## 5.8 DICOFOL (026)

### TOXICOLOGY

Dicofol is the International Organization for Standardization (ISO)–approved name of 2,2,2-trichloro-1,1-bis(4-chlorophenyl) ethanol (International Union of Pure and Applied Chemistry). Its Chemical Abstracts Service number is 115-32-2. Dicofol is structurally similar to dichlorodiphenyltrichloroethane (DDT). It is a non-systemic acaricide that acts by stimulating axonal transmission of nervous signals.

Dicofol was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues in 1968 and in 1992, when an acceptable daily intake (ADI) of 0–0.002 mg/kg body weight (bw) was established. It was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. Relevant parts of the most recent monograph have been incorporated into this toxicological evaluation. New studies on acute and short-term dermal toxicity, dermal hypersensitivity, skin and eye irritation, acute and subchronic neurotoxicity and reproductive toxicity, as well as supplementary studies on reproductive toxicity, carcinogenicity and mutagenicity, were provided and reviewed.

All pivotal studies with dicofol were certified as complying with good laboratory practice.

#### *Biochemical aspects*

Dicofol was almost completely and rapidly absorbed from the gastrointestinal tract within 24 hours following an oral dose in rats. Fat contained the highest concentration of dicofol, followed by adrenals, thyroid, liver and whole blood. After a single oral dose, the maximum tissue levels were reached 24–48 hours post-dosing, and the maximum excretion level was attained between 24 and 96 hours after dosing (half-life of 30 hours). The elimination of dicofol from the body was relatively slow, with greater than 65% of administered radioactivity still present in the carcass after 48 hours. Excretion occurred via faeces and urine, faeces being the main route of elimination. There is some indication of accumulation of dicofol in fat. Although dicofol accounts for most of the radioactivity in fat, it is only a minor component of the radioactivity in urine and faeces, indicating extensive metabolism after mobilization. The main metabolites are dichlorobenzhydrol (in males), FW-152 and hydroxyl dichlorobenzophenone (especially in females). Significant conjugation with glycine also occurs.

#### *Toxicological data*

The acute oral toxicity (median lethal dose [$LD_{50}$]) was 669 mg/kg bw in mice, 578 mg/kg bw in rats, 1810 mg/kg bw in rabbits and greater than 4000 mg/kg bw in dogs. Clinical signs of toxicity include decreased spontaneous motor activity, ataxia, passiveness, somnolence, prostration and occasionally tremors. In rabbits, dicofol was a slight to moderate irritant for skin and eyes. It gave a positive response for skin sensitization in a modified Buehler test in guinea-pigs.

In a single-dose gavage study in rats, the no-observed-adverse-effect level (NOAEL) was 15 mg/kg bw, based on decreased feed intake and hypertrophy of the adrenal zona fasciculata at 75 mg/kg bw.

The primary effects of dicofol after short- or long-term exposure were body weight reduction associated with decreased feed intake and increased liver weight accompanied by increased hepatic mixed-function oxidase (MFO) activity and liver hypertrophy in mice, rats and dogs, increased serum alanine aminotransferase (ALT) and serum alkaline phosphatase (AP) activities and hepatocellular necrosis at higher doses. Increases in hepatocyte hypertrophy and liver weight with no other effects were considered to be adaptive and not treated as adverse effects; other histopathological findings in the liver, such as fatty vacuolation and necrosis, were treated as adverse. At high doses, changes in the kidneys, adrenals, heart and testes were also observed in rodents. Reduced serum cortisone levels were seen in dogs, indicating disturbances in adrenocorticoid metabolism.

The short-term effects of dicofol were studied in 90-day feeding studies in mice, rats and dogs and in a 1-year feeding study in dogs.

In a 13-week dietary study in mice, slightly reduced final body weights in both sexes, increased hepatic MFO activity in both sexes and increased absolute and relative liver weights in females (by 20% and 25%) were observed at 125 ppm. At a dose of 250 ppm and above, dicofol induced hepatocellular hypertrophy in both sexes, increased ALT activity in males and females (by 68% and 78%, respectively) and decreased absolute kidney weight in females by 10%. At 500 and 1000 ppm, degenerative changes in the kidney of females, adrenal cortical hypertrophy and hepatocellular necrosis and vacuolation were observed. The no-observed-adverse-effect level (NOAEL) was 125 ppm (equal to 18 mg/kg bw per day), based on increased ALT activity and other liver effects at 250 ppm (equal to 38 mg/kg bw per day).

