytes	2, 10, 20 µg/ml in DMSO	97.5	Negative ^p	Allen et al. (1988)
<i>In vivo</i> Recessive lethal mutation	<i>Drosophila melanogaster</i> Canton-S	Feeding at 1250 and 1389 ppm in 5% ethanol:5% Tween 80	98	Equivocal	Woodruff et al. (1985)

Table 2 (contd)

End-point	Test object	Concentration	Purity (%)	Result	Reference
<i>In vivo</i> (contd) Recessive lethal mutation	<i>Drosophila melanogaster</i> Canton-S	Re-analysis of data of Woodruff et al. (1985)	NR	Equivocal	Mason et al. (1992)

NR, not reported; DMSO, dimethyl sulfoxide; NRF, nitroreductase-free

^a With and without metabolic activation

^b The positive controls, cyclophosphamide, 6-aminochrysene, and 2-aminofluorene, gave the expected results.

^c Without metabolic activation

^d The positive control nitroquinoline *N*-oxide gave the expected result.

^e With metabolic activation

^f The positive control sterigmatocystin gave the expected result.

^g Positive in TA98 (≥ 100 $\mu\text{g}/\text{plate}$) only without metabolic activation

^h Positive in TA98 and TA1538 (≥ 500 $\mu\text{g}/\text{plate}$) with and without metabolic activation and in TA100 without metabolic activation. The positive controls, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine, 9-aminoacridine, 2-aminoanthracene, and 2-nitrofluorene, gave the expected results.

ⁱ Positive in TA98 (≥ 100 $\mu\text{g}/\text{plate}$) with and without metabolic activation; positive control unknown

^j The positive control ethyl methanesulfonate gave the expected result.

^k The positive controls, 2-aminoanthracene and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, gave the expected results.

^l This test system has not been fully validated. The positive control *para*-fluorophenylaniline gave the expected result.

^m The positive control methyl methanesulfoxide gave the expected result.

ⁿ The positive control, Mischler's ketone [4,4'-bis(dimethylaminobenzophenone) in ethanol], gave the expected result.

^o A 2–2.5-fold increase in mitotic recombination. This test system has not been fully validated.

^p The positive controls, ethyl methanesulfonate and cyclophosphamide, gave the expected results.

the greatest difference occurring during the first week of gestation (64% of control values in F_0 females and 86% in F_1). There were no apparent effects on the food consumption of F_0 or F_1 males or females during pre-mating and gestation; however, the food consumption of females of both generations at the high dose was 11% lower during the first week of lactation, and these animals weighed more than controls and those given lower doses at the end of lactation, indicating that pups in the high-dose group were less demanding with respect to suckling than pups in the other groups.

At the end of the study, a greater proportion of F_0 and F_1 females at the high dose were in proestrus (4/28 F_0 controls versus 19/28 at the high dose; 4/24 F_1 controls versus 11/24 at the high dose). Since there was no indication of altered cycling, this result was considered to be of no toxicological concern. Sperm-stage analysis indicated no difference between control F_0 and F_1 males and those at the high dose with respect to the number of testicular tubules at various spermatogenic stages or in tubule diameter. There was a dose-related increase in the incidence of abnormal sperm, particularly with respect to tail defects, in F_0 males (0.25 ± 0.26 and $0.29 \pm 0.31\%$ in the two control groups, $0.59 \pm 0.96\%$ at the low dose, $0.6 \pm 0.65\%$ at the intermediate dose, and $0.67 \pm 0.58\%$ at the high dose); however, no such increase was observed in F_1 males at the high dose (0.21% versus 0.23% in controls). Furthermore, the mean values for F_0 animals were within the historical control range, 0.0–0.8%. Thus, these findings were considered incidental. The fertility and gestational indices of control and treated F_0 and F_1 females were similar. The male fertility indices of F_0 males at the intermediate and high doses were lower than that of controls (82 and 86%, respectively), as was that of F_1 males at the low dose (75%). Since the fertility index of F_1 males at the intermediate and high doses approached 100%, the lower fertility indices were considered incidental.

