

**DICOFOL**

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**EXPLANATION**

Dicofol is an acaracide which is structurally similar to DDT. Dicofol was previously evaluated by the Joint Meeting in 1968 (Annex 1, reference 10). An ADI of 0-0.025 mg/kg bw was allocated, based on a NOAEL of 50 ppm in the diet, equivalent to 2.5 mg/kg bw/day in the rat.

Since the last review a number of studies have been submitted including studies using a purer form of dicofol corresponding to current product purity. The purer form is generally > 95% dicofol (80-85% p,p'-dicofol and 15-20% o,p'-dicofol) which contains less than 0.1% DDT-related (DDTr) impurities (i.e. DDT,  $\alpha$ -chloro-DDT, DDE, and DDD). Relevant portions of the previous monograph have been incorporated into this toxicological monograph.

**EVALUATION FOR ACCEPTABLE DAILY INTAKE****BIOLOGICAL DATA****Biochemical aspects****Absorption, distribution, and excretion****Mice**

The disposition of dicofol was studied using groups of 3 male NIH mice given a single oral dose of 25 mg/kg bw of <sup>3</sup>H-p,p'-dicofol (<sup>3</sup>H-ring labeled; isomer composition unknown). Blood, urine, faeces, and tissues (fat, liver, kidney, lung, brain, spleen, heart) were collected over 4 days. Approximately 60% of the administered dose was eliminated within 4 days primarily in the faeces. Faecal excretion accounted for 40% of the administered dose whereas urinary excretion accounted for 20%. Peak tissue concentrations were reached within 24-48 h. The highest concentrations of radiolabel were found in adipose tissue followed by liver, kidney, lung, heart, blood plasma, brain, whole blood, and spleen. Concentrations dropped rapidly over 4 days except in adipose tissue (Kaneshima *et al.*, 1980).

## Rats

Upon ingestion of dicofol by mammals, storage of the compound occurs in the adipose tissue. Rats were fed dicofol at a level of 32 ppm in their diet for 12 weeks. After eight weeks the level of the compound in the fat had reached equilibrium at concentrations of 25 ppm for the males and 70 ppm for the females. After 12 weeks, dicofol was withdrawn from the diet and the level of stored material declined. The rate of decline was greater for the male animals than for the females. By 14 weeks after withdrawal the level of dicofol in the fat was zero for the males but still remained at about 6 ppm for the females. Feeding with higher or lower dose levels also showed that dicofol was stored in the fat of the female rat to a greater degree than in the male (Smith *et al.*, 1959).

The pharmacokinetics of p,p'-dicofol and o,p'-dicofol were studied in female Crl:CD BR rats (groups of 4) given a single oral dose of 50 mg/kg bw of <sup>14</sup>C-o,p'-dicofol or <sup>14</sup>C-p,p'-dicofol (uniformly ring labelled). Blood, urine, faeces, and tissues (fat, liver, adrenal gland, thyroid) were collected over 10 days. The two isomers showed similar distribution and excretion patterns, but p,p'-dicofol was much more persistent in the body than o,p'-dicofol. Both isomers were excreted primarily in the faeces, but o,p'-dicofol was excreted more rapidly. Over 90% of the o,p'-dicofol administered dose was excreted within 2 days (and essentially all eliminated by 10 days) compared to 40% of the p,p'-dicofol administered dose (80% excreted in 10 days).

Peak tissue concentrations were reached within 6 h in most tissues and after 1-2 days in fat. Both isomers showed a high affinity for adipose tissue. At the time of peak concentrations, approximately 51% of p,p'-dicofol radiolabel and 26% of o,p'-dicofol radiolabel were in body fat (assuming fat is 7% of body-weight). Tissue concentrations of both isomers were similar initially, but concentrations of o,p'-dicofol radiolabel declined more rapidly than p,p'-dicofol radiolabel. After 10 days, concentrations of p,p'-dicofol radiolabel were: fat, 144 ppm; adrenal gland, 30 ppm; thyroid, 16 ppm; liver, 6 ppm; whole blood, 1 ppm. In comparison, o,p'-dicofol radiolabel concentrations were: fat, 3 ppm; adrenal gland and thyroid, 1 ppm; blood, 0.6 ppm; liver, 0.5 ppm. Elimination half-lives were estimated to be 1.5-4 day for o,p'-dicofol and 4-7 day for p,p'-dicofol (DiDonato *et al.*, 1987).

In a study of similar design, the disposition of p,p'-dicofol was studied in male and female Sprague-Dawley rats (groups of 4/sex) given a single oral dose of 50 mg <sup>14</sup>C-p,p'-dicofol/kg bw (uniformly labelled ring). Blood, urine, faeces, and tissues (liver, kidneys, fat) were collected over 7 days. Males and females excreted 78% and 51%, respectively, of the administered dose in 7 days. Faecal excretion accounted for 32-61% of the administered dose with the remainder (16-19%) excreted in urine. Adipose tissue contained the highest concentration of radiolabel followed by liver, kidneys, and blood. Tissue concentrations were much higher in females than males. Adipose tissue concentrations were 5-7 times higher and liver

concentrations 3-4 times higher in females than males. After 7 days, fat concentrations were 148 ppm in females versus 30 ppm in males, and liver concentrations were 8 ppm in females versus 2 ppm in males (Tillman & Mazza, 1986).

The disposition of dicofol and DDT were compared following a single oral dose. Male and female Sprague-Dawley rats (groups of 1-4/sex) were given a single dose of 50 mg  $^{14}\text{C}$ -p,p'-dicofol or  $^{14}\text{C}$ -p,p'-DDT/kg bw (uniformly ring labelled). Blood, urine, faeces, and tissues were collected over 8 days. DDT and dicofol radiolabel showed qualitatively similar distribution and elimination patterns, but DDT was more persistent in the body than dicofol. Both distributed preferentially to adipose tissue and were eliminated mainly in the faeces. Essentially all of the dicofol dose was excreted within 8 days compared to 80% of the DDT dose. The highest concentrations of both compounds were found in adipose tissue and adrenal glands. After 8 days, DDT-radiolabel in fat was 275 ppm whereas dicofol-radiolabel was negligible. Dicofol-derived radiolabel was eliminated from the tissues more rapidly than DDT-derived radiolabel. Females generally had higher tissue levels than males. Elimination half-lives were estimated to be 30 h for dicofol in males and females and 55-95 h for DDT (Steigerwalt *et al.*, 1984a).

The overall excretion rate of dicofol in this study was considerably more rapid than the overall excretion reported in two other single dose studies in rats (DiDonato *et al.*, 1986; Tillman & Mazza, 1986).

The disposition of dicofol and DDT were compared following multiple oral doses using female Sprague-Dawley rats (groups of 1-2) given daily doses of 0.5 mg  $^{14}\text{C}$ -p,p'-dicofol or  $^{14}\text{C}$ -p,p'-DDT/kg bw (uniformly ring labelled) for 16 consecutive days. Blood, urine, faeces, and tissues were collected during treatment and over 16 days after exposure. As in the single dose comparison study, DDT and dicofol radiolabel showed qualitatively similar distribution and elimination patterns, but DDT was more persistent. Dicofol radiolabel was excreted approximately twice as fast as DDT radiolabel. Approximately 75% of the dicofol dose was excreted within 16 days compared to 40% of the DDT dose. Both were eliminated mainly in the faeces. Concentrations in tissues, such as fat, liver, and adrenal glands were comparable during treatment, but dicofol radiolabel was eliminated from these tissues more rapidly. Fat concentrations of DDT radiolabel increased and peaked post-exposure whereas dicofol radiolabel began declining when exposure ceased. After 16 days, DDT-radiolabel in fat was twice that of dicofol-radiolabel (38 vs. 13 ppm). Elimination half-lives were estimated to be 6-14 days for dicofol and 7-24 days for DDT (Steigerwalt *et al.*, 1984b).