There were significant increases in absolute and body weight-adjusted mean liver weights in F_1 males and females at the high dose; no data were collected for F_0 animals. The weights of the kidneys of F_1 animals at the high dose were increased, and the increase was significant in males. Brain weights were significantly lower in F_1 females at the high dose, and thymus weights were significantly lower in F_1 males and females at the high dose relative to controls. No microscopic examinations were conducted on these tissues. The mean absolute epididymal weights of F_0 males at the high dose and the absolute and body weight-adjusted epididymal weights of F_1 males at the intermediate and high doses were significantly lower than those of controls. The authors indicated that the values for the F_1 males were similar to 'recent control values for animals of similar age'; however, data on these controls were not provided. The mean absolute and body weight-adjusted testicular weights were statistically significantly increased in F_1 males at the high dose and non-significantly increased in F_0 males at this dose. There was a dose-related decrease in mean ovarian

weights in F₁ females, which was statistically significant in the high-dose group. No treatment-associated histopathological lesions were seen in the reproductive tissues that were examined. In the absence of histological data on non-reproductive tissues, the toxicological significance of the observed changes in organ weights in these tissues is equivocal.

No difference was observed in the mean number of implantation sites per litter, litter size, sex ratio, or litter indices in control and treated groups. A slight increase in the number of F₁ pups that died during days 4–21 of lactation was not observed in the F₂ generation and was considered to be incidental. The group mean weights of F₁ and F₂ litters were decreased throughout the lactation period, as reflected in the lower mean pup body weights at this dose. Although the magnitude of the difference was not as great in F₂ pups as in F₁ pups, these observations were consistent and considered to be related to treatment. The mean litter weights of dams at the low and intermediate doses were lower than those of controls on day 1 of gestation, but the mean pup weights were comparable to those of controls on days 1–21 of lactation, in contrast to the high-dose group, and were therefore considered not to be related to treatment. There were no treatment-related clinical observations or malformations.

The NOAEL for systemic toxicity was 250 ppm, equal to 21 mg/kg bw per day, on the basis of reduced body-weight gain at 1250 ppm in females during pre-mating and gestation and increased liver and kidney weights in males and females at this dose. The NOAEL for reproductive and developmental toxicity was also 250 ppm, equal to 21 mg/kg bw per day, on the basis of lower F₁ and F₂ pup weights at the next highest dose. There were no histopathological lesions in the reproductive tissues that would indicate that the changes in organ weights should be considered adverse (Barton & Wilcox, 1997).

In a three-generation study, with two litters per generation, dicloran (purity unspecified) was administered to groups of 20 rats (strain unspecified) of each sex at 0 or 100 ppm, equivalent to 5 mg/kg bw per day, in the diet. On the basis of the reproduction parameters examined, including the numbers of litters, stillbirths, live births, birth weight, and lactation indices, dicloran had no effect on reproduction (Lobdell & Johnston, 1965).

(iii) *Developmental toxicity*

Rats

Groups of 24 pregnant Sprague-Dawley rats (Upj:TUC (SD) SPF) were dosed by gavage with dicloran (purity, 93.7%) in 0.25% carboxymethyl cellulose and 0.5% Tween 20 at doses of 0, 100, 200, or 400 mg/kg bw per day on days 6–15 of gestation, the day of mating being considered gestation day 0. These doses were derived from the results of a range-finding study in which 5/5 rats died at doses of 2000 and 4000 mg/kg bw per day, and 3/5 at 1000 mg/kg bw per day. Neither of the two survivors treated with 1000 mg/kg bw per day had pups, and one had 15 early resorptions. The mean body-weight gain of females treated with 500 mg/kg bw per day was 25% less than that of controls.

The dams were sacrificed on day 20 of gestation, and the uterine contents were examined; the uteri of non-pregnant dams were stained with ammonium sulfide, and all fetuses were assessed for viability, sex, weight, and external malformations and variations. Equal numbers of fetuses from each group were assigned to either visceral or skeletal examination. There were no deaths. The treatment-related clinical signs included yellow staining of the urogenital region in some animals at each dose due to the colour of the test material, and slight central nervous system depression throughout the treatment period in animals at 200 and 400 mg/kg bw per day. The mean body-weight gain (corrected for gravid uterine weights) of treated animals was significantly lower (16–33% less) than that of the controls. The incidence of dams with totally resorbed litters (early implants only) was increased in all treated groups (1, 5, 9, 10; control to high dose, respectively); however, there were no differences in the mean numbers of implants or resorptions or in the mean litter sizes of dams with live litters. The fetal weights were significantly lower at 200 and 400 mg/kg bw per day (7 and 20% less than control, respectively), and a significant trend for an increased incidence of delayed or reduced ossification was noted in these two groups.

Increased incidences of other skeletal variations (bipartite sternabrae) were also seen at the high dose, but there were no teratogenic effects. (The authors maintained that the low dose was not maternally or embryotoxic. The conclusion concerning embryotoxicity was based on statistical significance rather than biological significance, and an erroneously large control value appears to have been used for statistical comparison.) On the basis of the significantly lower mean body-weight gains and the higher incidences of dams with totally resorbed litters (i.e. early implants only) in all treated groups, an NOAEL was not identified for maternal or developmental toxicity. (The authors indicated that sialodacryoadenitis viral infection was present during much of the dosing period and indicated that this may have had some effect on weight gain; however, in previous studies this virus had no teratogenic effects. If this is a valid conclusion, the increased incidence of total resorption of litters at all doses should be considered treatment-related.) (Marks et al., 1982).

Rabbits

Groups of 10, 12, and 14 pregnant New Zealand white rabbits were fed dicloran (purity unspecified) in the diet at 0, 100, or 1000 ppm, equivalent to 0, 3, and 30 mg/kg bw per day, on days 8–16 of gestation, the day of mating being considered gestation day 1. Pregnant females were allowed to deliver naturally, and the pups and parental females were sacrificed on post-natal day 21. There were no effects on maternal body weight during treatment. The mean litter size was slightly lower in the group at the high dose than in controls, with values of 7.1, 6.6, and 6.1 for the control, low, and high dose, respectively. In the absence of data on resorption, however, it could not be determined whether this effect was treatment-related. The pup weights at birth were not provided, but the mean pup weights at weaning (day 21) were comparable in all groups. There were no malformations and no increase in the incidence of skeletal malformations in treated pups (Wazeter, 1966).

Groups of 15–16 naturally mated New Zealand white rabbits were dosed by gavage with dicloran (purity, 98.3%) in 1% carboxymethyl cellulose at 0, 8, 20, or 50 mg/kg bw per day on days 6–18 of gestation, the day of mating being considered gestation day 0. These doses were based on the results of a range-finding study in which a dose of 100 mg/kg bw per day caused two deaths, marked reduction in food intake, reduced body-weight gain during treatment, slightly increased post-implantation loss, and reduced litter and mean fetal weights, with gross malformations in 4/11 fetuses from one litter. The dose of 50 mg/kg bw per day resulted in reduced food intake and lower body-weight gains throughout treatment, and at 20 mg/kg bw per day there was a slight, transient reduction in food intake, with reduced body-weight gain and slight body-weight loss on days 9–11 of gestation (James & Brennan, 1991).

The dams were sacrificed on day 29 of gestation, the uterine contents were examined, and all fetuses were assessed for viability, sex, and external, visceral, and skeletal malformations and variations. There were no treatment-related effects on mortality, clinical signs, or food consumption. The mean body weights and body-weight gain were slightly but consistently lower than those of controls in females at the intermediate and high doses throughout both the treatment and post-treatment periods. This resulted in an overall mean body-weight gain in these animals that was 17–32% less than that of controls during treatment and at termination. The mean gravid uterine weights were also lower in these animals than in controls, whereas the carcass weights were similar in all groups, indicating that the weight changes were attributable primarily to effects on the litters rather than the dams. The lower body-weight gain of dams at the intermediate dose in the latter stages of gestation may be due partially to the smaller litter sizes of this group, which were not treatment-related; however, the effects seen in females at the intermediate dose early during treatment (days 6–9) may indicate maternal toxicity. The differences in body-weight gain in animals at the high dose were also considered to be toxicologically significant. Dams at the two higher doses had a slight increase in the frequency of post-implantation loss (7.4, 9, 4.8, and 10% for control to high dose, respectively). There were slight increases in the incidence of minor anomalies of the gall-bladder in three fetuses in three litters and delays in ossification of all limb epiphyseal sites in four fetuses in three litters (with none in controls) at 50 mg/kg bw per day. The

NOAEL for maternal toxicity was 8 mg/kg bw per day on the basis of lower body-weight gain early in the treatment period at 20 and 50 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw per day (Barton & Wilcox, 1996).