Groups of 10 male Wistar rats were given daily oral doses of 63 mg/kg bw (1/20 LD<sub>50</sub> of dicofol (84.8% purity)) for 40 days. Blood, urine, faeces, and tissues were collected over the treatment period and analyzed by TLC and GC. Twenty-eight percent of the administered dose was eliminated as dicofol and 99% of

dicofol was excreted in the faeces. At day 40, adipose tissue contained the highest concentration of dicofol (69 ppm) with lesser amounts in muscle (17 ppm), lung (16 ppm), testes (13 ppm), liver (9 ppm), kidney (9 ppm), brain (8 ppm), and heart (7 ppm) (Brown *et al.*, 1969; Brown & Casida, 1987).

Groups of 5 male Wistar rats were given a single i.p. dose of 376 mg/kg bw (1/5 LD<sub>50</sub> of dicofol (84.8% purity)). Blood, tissues, urine, and faeces were collected over 4 days. Peak tissue concentrations were reached within 32-40 h except for adipose tissue which had not reached its peak concentration. After 4 days, adipose tissue contained the highest concentration (77 ppm) of dicofol followed by testes (8 ppm), liver (7 ppm), muscle (7 ppm), kidney (4 ppm), lung (4 ppm), heart (3 ppm), blood (1.5 ppm), and brain (0.9 ppm) (Brown *et al.*, 1969; Brown & Casida, 1987).

### Humans

The dicofol metabolite dichlorobenzilic acid (DCBA) was measured in the urine of 4 workers involved in the mixing/loading or application of dicofol (3.0 pounds/acre in 500 gallons of water) to citrus crops for 10 consecutive days. Urine samples were obtained over 4 days beginning 6 days after exposure. Because of previous use of chlorobenzilate, pre-exposure DCBA excretion rates were not zero. Mean daily DCBA excretion was 19-42 µg/day over the exposure period. The variation correlated with the difference in estimated dermal dose (2.7-13 mg/day). The percent dermal dose excreted as DCBA was estimated to be 0.25%. The half-life for DCBA excretion in the urine was estimated to be 7 days (Nigg *et al.*, 1991).

### Biotransformation

#### Mice

Groups of 2-3 male Swiss-Webster mice were administered a single intraperitoneal dose of 30 mg/kg bw of radiolabelled dicofol,  $\alpha$ -chloro-DDT, dichlorobenzidine (DCB), or DDT (phenyl C<sup>14</sup>, high chemical purity). One hour later, mice were sacrificed and tissues were collected and analyzed for metabolites. The proposed metabolic scheme in mice is consistent with the scheme shown for rats in Figure 1. In mice, dicofol was converted to DCD (same as FW-152), dichlorobenzophenone (DCBP), and dichlorobenzhydrol (DCBH) based on analyses of brain, fat, and liver. These three metabolites represented 33%, 30%, and 7%, respectively, of the radiolabel in the liver. Administered DCD was also metabolized to DCBP and DCBH. The reduction of DCBP to DCBH was suggested to be the rate-limiting step in dicofol metabolism. The metabolic pattern observed in the mouse *in vivo* was similar to results obtained with rat liver microsomes under anaerobic conditions and in the presence of NADPH. DDE (1,1-dichloro-2'2-bis(p-chlorophenyl ethylene) was not detected as a metabolite of dicofol or DCD.

Under the same experimental conditions,  $\alpha$ -chloro-DDT, an impurity of technical dicofol, was metabolically dechlorinated to DDE in mouse liver (50% of radiolabel in liver) and rat liver microsomes. The conversion of  $\alpha$ -chloro-DDT to DDE also occurred *in vitro* in the presence of reduced haematin. The  $\alpha$ -chloro-DDT impurity in technical dicofol may be a source of DDE detected in tissues. The authors proposed that *in vivo* metabolic dechlorination of dicofol and  $\alpha$ -chloro-DDT involves a reduced porphyrin in liver microsomes (Brown & Casida, 1987).

## Rats

The metabolism of dicofol was studied in Sprague-Dawley rats (groups of 4/sex) given a single oral dose of 50 mg/kg bw of  $^{14}\text{C}$ -p,p'-dicofol (uniformly labelled ring). The proposed metabolic scheme for dicofol in rats is shown in Figure 1. In the faeces, most of the extracted radiolabel was present as FW-152 and DCBH in males (50-70% combined) and FW-152 and OH-DCBP in females (50-60% combined). Faeces contained lesser amounts of dicofol and OH-DCBH/DCBA-glycine. In urine, the radiolabel was mostly DCBH-glycine and OH-DCBP/DCBH (25-40% combined) in both sexes. Urine contained smaller amounts of CBA-glycine, DCBA, OH-DCBH/CBA. About 20% of faeces radiolabel and 30-40% of urine radiolabel were unidentified.

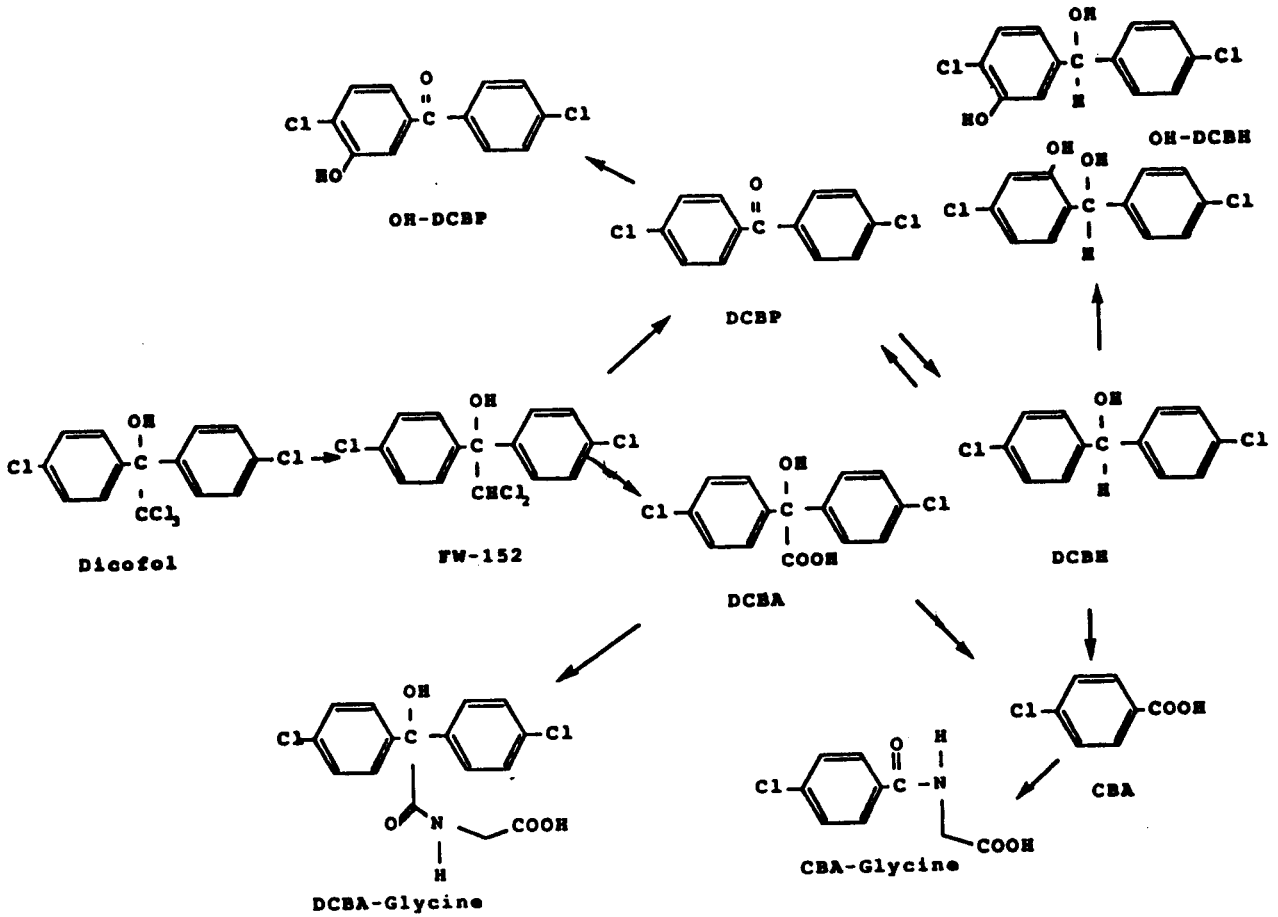
In adipose tissue, most of the extracted radiolabel was present as the parent compound (80-90%), with smaller amounts of DCBP and FW-152 identified in both sexes. In the liver, most of the extracted radiolabel was FW-152 (70-80%) in both sexes with lesser amounts of DCBP, dicofol, and DCBH.