(f) *Special studies*

(i) *Cataractogenicity*

Dogs have been shown to develop lesions of the cornea and lens after prolonged oral administration of dicloran. It has been suggested that a photochemical product reaction is responsible for the lesion as it occurs only when dogs are exposed to sunlight. Groups of four to eight beagle dogs and six Hormel-Hanford miniature swine were fed dicloran (purity, 95.8%) at concentrations of 0, 0.75, 6, 24, 48, or 192 mg/kg bw per day for periods varying from 50 to 306 days. Corneal opacity appeared in dogs within 53–104 days after administration of 24 or 48 mg/kg bw per day, when they were exposed to sunlight. Dogs that were not exposed to sunlight and eyes that had been sutured closed did not develop lesions. Dogs given 192 mg/kg bw per day refused to eat after 38 days and were given dicloran by capsule. All of these animals died 49–53 days after the study began; no eye lesions were detected. In several dogs with eye damage that were maintained for four months to one year after dicloran administration had ceased, the pathological changes seen in the cornea and lens were not reversed. Dicloran did not appear to affect miniature swine at any concentration, and no histopathological changes were seen in the eyes. A dose-related increase in the presence of Heinz bodies was seen in the blood of both dogs and swine, with at least one dog affected per dose and pigs affected at doses of 48 mg/kg bw per day or more. No methaemoglobin formation was found in dogs or pigs. Administration of dicloran as a dust (0.1 mg) or a 5% solution directly into the eyes of dogs for three months had no effect on corneal opacity or irritation of the conjunctivae (Curtis et al., 1968; Bernstein et al, 1970; Earl et al., 1971).

Ocular toxicity was not observed in rats or rhesus monkeys (Serrone et al., 1967), and administration of 0.143 mg/kg bw per day for three months to 20 human volunteers produced no ocular symptoms (Strough, 1962).

The 1977 Joint Meeting (Annex 1, reference 28) reviewed additional data requested by the 1974 JMPR and concluded that a species difference in the kinetics of dicloran may partly explain the photosensitive ocular toxicity observed in dogs, but not in other mammals. ¹⁴C-Dicloran was administered orally at 100 mg/kg bw per day for five days to four beagle dogs, four pigs (breed unspecified), and eight Wistar rats. The concentration of radiolabelled residues was highest in the dogs and slightly lower in pig tissues, but the tissue residues in both species were 2.4–11-fold higher than those in liver or plasma residues of rats. All three species had a high concentration of residue in the liver, but in dogs and pigs the highest concentrations were found in the pigmented tissues of the eye, the dog having two to three times more residue in the iris and pigmented retina than the pig. Nonpigmented eye tissues (cornea and lens) had low concentrations. There was no correlation between ocular toxicity and tissue residues of dicloran and its metabolites. The plasma concentrations of radiolabel increased more rapidly in the dogs, but 24 h after three days of dosing the plateau plasma concentrations in the pigs and dogs were similar (10–15 mg/kg bw). High concentrations of radiolabel were excreted in the bile of dogs and pigs (Hamilton, 1977).

(ii) *Haematological effects*

Groups of 10 or 15 rats (strain unspecified) of each sex were treated with dicloran at concentrations of 0, 5, 20, or 100 mg/kg bw per day by gavage for four months or in the diet at a concentration of 0 or 20 ppm, equivalent to 1 mg/kg bw per day for four months. Measurements of red blood cells, total and differential leukocyte counts, platelet count, haematocrit, haemoglobin concentration, glucose, growth, and food consumption indicated no significant effect attributable to dicloran at any dose by either route (Evans et al., 1963).