Small amounts of material in faeces co-chromatographed with DDE. Additional analyses by HPLC determined that 0.27% of the extracted radiolabel in faeces was actually DDE. Additional analyses of fat and liver detected small amounts of DDE in one fat sample (0.2% of extract, 0.34 ppm tissue concentration) and two liver samples (0.25%-0.34% of extract, 0.018-0.29 ppm tissue concentration). The  $^{14}\text{C}$  dosing solution was reported to contain 0.01% DDE (Tillman & Mazza, 1986).

DDE, DDT, and  $\alpha$ -chloro-DDT impurities may account for the small amounts of DDE in tissues. The latter impurity has been shown to be converted to DDE in rat liver microsomes and mouse liver *in vivo* (Brown & Casida, 1987).

Following a single i.p. dose of 376 mg/kg bw of technical dicofol (84.8% pure) in male Wistar rats, the parent compound and a metabolite, DCBP, were quantified in blood. 4,4-Dichlorobenzhydrol (DCBH) was detected but not quantified. DCBP was also detected in tissues following exposure for 40 days to 63 mg/kg bw/day. Small amounts of DDE were detected (Brown *et al.*, 1969, 1971).

Figure 1. Dicofol metabolism in rats



## Effects on enzymes and other biochemical parameters

### Mice

The hepatic mixed-function oxidase (MFO)-inducing effect of dicofol was studied. Groups of 4 male and 4 female CD-1 mice were administered 3 daily oral doses of 1.4, 4.4, 14.9, 42.8, or 151 mg/kg bw of technical dicofol (87.6% purity). MFO activity in liver microsomal cell fractions was determined by O-demethylation of p-nitroanisole. Relative liver weight was increased at the high-dose in both sexes and at 42.8 mg/kg bw in males. MFO activity was increased 22%-43% in females at 14.9 mg/kg bw and above. MFO activity was unaffected in males (Steigerwalt *et al.*, 1984c).

The MFO-inducing effects of technical dicofol, dicofol isomers, and technical dicofol impurities were compared in B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> male mice. Groups of 4 mice were administered the test material in the diet daily for two weeks. Technical dicofol (87.5% purity) was administered at 0, 8, 25, 80, 250, or 800 ppm. The two highest doses bracketed the doses producing liver tumours in male B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice. p,p'-Dicofol was administered at 0, 6, 20, 63, 195, or 625 ppm. Liver MFO activity was measured by the following enzyme assays: p-nitroanisole O-demethylation, aminopyrine N-demethylation, and aniline hydroxylation. Technical dicofol depressed body-weight at 80 ppm and above and increased liver weight at 250 ppm and above. p,p'-Dicofol depressed body-weight and increased liver weight at 625 ppm. Technical dicofol increased MFO activity at 250 and 800 ppm. p,p'-Dicofol increased MFO activity at 63 ppm and above. A comparison of dose-response curves indicated p,p'-dicofol was equal to or slightly less potent than technical dicofol at comparable concentrations of active ingredient. Administration of 37 ppm of o,p'-dicofol, ED-8 isomers, DDE isomers, and up to 195 ppm of DCBP isomers produced no toxicity and had no effect on MFO activity indicating these constituents of technical dicofol do not play a disproportionate role in induction of MFO activity. The authors concluded that p,p'-dicofol was responsible for a large majority but not all the induction of liver MFO activity produced by technical dicofol (Steigerwalt *et al.*, 1984d).

### Rats

MFO induction activity was studied using groups of 6 male Sprague-Dawley rats given 4 daily intraperitoneal doses of pure dicofol (98.8% pure; 81.4% p,p'-, 18.6% o,p'-), technical dicofol (85% pure; 69.2% p,p', 15.8% o,p'; 15% impurities including DDTr), pure DDT (99% pure; 81.4% p,p'-, 18.6% o,p'-), phenobarbital, or  $\beta$ -naphthoflavone. Dose concentrations ranged from 1.5 to 59 mM (2.2 to 103 mg/kg bw). Liver MFO activity was measured by the following enzyme assays: cytochrome C reductase, aminopyrine N-demethylase, ethoxycoumarin O-deethylase, microsomal epoxide hydrolase, cytosolic epoxide hydrolase, and glutathione-S-transferase. Technical and pure dicofol and DDT induced MFO

activity in a pattern consistent with phenobarbital-type induction. At a concentration of 59 mM (87.4 mg/kg bw), pure dicofol increased microsomal protein 1.7-fold and cytochrome P-450 activities 2- to 3-fold. Equimolar doses of technical dicofol and pure dicofol produced comparable responses, and dicofol was equal in potency to DDT of equivalent isomer composition (Narloch *et al.*, 1987).

MFO induction activity was assessed using groups of 6 male Wistar rats administered dicofol (described as "pure") daily in the diet for two weeks at 0, 2, 5, 10, 20, 50, or 200 ppm. MFO activity in liver microsomal cell fractions was determined by aniline hydroxylase, aminopyrine demethylase, and hexobarbital oxidase activities. Dicofol at concentrations of 10 ppm and above increased MFO activity. Aminopyrine demethylase activity showed the greatest induction with activity increased 2- to 5.7-fold. *p,p'*-DDT increased the activity of this enzyme 2.4- to 7.6-fold over the same dose range. Dicofol ranked after heptachlor, DDT, chlorfenson, and dieldrin in capacity for inducing MFO enzymes (Den Tonkelaar & Van Esch, 1974).

Dicofol inhibited gap junctional intercellular communication in two systems: Chinese hamster V79 metabolic cooperation assay and scrape-loading/dye transfer assay in WB-F344 rat liver epithelial cells. Dicofol (1000 ppm in the diet for 11 weeks) enhanced the development of gamma-glutamyltranspeptidase-positive hepatic foci in nitrosamine-initiated male Sprague-Dawley rats (Flodstrom *et al.*, 1990).