Because of the known ability of 4-nitroaniline to induce specific blood dyscrasias, short-term studies were performed to compare dicloran and 4-nitroaniline. Groups of five weanling rats (strain unspecified) of each sex were given dicloran by gavage at 0 or 400 mg/kg bw per day, five days/week, for four weeks, and 4-nitroaniline was administered at 200 or 400 mg/kg bw per day to two comparable groups over the same interval. In a second experiment, groups of six male weanling rats were given dicloran by gavage at 0 or 800 mg/kg bw per day or 4-nitroaniline at 400 or 800 mg/kg bw per day, five days per week, for two weeks. Haematological parameters were normal in rats treated at 400 mg/kg bw per day—no Heinz bodies were detected and the reticulocyte counts were normal—although their body-weight gain was 50–60% of that of controls. At 800 mg/kg bw per day, there was slight weight loss. Red blood cell count, packed cell volume, and haemoglobin measurements were normal, while the lymphocyte counts were reduced to 65% of that of controls. At the end of the study, no effects were seen on the spleen. In contrast, 4-nitroaniline had definitive effects on growth at 200 mg/kg bw per day (70% of control), Heinz bodies were identified in the blood, and the reticulocyte count was markedly elevated to two to four times the control value. The bone marrow was not affected. At 400 mg/kg bw per day, growth was reduced to 60% of the control value, and a reduced red blood cell count (65% of control with polychromasia and nucleation) was seen, accompanied by increased spleen weight (200% of control). These effects were more pronounced at 800 mg/kg bw per day where, in addition, the haemoglobin concentration was reduced and the lymphocyte count greatly increased. Although the high doses of dicloran caused lymphopenia, the other haemotoxic effects seen with 4-nitroaniline were not observed with dicloran (Boots Pure Drug Co., 1962).

In studies to substantiate the difference between the effects of 4-nitroaniline and dicloran, a single oral dose of 500 mg/kg bw dicloran was given to cats. No methaemoglobin was noted at any time between 1 and 48 h after dosing; however, administration of 4-nitroaniline as a single dose of 100 mg/kg bw resulted in methaemoglobin over the same time. In addition, the cats given 4-nitroaniline were cyanotic and showed extensive muscle weakness (Gurd, 1974). Although methaemoglobin was not assessed in all studies, it is noteworthy that the 60-day study in mice was the only one that showed an association between dicloran and significantly elevated methaemoglobin concentrations in the blood at doses of 100 and 200 mg/kg bw per day (Mallyon et al., 1986). No effect on methaemoglobin was seen in the two-year study in dogs (Woodard et al., 1964).

3. Observations in humans

In a double-blind clinical study, 20 male volunteers were given 10 mg of dicloran (purity unspecified), equivalent to 0.14 mg/kg bw per day, and 10 received a placebo, once daily, for 90 days. The dose and duration were based on the estimated maximum residues obtained from consumption of fruits and vegetables over one year. Haematological examinations and tests for liver and kidney function were performed at various intervals over the course of the study; the results were found to be normal (Strough, 1962).

Extensive examinations were made on an industrial worker who had been occupationally exposed to dicloran for three years, with considerable exposure by inhalation and dermal contact for about 60 days per year. No adverse effects were observed (Brooks & Boyack, 1963).

Comments

Dicloran is rapidly absorbed, metabolized, and eliminated, mainly in the urine, after oral administration to rats, goats, and humans; it is also rapidly excreted by hens. Rats excreted most of the radiolabel (90–96%) within 24 h, with > 70% in the urine and an additional 13–22% in the faeces, depending on the dose. Elimination was essentially complete (91–97% of the administered dose) by 48 h, with total tissue residues accounting for 0.2–0.3%. The highest tissue concentrations were found in the liver (0.05–0.06 mg/kg) and kidneys (0.02 mg/kg). Unchanged parent compound

was detected in the faeces of animals at the high dose only. The major urinary metabolites were the sulfate and glucuronide conjugates of 2,6-dichloro-4-hydroxyaniline, which accounted for 55–79% of the total administered radiolabel, and the major faecal metabolites were derivatives of glutathione conjugates. The parent compound and its glutathione conjugates were the major residues in plants. None of the metabolites was of toxicological concern. The rapid excretion of dicloran as a conjugate indicates that it is readily metabolized *in vivo*.