## Dogs

The effect of dicofol on plasma 17-hydroxy-corticosteroids in the dog was determined in two dogs which were fed 300 ppm or 900 ppm dicofol over two separate periods of one to two months' duration. The ability of the adrenal cortex to elaborate 17-hydroxy-corticosteroids in response to ACTH stimulation was slightly reduced at the 300 ppm level and markedly reduced at the 900 ppm level. The results also showed that following this treatment with dicofol, the ability of the adrenal gland to return to the pre-treatment level of response to ACTH proceeded slowly and, possibly, incompletely (Smith *et al.*, 1959).

## Toxicological studies

### Acute toxicity studies

The acute toxicity of technical dicofol is summarized in Table 1. Common signs of toxicity include decreased spontaneous motor activity, ataxia, passiveness, somnolence, prostration, and occasionally tremors. In cats, dicofol given i.v. had no convulsive activity but produced cardiovascular effects consisting of prolonged arrhythmia and hypertension at sublethal doses and ventricular fibrillation at a lethal dose.

**Table 1. Acute toxicity of dicofol**

Species	Strain	Sex	Route	LD <sub>50</sub> (mg/kg bw)	LC <sub>50</sub> (mg/l)	Reference
Mouse	CRJ:CD-1 (ICR)	M	oral	669		Onishi (1989)
		F		675 <sup>1</sup>		
Rat	Charles River CD	M	oral	595		Krzywicki & Bonin (1985a)
		F		587 <sup>1</sup>		
	?	M	oral	809		Smith <i>et al.</i> (1959); Annex I: 11
		F		684 <sup>2</sup>		
	Wistar	M	oral	1495 <sup>2,4</sup>		Brown <i>et al.</i> 1969
	Charles River CD	M	dermal (24-hr exp)	>5000		Krzywicki & Bonin, (1985b)
	F	>5000 <sup>1</sup>				
Wistar	M&F	i.p.	1115 <sup>3</sup> -1150 <sup>2,4</sup>		deGroot (1974); Brown <i>et al.</i> (1969)	
	Crl:CDBR	M	inhalation (4 hr exp)		>5	Fisher & Hagan, (1987)
		F			>5 <sup>1</sup>	
Rabbit	?	M	oral	1810 <sup>2</sup>		Smith <i>et al.</i> 1959; Annex I: 11
	New Zealand, white	F	dermal (24-hr exp)	>2500 <sup>1</sup>		Krzywicki & Bonin (1985b)
Cat	?	M	i.v.	<20 <sup>3</sup>		Joy (1976)
Dog	?	M&F	oral	>4000 <sup>2</sup>		Smith <i>et al.</i> (1959); Annex I: 11

<sup>1</sup> Purity of technical dicofol was 94-96%, <0.1% DDT.

<sup>2</sup> Purity of technical dicofol was 80-85%.

<sup>3</sup> Purity of technical dicofol was unspecified.

<sup>4</sup> The observation period was 7 days only.

## Short-term toxicity studies

### Mice

Groups of 10 CD-1 (ICR) mice/sex received technical dicofol (95.6% pure; < 0.1% DDT<sub>r</sub>) in the diet daily for 13 weeks at 0, 10, 125, 250, 500, or 1000 ppm, (equal to 1.6, 18, 38, 84, and 180 mg/kg bw/day for males and 2.1, 29, 56, 110, and 190 mg/kg bw/day for females). At 125 ppm, final body-weight was reduced in females, hepatic mixed function oxidase (MFO) activity was increased in both sexes, and absolute and relative liver weight was increased in females. Liver cell hypertrophy in both sexes, SGPT in females, and kidney weight in females were increased at 250 ppm. Findings at 500 and 1000 ppm only included increased plasma proteins and lipids, degenerative changes in the kidney of females, adrenal cortex hypertrophy, and hepatocellular necrosis and vacuolation. The NOAEL was 10 ppm, equal to 2.1 mg/kg bw/day based on reduced weight, liver enlargement, and increased hepatic MFO activity at 125 ppm (Goldman & Harris, 1986).

In a dose-range finding study for a carcinogenicity study, groups of 10 male B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice received technical dicofol (> 95% pure) in the diet daily for 13 weeks at 0, 250, 500, or 750 ppm (equivalent to 36, 71, or 107 mg/kg bw/day). At 500 and 750 ppm, final body-weight, overall food consumption, and heart weight were reduced. Liver histopathology was evident at all dose levels. Hepatic changes were characterized by centrilobular hypertrophy, eosinophilic and vitreous liver cells, and polynuclear cells. In the high-dose group, entire liver lobules were vitreous in some cases. A NOAEL was not identified in this study. Histological changes in the liver were observed at all dose levels (Sato *et al.*, 1987).

### Rats

Dicofol was fed to groups, each containing 10 male and 10 female rats, for 90 days at dietary concentrations of 0, 20, 100, 500, 1250 or 2500 ppm. Survival was adversely affected at 1250 ppm and above. Growth was inhibited at 100 ppm and higher in the females but only at 1250 ppm in the males. Increased liver to body weight ratios occurred in the survivors in both sexes. Liver lesions were the most consistent histopathological finding, but were only of scattered incidence at dose levels below 1250 ppm (Smith *et al.*, 1959).

Groups of 10 Crl-CD(SD) rats/sex received technical dicofol (95.6% pure, < 0.1% DDT<sub>r</sub>) in the diet daily for 13 weeks at 0, 1, 10, 100, 500, or 1500 ppm. (equal to 0.07, 0.64, 6.5, 32, or 96 mg/kg bw/day for males and 0.08, 0.78, 7.8, 36, or 110 mg/kg bw/day for females). The highest dose of 1500 ppm produced mortality, ataxia, and lethargy. At 500 ppm and above in both sexes, body-weight and overall food consumption were reduced, liver weight was increased, blood corticosterone levels were decreased, and the incidence of adrenal cortex vacuolation was increased. At 100 ppm, hepatic MFO activity and the incidence

of liver hypertrophy were increased. The incidence and severity of thyroid follicular cell hypertrophy (minimal to marked) was increased in males at 10 ppm and above and in females at 500 and 1500 ppm. The pathologist considered the thyroid finding of uncertain significance because it is a relatively non-specific change that has been associated with environmental factors such as low temperature and stress. The NOAEL was 1 ppm, equal to 0.07 mg/kg bw/day based on the increase in thyroid follicular epithelial hypertrophy in males (Goldman *et al.*, 1986).

Groups of 10 Wistar rats/sex received technical dicofol (74% pure) in the diet daily for 13 weeks at 0, 50, 200, 1000, or 3000 ppm (equivalent to 2.5, 10, 50, or 150 mg/kg bw/day). All animals receiving the high-dose died within five weeks. All dose levels adversely affected body-weight (final weight reduced 10-40%). Food consumption was not measured. Absolute liver weight was increased in high-dose males and in females receiving 200 ppm and higher. Histopathological changes in the liver, described as SER whorls and V101-cells, were observed in males and females at 200 and 1000 ppm. V101-cells were described as enlarged hepatocytes with enlarged nuclei, some hyperchromatic or with unbalanced chromatic distribution. A basophilic granulation was usually seen in the periphery of the enlarged cell with the remainder of the cytoplasm containing fine granules and having eosinophilic character. An additional observation in high-dose females was increased thyroid weight. No microscopic changes in the thyroid were found. A NOAEL was not identified in this study (Verschuuren *et al.*, 1973).

Groups of 6 Crl:CD BR rats/sex received dermal applications (6 h/day, 5 days/week) of the formulation Kelthane MF-B (44.8% dicofol) at doses of 1, 2.5, 4, or 40 mg active ingredient/kg bw/day for 4 weeks. Control groups received either dermal application of distilled water or the formulation vehicle (vehicle dose of 53 mg/kg bw). The vehicle and all dose levels caused skin irritation attributable to the formulation vehicle. During the third week of treatment, males receiving 40 mg/kg bw/day experienced a reduction in absolute body-weight (10%) and body-weight gain (20%). Effects on the liver were observed at the high-dose in both sexes. SGPT was slightly elevated in high-dose males. Liver weight relative to body-weight was increased for high-dose males and females. Minimal hypertrophy of centrilobular hepatocytes was observed in 5/6 males and 6/6 females receiving 40 mg/kg bw/day compared to none in controls. The enlarged hepatocytes were characterized by eosinophilic cytoplasm. High-dose males also showed increased severity of multifocal inflammation of the liver. Single-cell necrosis was observed in some foci. A systemic NOAEL of 4 mg/kg bw/day was determined based on reduced body-weight and liver effects at 40 mg/kg bw/day (Lampe & Baldwin, 1990).

## Dogs

Groups of six beagle dogs/sex received technical dicofol (93.3% pure; < 0.1% DDTr) in the diet daily for 13 weeks at 0, 10, 100, 300, or 1000 ppm equal to 0.29, 3.3, 9.9, or 26 mg/kg bw/day for males and 0.31, 3.4, 9.8, and 27 mg/kg bw/day for females. Clinical laboratory tests on blood, urinalysis, and physiological measurements (i.e. electrocardiogram, heart rate, and body temperature) were conducted prior to treatment, after 4 weeks, and prior to study termination. The high-dose produced mortality in 5/6 males and 5/6 females. Both sexes receiving 300 and 1000 ppm exhibited signs of toxicity such as laboured breathing, excessive salivation, inactivity, incoordination, dehydration, and red-tinged diarrhoea. Body-weight and food consumption were unaffected at 300 ppm and below. Clinical chemistry findings were consistent with liver injury at 300 and 1000 ppm and there were effects on adrenal gland function at 100 ppm and above. In both sexes receiving 1000 ppm, serum enzymes (SAP, SGPT) were increased and serum proteins (albumin, total protein) were decreased. In 300 ppm females, alkaline phosphatase was increased four-fold and albumin was slightly decreased. Baseline cortisol blood levels were normal, but cortisol response to ACTH challenge (20 units of ACTH; cortisol measured 30 and 90 minutes after challenge) was markedly decreased (50-75%) in both sexes at 100 ppm and above. Electrocardiograms suggested treatment-related prolongation of the QT and PR intervals in dogs receiving 300 or 1000 ppm. Liver weight was increased in males at 300 ppm and in females at 1000 ppm. Microscopic changes were notable only at the high-dose. Findings consisted of single cell necrosis and mononuclear cell infiltrates in the liver of both sexes, gastrointestinal haemorrhagic enteritis and congestion in both sexes, and myocardial necrosis in one male. An additional observation was oligospermatogenesis observed in three middle-dose (300 ppm) males and five high-dose (1000 ppm) males. The NOAEL was 10 ppm, equal to 0.29 mg/kg bw/day, based on reduced cortisol response to ACTH challenge at 100 ppm (Shellenberger, 1986).

Groups of six beagle dogs/sex received technical dicofol (93.3% pure; < 0.1% DDTr) in the diet daily for 52 weeks at 0, 5, 30, or 180 ppm (equal to 0.12, 0.82, or 5.7 mg/kg bw/day for males and 0.13, 0.85, or 5.4 mg/kg bw/day for females). Adverse findings occurred only at the high dose and were confined to the liver and adrenal glands. Slightly elevated serum alkaline phosphatase and reduced albumin were suggestive of mild liver injury in both sexes at the high dose. Baseline cortisol blood levels were normal, but cortisol response to ACTH challenge (20 units of ACTH; cortisol measured 30 and 90 minutes after challenge) was markedly decreased (about 50%) in high-dose males and females. Liver weight relative to body-weight and brain weight was increased in males. Minimal to mild hepatocellular hypertrophy was observed in 5/6 males and 5/6 females receiving the high-dose compared to none in control or lower-dose groups. No treatment-related microscopic changes in the adrenal gland were found. The NOAEL was 30 ppm, equal to 0.82 mg/kg bw/day based on histological and

clinical chemistry indices of an effect on the liver and reduced cortisol response to ACTH challenge at 180 ppm (Tegeris & Shellenberger, 1988).

Groups each containing three dogs were given dicofol at 100, 300 or 900 ppm for one year. Survival was affected only at 900 ppm. Body-weight gain was normal and haematological and histological observations revealed no pathological effects (Smith *et al.*, 1959).

### Rabbits

The formulation Kelthane MF (40.7% dicofol) was tested in rabbits by the dermal route. Groups of 6 male and 6 female New Zealand white rabbits received dermal applications (6 h/day, 5 days/week) of the formulation at doses of 4.1, 10.2, or 61.1 mg active ingredient/kg bw for 4 weeks. Control groups (6/sex) received dermal applications of distilled water or the formulation vehicle (concentration equal to the vehicle concentration of the high dose). The vehicle and all dose levels of the test material caused dermal irritation attributable to the formulation vehicle. Reduced body-weight at the high- (males and females) and middle-doses (males) was the only other sign of toxicity. Overall body-weight gain was reduced 60-65% in high-dose males and females and reduced 56% in middle-dose males compared to water controls. These groups also showed consistently lower weight gain than vehicle controls. A NOAEL of 4.1 mg/kg bw based on reduced weight gain at 10.2 mg/kg bw and above (Bonin *et al.*, 1986).

### Long-term toxicity/carcinogenicity studies

#### Mice

Groups of 50 B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice/sex were administered technical dicofol (90% pure, < 1% DDT<sub>r</sub>) in the diet daily for 78 weeks and the basal diet for an additional 14 weeks. Purity of the test material was initially reported as 40-60% but later analyses of the test material (and a lot sample) indicated 87-93% purity (A.M. Rothman, 1981. Personal communication). Male mice received time-weighted average diet concentrations of 260 or 530 ppm and female mice received time-weighted average concentrations of 120 or 240 ppm, equivalent to 40 or 80 mg/kg bw/day for males and 18 or 36 mg/kg bw/day for females. Groups of 20 male and 20 female control mice received untreated diets for 91 weeks. At the end of the study, survival rates were 35, 76, and 76% for males and 95, 84, and 96% for females administered the control, low-dose, and high-dose, respectively. Body-weights of treated males were comparable to that of controls but weights of low- and high-dose females were lower than controls from week 40 to the end of the study. Food consumption data were not reported. No clinical signs or non-neoplastic lesions were related to dicofol treatment. A dose-related increase in the incidence of liver adenomas was observed in male mice. Based on a re-read of the slides using updated diagnostic criteria, the incidence of liver tumours for the control, low-dose, and high-dose groups were 0, 27, and 49% for hepatocellular

adenomas, respectively; 11, 25, and 19% for hepatocellular carcinomas, respectively; and 11, 52, and 68% for hepatocellular adenomas and carcinomas combined, respectively (R.R. Maronpot, Personal communication). The re-read resulted in reclassification of a large number (about 50% at low-dose and 75% at high-dose) of carcinomas as adenomas. The majority of tumours reported in the 1978 by NCI were carcinomas (NCI, 1978).

## Rats

Groups containing equal numbers of male and female rats were fed 0, 2, 5, 10, 15 or 20 ppm dicofol in their diets for 55 weeks. Growth, survival and liver to body weight ratios were not affected at any dose level (Smith *et al.*, 1959).