The major metabolite in rat urine (2,6-dichloro-4-hydroxyaniline) was not present in goat urine, which contained more polar conjugates that could not be hydrolysed by glucuronidase or sulfatase. In goats, dicloran is reduced to 4-amino-2,6-dichloro-aniline and acetylated to 4-amino-3,5-dichloroacetanilide (4–6% in urine), which is rapidly metabolized and excreted in the urine and faeces, although a reactive intermediate is formed in goat liver and bound covalently to macromolecules. Species differences in dicloran metabolism were also apparent in dogs and mice, which partly explain the photosensitive oculotoxic effects observed in dogs and the methaemoglobinaemia noted in mice, which are not seen in other mammals. Preliminary studies suggested that the metabolites of dicloran in humans are similar to those in rats. Although recovery was considered complete, excretion was somewhat slower in humans than in rats, most of the material being excreted within 1.5 days.

Dicloran has low or slight toxicity when administered by the oral route, depending on species. It has low dermal toxicity, is not irritating to the skin, is a mild eye irritant, and is not a skin sensitizer.

WHO has classified dicloran as unlikely to present an acute hazard in normal use (WHO, 1996).

The results of short-term studies of toxicity in rabbits treated dermally and in mice and rats treated in the diet or by gavage and of long-term studies of toxicity in mice, rats, and dogs treated in the diet indicate that the liver is the primary target organ. Increased liver weights and centrilobular hepatic hypertrophy were observed consistently in all species treated orally, and increased splenic activity and splenic extramedullary haematopoiesis were noted in short- and long-term studies in mice.

When dicloran was fed to mice at 0, 300, 600, or 1200 ppm for 60 days, the NOAEL was 300 ppm (equal to 49 mg/kg per day), on the basis of hepatic lesions, splenic extramedullary haematopoiesis, and increased methaemoglobin at doses of 600 ppm and above.

In a 90-day study in mice treated by gavage with 0, 15, 45, 135, 400, or 600 mg/kg bw per day, the NOAEL was 15 mg/kg bw per day on the basis of significant polycythaemia in males and hypercholesterolaemia and a significant increase in the incidence of splenic extramedullary haematopoiesis in females at 45 mg/kg bw per day and above.

A NOAEL was not identified in a 90-day study in rats fed diets containing 0, 1000, 3000, or 5000 ppm dicloran because of significantly reduced body-weight gain in conjunction with effects on the liver and thyroid in all treated groups. In an eight-week study in which rats were given diets containing dicloran at doses of 0, 500, or 750 ppm, the NOAEL was 500 ppm (equal to 44 mg/kg bw per day) on the basis of reduced body-weight gain, decreased food consumption, and increased liver weights with associated hepatic histopathological manifestations at 750 ppm.

In a study in which dicloran was fed to rats for six months at 0, 30, 300, or 3000 ppm, the NOAEL was 300 ppm (equal to 22 mg/kg bw per day) on the basis of reduced body weights and increased liver and spleen weights at 3000 ppm.

In a four-week study in rats given a dose of 0, 35, 140, or 350 mg/kg bw per day by gavage, the NOAEL was 35 mg/kg bw per day on the basis of growth depression and hepatic hypertrophy and vacuolation at 140 mg/kg bw per day.

In an 18-month study of carcinogenicity in mice at dietary concentrations of 0, 50, 175, or 600 ppm, the NOAEL was 175 ppm (equal to 25 mg/kg bw per day) on the basis of increased liver weights, centrilobular hepatocyte enlargement, centrilobular haemosiderosis, focal and single-cell liver necrosis, and vacuolation of centrilobular hepatocytes. There was no evidence of carcinogenicity in mice.

Two studies were conducted in rats to assess toxicity over a two-year period of exposure. Rats were fed diets containing dicloran at concentrations of 0, 20, 100, or 3000 ppm or 0 or 1000 ppm. The overall NOAEL was 1000 ppm (equal to 59 mg/kg bw per day), on the basis of changes in

body-weight, food consumption, and haematological parameters, spleen pigmentation, increased liver weights, centrilobular hepatocyte enlargement, and thyroid hypertrophy at 3000 ppm. There was no evidence of carcinogenicity in these studies, but they were considered inadequate for complete evaluation of the carcinogenetic potential of dicloran.