Dicofol was fed to 60 groups, each containing 10 male and 10 female rats, at dietary levels of 0, 20, 100, 250, 500 or 1000 ppm dicofol for two years. Growth depression occurred in male rats at 500 and 1000 ppm, and in female rats progressively with increasing dietary concentrations at 250, 500 and 1000 ppm. Growth depression after three months, recorded in female rats at 20 ppm (but not at 100 ppm), was not observed at a later time. Absolute organ weights showed no significant differences from the controls, with the exception of an increase in the case of the livers and kidneys of the female rats fed 1000 ppm. Organ to body weight ratios were significantly increased for the liver at 250 ppm and for the liver, kidney and heart at 500 ppm in females, but only for the liver at 500 ppm in males. Histopathological findings were confined to hydropic changes in the liver which were regarded as reversible (Larson, 1957).

Groups of 60 Crl-CD BR rats/sex received technical dicofol (93.3% pure, < 0.1% DDT) in the diet daily for 24 months at 0, 5, 50, or 250 ppm (equal to 0.22, 2.2 or 11 mg/kg bw/day for males and 0.27, 2.7 or 14 mg/kg bw/day for females). Additional groups (10/sex/dose) were treated for 3, 12, and 18 months. Survival was unaffected, and no clinical signs were related to treatment. Body-weight was reduced 15-25% at 250 ppm in both sexes. Overall food consumption was reduced 12% in females receiving 250 ppm. Hepatic MFO activity, measured by aminopyrine N-demethylation after 3 and 12 months, was increased at 50 and 250 ppm. Blood levels of corticosterone and thyroid hormones (T3, T4, TSH) were normal. Relative liver weight was increased 19% at 50 and 250 ppm in males and 35% at 250 ppm in females. Gross changes in the liver (i.e. prominent lobular architecture, focal discoloration) were seen at 50 and 250 ppm. At the terminal sacrifice, the incidence and severity of histopathological changes in the liver and adrenal gland were increased at 50 and 250 ppm. Liver cell changes included minimal to marked centrilobular hypertrophy, centrilobular and mid-zonal vacuolation, and cellular alteration of the eosinophilic type. The incidences of centrilobular hypertrophy were 0/58, 0/57, 35/60, and 52/58 in males and 0/59, 0/61, 42/60, and 56/59 in females at the 0, 5, 50, and 250 ppm dose levels, respectively. Eosinophilic cellular alteration appeared to be increased in low-dose females at the 24-month sacrifice; however, this was unaccompanied by

hypertrophic cells observed at the higher doses. Focal hepatocellular hyperplasia was increased in high-dose females. Diffuse vacuolation of adrenal cortical cells in the *zona fasciculata* and *zona reticularis* was increased primarily at the 250 ppm dose level at the terminal sacrifice and at 50 and 250 ppm at the 18-month sacrifice. At the terminal sacrifice an increase in chronic cystitis of the urinary bladder was noted in high-dose females. In the liver and adrenal gland, microscopic changes were observed at all sacrifice times. No treatment-related changes in the thyroid were observed at any time point. No neoplastic lesions were associated with dicofol treatment. The NOAEL was 5 ppm, equal to 0.22 mg/kg bw/day, based on histopathological changes in the liver and adrenal gland at 50 and 250 ppm (Hazelton & Harris, 1989).

Groups of 50 Osborne-Mendel rats/sex were administered technical dicofol (90% pure, < 1% DDT<sub>r</sub>) in the diet daily for 78 weeks then a basal diet during a 34-week observation period. Purity of the test material was initially reported as 40-60% but later analyses of the test material (and a lot sample) indicated 87-93% purity (A.M. Rothman, Personal communication, 1981). Male rats received time-weighted average diet concentrations of 470 or 940 ppm and female rats received constant diet concentrations of 380 or 760 ppm equivalent to 24 or 47 mg/kg bw/day for males and 19 or 38 mg/kg bw/day for females. Groups of 20 male and 20 female control rats received untreated diets for 110 weeks. Survival rates at 100 weeks were 55, 64, and 72% for males and 80, 92, and 88% for females administered the control, low-dose, and high-dose, respectively. Body-weights of low- and high-dose males and females were lower than control weights throughout the treatment period. Food consumption data were not reported. No treatment-related clinical signs were observed. No neoplastic or nonneoplastic lesions were associated with dicofol treatment (NCI, 1978).

## Reproduction studies

### Mice

Groups of varying numbers of mice were maintained throughout five generations on dietary levels of 0, 7, 25, 100, 225 or 500 ppm dicofol. At 500 ppm the litter sizes, average weight of the pups and the fertility, viability and lactation indices were lower than for the control group. However, all these parameters were normal at 225 ppm and below (Brown, 1967a).

### Rats

Four groups each of 27 male and 27 female rats were fed dietary levels of 0, 100, 500 or 1000 ppm dicofol in a two-generation reproduction study. There were no F<sub>1b</sub> pups surviving at 21 days when the original parents were fed 500 or 1000 ppm dicofol. Litter size from the 1000 ppm group was similar to the control, but overall mortality in the pups was greater. Considerable reduction in fertility

of the animals fed 500 and 1000 ppm dicofol was evident. No congenital defects were observed in any of the F<sub>2a</sub> or F<sub>2b</sub> animals (Brown, 1965).

Groups of rats were maintained on diets containing 25 or 75 ppm dicofol through a three-generation study. The average number of pups born per litter to parents receiving 75 ppm was slightly lower than for the controls. There were no compound-related effects relative to body weight, fertility, gestation, viability or lactation indices at either level, nor were there any congenital abnormalities evident in either the viable or the still-born pups (Brown, 1967b).

Dicofol technical (93.3% pure) was administered to Crl:CD BR rats over two generations (one-two litter study) at 5, 25, 125, or 250 ppm in the diet equal to 0.5, 2.1, 10 or 21 mg/kg bw/day for males and 0.5, 2.2, 11 or 18 mg/kg bw/day for females. The first parental (P<sub>1</sub>) animals were treated for 10 weeks prior to mating, during mating, during pregnancy, and through weaning of the F<sub>1</sub> offspring. Selected F<sub>1</sub> offspring (P<sub>2</sub>) were treated during growth, mating, the production of two F<sub>2</sub> litters (F<sub>2a</sub>, F<sub>2b</sub>), and until the second F<sub>2</sub> litter was weaned. During the pre-mating period and gestation, P<sub>1</sub> females receiving 125 or 250 ppm showed reduced body-weight gain and food consumption. Treatment-related histological changes were observed in the liver, ovaries, and adrenal glands of P<sub>1</sub> and P<sub>2</sub> rats. The most prominent liver change was minimal to moderately severe hypertrophy of centrilobular hepatocytes accompanied by centrilobular to mid-zonal vacuolation in P<sub>1</sub> and P<sub>2</sub> males and females. The response was more severe in males than females. The incidence in P<sub>2</sub> males was 0/25, 1/25, 14/25, 24/25, and 25/25 in 0, 5, 25, 125, and 250 ppm groups, respectively. Focal eosinophilic cellular alteration was increased in P<sub>2</sub> male (6/25) and female (8/25) rats at 250 ppm and P<sub>2</sub> females at 125 ppm (6/25) compared to controls (1/25 in males; 0/25 in females). At 250 ppm, there was an increase in bile duct hyperplasia in P<sub>1</sub> and P<sub>2</sub> females. Vacuolation of the ovary was increased at 250 ppm in P<sub>1</sub> females and at 25 ppm and above in P<sub>2</sub> females. The incidences in P<sub>2</sub> females were 1/25, 1/25, 6/25, 5/25, and 18/25 in 0, 5, 25, 125, and 250 ppm groups, respectively. The change was characterized by an increase in the size and/or number of vacuoles in the cytoplasm of ovarian stromal cells. The morphological change was described as compatible with enhanced steroidogenic activity. The incidence of hypertrophy and/or vacuolation of the adrenal cortex was increased in P<sub>1</sub> and P<sub>2</sub> females receiving 125 ppm (P<sub>1</sub>, 7/25; P<sub>2</sub>, 8/25) and 250 ppm (P<sub>1</sub>, 23/25; P<sub>2</sub>, 25/25) compared to controls (P<sub>1</sub> and P<sub>2</sub>, 0/25). The change was characterized by diffuse enlargement and increased amounts of finely vacuolated cytoplasm or prominent large vacuoles in the cells of the inner cortex.