Dogs fed dicloran at dietary concentrations of 0, 20, 100, or 3000 ppm for two years had treatment-related changes in haematological and clinical chemical parameters and significant increases in liver, spleen, and kidney weights, accompanied by histological changes at 3000 ppm that included irregular hepatic-cell size, moderate hepatic-cell hypertrophy, and increased pigmentation of the liver and spleen. The NOAEL was 100 ppm, equal to 1.7 mg/kg bw per day.

Dicloran was not mutagenic in most assays, although occasional positive responses were seen in more recent tests for reverse mutation and in a test for mitotic recombination. The results of an assay for sex-linked recessive mutation in *Drosophila* were equivocal. The Meeting concluded that dicloran is unlikely to be genotoxic.

Studies of reproductive and developmental toxicity indicated that dicloran is not a reproductive toxicant and is not teratogenic in rats or rabbits. Dicloran was embryotoxic at maternally toxic doses in rabbits, but an NOAEL for maternal or developmental toxicity was not identified in rats.

In a two-generation study of reproductive toxicity in rats (one litter per generation) at dietary concentrations of 0, 50, 250, or 1250 ppm, the NOAEL for systemic toxicity was 250 ppm (equal to 21 mg/kg bw per day) on the basis of reduced body-weight gains and increased liver weights at 1250 ppm. The NOAEL for reproductive and developmental toxicity was 250 ppm (equal to 21 mg/kg bw per day) on the basis of reduced weights of F₁ and F₂ pups at 1250 ppm.

In a study of developmental toxicity, dicloran was administered by gavage to rats on days 6–15 of gestation at doses of 0, 100, 200, or 400 mg/kg bw per day. Because of significantly reduced mean maternal body-weight gains and higher incidences of totally resorbed litters in all treated groups, NOAEL values for maternal or developmental toxicity were not identified. Dicloran was not teratogenic in this study.

In a study of developmental toxicity in rabbits, dicloran was administered by gavage at doses of 0, 8, 20, or 50 mg/kg bw per day on days 6–18 of gestation. The NOAEL for maternal toxicity was 8 mg/kg bw per day, on the basis of reduced maternal body-weight gains early during treatment with 20 or 50 mg/kg bw per day. The NOAEL for developmental toxicity was 20 mg/kg bw per day on the basis of a slight increase in post-implantation losses, a slight increase in the incidence of minor anomalies of the gall-bladder, and delays in ossification of all limb epiphyseal sites in fetuses at 50 mg/kg bw per day.

Dogs have been shown to develop lesions in the cornea and lens after prolonged oral administration of dicloran. The 1977 Joint Meeting reviewed additional data requested by the 1974 Meeting and concluded that the photosensitive oculotoxic effects observed in dogs but not in other mammals were due partly to a species difference in the kinetics of dicloran. No oculotoxic effects were seen in any of the more recent studies, although no further studies have been conducted in dogs.

In a double-blind clinical study, 20 men were given 10 mg dicloran (equivalent to 0.14 mg/kg bw per day) and 10 received a placebo once daily for 90 days. The dose and duration were chosen on the basis of the maximum residues estimated to be derived from consumption of fruits and vegetables over one year. There was no indication that administration of dicloran at this dosage had any adverse effect.

An ADI of 0–0.01 mg/kg bw per day was established on the basis of the NOAEL of 1.7 mg/kg bw per day for hepatic and haematological effects in the two-year study in dogs and a 200-fold safety factor. A larger than normal safety factor was used because of the inadequacy of the long-term studies in rats for assessing the carcinogenic potential of dicloran and because of the lack of a NOAEL for maternal and developmental toxicity in rats.

An acute RfD was not allocated because dicloran has low or slight toxicity when administered orally or dermally and because acute effects occur only at very high doses, resulting in a 10 000-fold difference between the ADI and the LOAEL for maternal and developmental toxicity in rats. Therefore, the Meeting concluded that the acute intake of residues is unlikely to present a risk to consumers.

Toxicological evaluation

Levels that cause no toxic effect

- Mouse: 300 ppm, equal to 49 mg/kg bw per day (lowest dose tested, eight-week study of toxicity)
15 mg/kg bw per day (13-week study of toxicity)
175 ppm, equal to 24 mg/kg bw per day (18-month study of carcinogenicity)
- Rat: 35 mg/kg bw per day (lowest dose tested, four-week study of toxicity)
500 ppm, equal to 44 mg/kg bw per day (lowest dose tested, eight-week study of toxicity)
300 ppm, equal to 22 mg/kg bw per day (six-month study of toxicity)
1000 ppm, equal to 59 mg/kg bw per day (two two-year studies of toxicity)
250 ppm, equal to 21 mg/kg bw per day (two-generation study of reproductive toxicity)
- Rabbit: 8 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)
20 mg/kg bw per day (developmental toxicity)
- Dog: 100 ppm, equal to 1.7 mg/kg bw per day (two-year study of toxicity)
- Human: 0.14 mg/kg bw per day (90-day study of toxicity)

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of an acute reference dose

Not allocated (unnecessary).

Studies that would provide information useful for continued evaluation of the compound

1. Combined study of toxicity and carcinogenicity in rats.
2. Study of developmental toxicity in rats at less than 100 mg/kg bw per day to establish clear NOAELs for maternal and developmental toxicity.
3. Assays for genotoxicity in mammals *in vivo*, such as an assay for micronucleus formation
4. Further observations in humans

List of end-points for setting guidance values for dietary and non-dietary exposure

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of absorption	Rapid/complete, > 70% urinary excretion within 24 h
Dermal absorption	No data
Distribution	Liver, kidney
Potential for accumulation	Minimal
Rate and extent of excretion	Rapid/complete, 90–96% in urine and faeces within 24 h
Metabolism in animals	Metabolites differ in rats and goats
Toxicologically significant compounds (animals, plants and environment)	Parent compound

Acute toxicity

Rat: LD ₅₀ oral	> 4000 mg/kg bw
Rabbit: LD ₅₀ dermal	> 2000 mg/kg bw

Rat: LC ₅₀ inhalation	No data		
Skin irritation	Not irritating		
Eye irritation	Minimally irritating		
Skin sensitization	Not sensitizing (Draize test)		
<i>Short term toxicity</i>			
Target/critical effect	Liver/centrilobular hepatotoxicity (mice, rats, dogs) Spleen/extramedullary haematopoiesis (mice)		
Lowest relevant oral NOAEL	Rat: 44 mg/kg bw/per day (diet); 15 mg/kg bw per day (gavage)		
Lowest relevant dermal NOAEL	Rabbit: 120 mg/kg bw per day		
Lowest relevant inhalation NOAEL	Poor study: data on actual intake, particle size not provided		
<i>Genotoxicity</i>	Unlikely to be genotoxic; no study of genotoxicity in mammals <i>in vivo</i>		
<i>Long term toxicity and carcinogenicity</i>			
Target/critical effect	Liver/centrilobular hepatotoxicity (mice, rats, dogs) Spleen/extramedullary haematopoiesis (mice)		
Lowest relevant NOAEL	Dog: 1.7 mg/kg bw per day (2-year study)		
Carcinogenicity	No evidence of carcinogenicity in mice. The study in rats was inadequate.		
<i>Reproductive toxicity</i>			
Reproduction target/critical effect	Increased ovary/testis weights (no histological findings)		
Lowest relevant reproductive NOAEL	Rat: 21 mg/kg bw per day (reduced body-weight gain, increased liver weight)		
Developmental target/critical effect	Increased resorptions, delayed ossification, not teratogenic		
Lowest relevant developmental NOAEL	20 mg/kg bw per day in rabbits; no NOAEL in rats. A 10 000-fold difference exists between the ADI and the LOAEL in rats.		
<i>Neurotoxicity/Delayed neurotoxicity</i>	No data		
<i>Other toxicological studies</i>			
Cataractogenicity	Photosensitive oculotoxic effects observed in dogs are not seen in other mammals		
<i>Medical data</i>	No indication that administration of dicloran at 10 mg/day to men for 90 days had any adverse effect. Extensive examinations were made on one industrial worker occupationally exposed to dicloran over three years, with considerable inhalation and dermal exposure for about 60 days/year. No adverse effects were observed		
Summary	Value Study Safety factor		
ADI	0–0.01 mg/kg bw	2-year study, dogs	200
Acute reference dose	Not allocated (unnecessary)		

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