Reproductive performance of P<sub>1</sub> and P<sub>2</sub> rats was unaffected. Offspring toxicity was observed in F<sub>1</sub> and F<sub>2</sub> pups at 125 and 250 ppm. Viability was reduced in F<sub>1</sub> pups at 250 ppm and F<sub>2</sub> pups at 125 and 250 ppm. Reduced survival was primarily due to deaths during days 0-4 of lactation. At 250 ppm, growth of F<sub>1</sub> and F<sub>2</sub> pups was reduced during lactation. The NOAEL based on reproductive parameters was 25 ppm, equal to 2.1 mg/kg bw/day. The NOAEL for parental

toxicity was 5 ppm equal to 0.5 mg/kg bw/day, based on histopathological changes in the liver and ovaries at 25 ppm and above. The ovarian effect was considered compatible with enhanced steroidogenic activity (Solomon & Kulwich, 1991).

### **Special studies on embryo/fetotoxicity**

#### **Rats**

The teratogenicity of dicofol was studied in Crl:COBS CD (SD)BR rats. Dicofol (95.6% pure) was administered on days 6-15 of gestation by oral gavage to groups of 25 mated females rats at doses of 0, 0.25, 2.5, or 25 mg/kg bw/day. Controls received corn oil. Rats were sacrificed on day 20. During the treatment period, a majority (21/25) of the high-dose group frequently exhibited excessive salivation as did one-fifth (5/25) of the middle-dose group (on one to three occasions). This clinical sign was not observed in the dose range-finding study in which 8 rats/sex/dose were given doses of 1, 5, 20, 60 or 180 mg/kg bw/day, except in one animal (180 mg/kg bw/day group) on one day. Body-weight gain and food consumption were reduced at the high-dose (25 mg/kg bw/day) during the treatment period; a rebound increase was observed post-treatment. Liver weight relative to body-weight was increased (7%) at the high-dose. A histological change in the liver, consisting of centrilobular hepatocyte hypertrophy (minimal to slight), was observed in 17/25 of the high-dose group versus none in the control or lower dose groups. Dicofol had no-observable-effect on the offspring.

The NOAEL for maternal toxicity was 0.25 mg/kg bw/day based on clinical signs of toxicity (salivation) at 2.5 mg/kg bw/day and above. The NOAEL for embryo-fetal toxicity and teratogenicity was 25 mg/kg bw/day based on no-observable-effect on the offspring at the highest dose tested (Hoberman & Christian, 1986b).

#### **Rabbits**

The teratogenicity of dicofol was studied in New Zealand white rabbits. Dicofol (95.6% pure) was administered on days 7-19 of gestation by oral gavage to groups of 20 artificially inseminated females at doses of 0, 0.4, 4, or 40 mg/kg bw/day. The control received the aqueous methylcellulose vehicle. Rabbits were sacrificed on day 29. Maternal toxicity was produced by the 4 and 40 mg/kg bw/day doses. The high-dose group experienced clinical signs (abnormal faeces), weight loss, and reduced food consumption during the treatment period. Although body-weight showed a rebound increase after treatment, overall body-weight gain was depressed (42%). Relative liver weight expressed to body-weight was increased (20%) at the high dose. The incidence of eosinophilic, hyaline material in centrilobular hepatocytes was increased at the 4 mg/kg bw/day (2/19) and 40 mg/kg bw/day (8/20) dose levels compared to controls (0/20). Diffuse vacuolation of hepatocytes was observed in 6/20 of the high-dose group compared to 0/20 of controls. An increased incidence of abortion was observed at the high

dose (high dose, 4/19; control, 1/18). Dicofol treatment had no other effect on the developing offspring.

The NOAEL for maternal toxicity was 0.4 mg/kg bw/day based on histopathological changes in the liver at 4 mg/kg bw/day and above. The NOAEL for teratogenicity was 40 mg/kg bw/day based on no observable effect on the offspring at the highest dose tested (Hoberman & Christian, 1986a). The incidence of abortion was increased at the high-dose (4/19) compared to concurrent controls (1/18) and historical controls (up to 1/14 to 2/15 with an outlier of 1/4). The high incidence may be related to maternal toxicity, but a direct developmental effect cannot be excluded. The NOAEL for embryo-fetal toxicity was therefore 4 mg/kg bw/day based on the increased incidence of abortion at 40 mg/kg bw/day.

### **Special studies on eye and skin irritation and hypersensitivity**

Technical dicofol is reported to be irritating to the skin but non-irritating to the eye (Baldwin & Hurt, 1985).

Technical dicofol produced delayed contact hypersensitivity in guinea-pigs (Bonin & Hazelton, 1987).

### **Special studies on genotoxicity**

Results of representative genotoxicity studies are shown in Table 2. Dicofol has been overwhelmingly negative in assays for point mutation, chromosomal aberration, unscheduled DNA synthesis, and sister chromatid exchange. Occasional positive findings have not been substantiated by other studies.

### **Observations in humans**

In 1979, 78 incidents of Kelthane® exposure were reported by the US Environmental Protection Agency Pesticide Incident Monitoring System. Fourteen cases involved dicofol alone and 8 of these reported symptoms. One case involved dicofol ingestion (amount unspecified) leading to nausea, dizziness, and vomiting. Three cases involved inhalation exposure resulting in dizziness, weakness, and vomiting in two cases and sinus congestion in the third. Two cases involved dermal exposure (amount unspecified) resulting in skin irritation in one case and rash (allergic reaction) in the other (USEPA, 1979).

In a case report, a 12-year-old male was accidentally exposed to dicofol when he fell from a bicycle into a puddle of spilled undiluted dicofol formulation (470 g/l; 50-gal. drum). The skin was abraded and clothing contaminated. The patient had initial symptoms of nausea, dizziness, disorientation, confusion, lethargy, and headache. The patient demonstrated horizontal nystagmus and impaired balance. These symptoms resolved within three weeks. Three weeks

Table 2. Results of genotoxicity assays on dicofol

Test system	Test object	Concentration of dicofol	Purity	Results	Reference
Ames test (1)	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	5-5000 µg/plate dissolved in DMSO	95.6%	Negative (2)	Higginbotham & Byers (1985)
	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	1-1000 µg/plate dissolved in DMSO	89.9%	Negative	Shirasu <i>et al.</i> (1980)
<i>E. coli</i> mutation assay (1)	<i>E. coli</i> . WP2 hcr	1-5000 µg/plate dissolved in DMSO	89.9%	Negative	Shirasu <i>et al.</i> (1980)
<i>B. subtilis</i> rec-assay	<i>B. subtilis</i> . H17. M45	20-2000 µg/disk dissolved in DMSO	89.9%	Negative	Shirasu <i>et al.</i> (1980)
CHO/HGPRT mutation assay (1)	Chinese hamster ovary cells (CHO-K <sub>1</sub> -BH <sub>4</sub> )	3-20 µg/ml dissolved in DMSO	95.6%	Negative	Foxall (1986)
Sex-linked recessive lethal mutation	<i>D. melanogaster</i>	10 000 ppm, feeding and injection	34.8%	Negative	Woodruff <i>et al.</i> (1985)
Unscheduled DNA synthesis	Male rat (F-344) primary culture hepatocytes	0.025-0.5 µg/ml in DMSO	95.6%	Negative (3)	Foxall & Byers (1986)
<i>In vitro</i> sister chromatid exchange (1)	Chinese hamster ovary cells (CHO-W-BI)	5-500 µg/ml	?	Negative	Galloway <i>et al.</i> (1987)
<i>In vitro</i> cytogenetics (1)	Chinese hamster ovary cells (CHO-WBL)	7.5-20 µg/ml dissolved in DMSO 50-500 µg/ml	95.6%	Negative	Ivett & Myhr (1986)
<i>In vivo</i> cytogenetics	Male: CRL:COBS-CD(SD) rat. bone marrow	47.8-478 mg/kg bw orally X I	89.6%	Negative (4)	Sames & Doolittle (1986)

(1) Both with and without metabolic activation

(2) No positive control in nonactivated assay

(3) Unable to verify cytotoxicity

(4) No evidence presented (e.g., mitotic index) to demonstrate test material reached the target tissue. A maximum tolerated dose may not have been used.

after the incident, serum dicofol levels were 1.1  $\mu\text{g}/\text{l}$  and adipose tissue levels were 0.153  $\mu\text{g}/\text{kg}$  (analytical methods unspecified). No dicofol was detected in serum 16 weeks after the exposure. Following persistent emotional difficulties, the patient underwent a neuropsychological evaluation eight months after the exposure, which showed impairment of certain cognitive functions including auditory attention, immediate memory, and ability to selectively inhibit inappropriate responses. A pre-exposure neuropsychological analysis was unavailable for comparison (Lessenger & Riley, 1991).

## COMMENTS

Dicofol was extensively absorbed from the gastrointestinal tract. At near steady-state conditions, the highest tissue concentrations were found in adipose tissue followed by the adrenal glands, thyroid, and liver. The p,p'-dicofol isomer, the main component of technical dicofol, was more persistent in the body than the o,p'-isomer. Female rats tended to retain dicofol to a greater extent than males. Dicofol and DDT showed a similar pattern of distribution and elimination. Dicofol is more polar and therefore less persistent in the body.

In rats, dicofol was excreted as polar metabolites, primarily in the faeces, but with lesser amounts in the urine. Metabolism involved dechlorination and oxidation of the ethanol moiety and hydroxylation of the aromatic rings. In adipose tissue, the parent compound was predominant. The metabolic profile was similar in mice.

Dicofol had moderate acute oral toxicity. It produces signs of toxicity consistent with CNS depression. WHO has classified dicofol as slightly hazardous (WHO, 1992).

In a 13-week study in mice using dietary concentrations of 0, 10, 125, 250, 500, or 1000 ppm in the diet, the NOAEL was 10 ppm, equal to 2.1 mg/kg bw/day, based on reduced body-weight, liver enlargement, and increased hepatic mixed function oxidase (MFO) activity. In another 13-week study in mice using dietary concentrations of 0, 250, 500, or 750 ppm, liver histopathology, including centrilobular hypertrophy and eosinophilia of hepatocytes, was observed at all dose levels.

In a 13-week study in rats at dietary concentrations of 0, 1, 10, 100, 500, or 1500 ppm, the NOAEL was 1 ppm, equal to 0.07 mg/kg bw/day. Although the incidence and severity of thyroid follicular epithelial hypertrophy was increased in males at 10 ppm and above, this thyroid effect was not found in a second 13-week study using dietary concentrations of 0, 50, 200, 1000, or 3000 ppm.

In a 13-week study in dogs using dietary concentrations of 0, 10, 100, 300, or 1000 ppm in the diet, the NOAEL was 10 ppm, equal to 0.29 mg/kg bw/day. At 100 ppm, equal to 3.3 mg/kg bw/day, cortisol response to ACTH was reduced.

A 1-year dog study used dietary levels of 0, 5, 30, or 180 ppm was performed to better define the NOAEL. The NOAEL was 30 ppm, equal to 0.82 mg/kg bw/day, based on liver changes and reduced cortisol response to ACTH at 180 ppm, equal to 5.7 mg/kg bw/day.

In a 78-week carcinogenicity study in mice using time-weighted average concentrations of 260 or 530 ppm for males and 120 or 240 ppm for females, dicofol produced an increased incidence of liver adenomas and adenomas/carcinomas combined in male mice at 260 and 530 ppm, equivalent to 40 and 80 mg/kg bw/day. Dicofol was not carcinogenic in female mice.

In a two-year study in rats using dietary concentrations of 0, 5, 50, or 250 ppm in the diet, the NOAEL was 5 ppm, equal to 0.22 mg/kg bw/day, based on histopathological changes in the liver and vacuolation of adrenal cortical cells at 50 ppm, equal to 2.2 mg/kg bw/day. No treatment-related changes in the thyroid or in the incidence of neoplasia were observed. There was no evidence of carcinogenicity in a 78-week carcinogenicity study in rats using time-weighted average concentrations of 470 or 940 ppm (24 or 47 mg/kg bw/day) for males and 380 or 760 ppm (19 or 38 mg/kg bw/day) for females. Dicofol was not carcinogenic in rats.

In a two-generation reproduction study in rats using dietary concentrations of 5, 25, 125, or 250 ppm in the diet, the NOAEL was 5 ppm, equal to 0.5 mg/kg bw/day, based on an increased incidence of ovarian stromal cell hypertrophy and hepatocellular changes at 25 ppm. Offspring viability was reduced at 125 and 250 ppm. The NOAEL for reproductive parameters was 25 ppm, equal to 2.1 mg/kg bw/day.

In a teratology study in rats using gavage doses of 0, 0.25, 2.5, or 25 mg/kg bw/day, the NOAEL for maternal toxicity was 0.25 mg/kg bw/day based on clinical signs of toxicity at 2.5 mg/kg bw/day. The NOAEL for embryofetal toxicity was 25 mg/kg bw/day. In a teratology study in rabbits using gavage doses of 0, 0.4, 4, or 40 mg/kg bw/day, the NOAEL for maternal toxicity was 0.4 mg/kg bw/day based on histopathological changes in the liver at 4 mg/kg bw/day. The NOAEL for embryofetal toxicity was 4 mg/kg bw/day based on an increased incidence of abortion at 40 mg/kg bw/day. Teratogenic effects were not found in these studies.

After reviewing the available genotoxicity data, the Meeting concluded that dicofol was not genotoxic.

The Meeting concluded, after consideration of the liver tumours in male mice found in the long-term studies together with the genotoxicity data, that dicofol did not present a carcinogenic hazard for humans.

The previous ADI was revised. A new ADI was allocated, based upon the NOAEL of 0.22 mg/kg bw/day in the long-term study in rats, using a safety factor of 100.

### TOXICOLOGICAL EVALUATION

#### Level causing no toxicological effect

Mouse:	10 ppm, equal to 2.1 mg/kg bw/day (13-week study)
Rat:	5 ppm, equal to 0.22 mg/kg bw/day in males (two-year study) 0.25 mg/kg bw/day (teratogenicity study, maternal toxicity)
Rabbit:	0.4 mg/kg bw/day (teratogenicity study, maternal toxicity)
Dog:	30 ppm, equal to 0.82 mg/kg bw/day (one-year study).

#### Estimate of acceptable daily intake for humans

0-0.002 mg/kg bw

#### Studies which will provide information valuable in the continued evaluation of the compound

Further observations in humans.

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