In a 13-week feeding study in rats, a dose of 1500 ppm caused death in 5 of 10 male and 8 of 10 female rats. The feed intake and mean body weights were significantly decreased in males and females fed diets containing 500 ppm and above. Clinical signs of scant droppings, soft faeces and/or faeces with mucus were seen in females at 500 ppm. Changes in haematology and clinical chemistry parameters and liver hypertrophy accompanied by increased liver weight were observed at 500 ppm and above. Kidney and adrenal weights were also significantly increased. The incidence and severity of thyroid follicular cell hypertrophy (minimal to marked) were increased in males at 10 ppm and above, but the end-point was considered to be of doubtful toxicological significance, as no changes in thyroid stimulating hormone, thyroxine or triiodothyronine were observed in a long-term toxicity study in rats. The NOAEL was 100 ppm (equal to 6.5 mg/kg bw per day), based on the reduction in mean body weights at 500 ppm (equal to 32 mg/kg bw per day) in both sexes.

In a 13-week dietary study in dogs, the highest tested concentration of 1000 ppm caused death in five of six dogs of each sex. Clinical signs of toxicity (laboured breathing, inactivity, dehydration, red-tinged diarrhoea, incoordination and excessive salivation) were observed at 300 ppm and above. ALT activity was significantly increased at 1000 ppm, and serum AP activity was increased at 300 ppm and above in females. Dicofol at 300 ppm and above caused prolongation of the QT interval. In male dogs fed dietary concentrations of 300 or 1000 ppm, a decrease in spermatogenesis (3/6 and 5/6 males, respectively) and an increase in mean hepatic weights were observed. Gross lesions, as well as microscopic lesions in the liver, testes and heart, were observed at 1000 ppm in both sexes. Dicofol at dietary concentrations of 100 ppm and above caused reduced cortisol response to adrenocorticotropic hormone (ACTH). The NOAEL was 10 ppm (equal to 0.29 mg/kg bw per day), based on reduced cortisol response to ACTH challenge at 100 ppm (equal to 3.3 mg/kg bw per day).

In a 52-week dietary study in dogs, adverse findings occurred only at the highest tested concentration of 180 ppm and were more prominent in males than in females. This concentration resulted in increased serum AP activity and cholesterol levels in males, decreased albumin in both sexes and a significant increase in lactate dehydrogenase activity in females at week 39. Relative (to body and brain weight) liver weights were increased in males but were unchanged in females. 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 in both sexes at 180 ppm. Minimal to mild hepatocellular hypertrophy was observed in five of six dogs of each sex. The NOAEL was 30 ppm (equal to 0.82 mg/kg bw per day), based on histological and clinical chemistry changes at 180 ppm (equal to 5.4 mg/kg bw per day).

The overall NOAEL for the two dog studies was considered to be 30 ppm (equal to 0.82 mg/kg bw per day), with an overall lowest-observed-adverse-effect level (LOAEL) of 100 ppm (equal to 3.3 mg/kg bw per day).

In a 78-week toxicity and carcinogenicity study in mice, using time-weighted average dietary concentrations of 264 or 528 ppm (equivalent to 40 or 80 mg/kg bw per day) in males and of 122 and 243 ppm (equivalent to 18 and 36 mg/kg bw per day) in females, an increased number of liver adenomas and carcinomas was observed in males at 264 and 528 ppm. The incidence of hepatocellular carcinomas was increased at both doses compared with controls, but there was no pair-wise or trend significance. No treatment-related tumours were observed in female mice at doses up to 243 ppm. Survival in male and female mice was not affected in this study. There was a decrease in the body weights of high-dose females. The body weights of male mice were not affected. The NOAEL in female mice was 122 ppm (equivalent to 18 mg/kg bw per day), based on decreased body weight at 243 ppm (equivalent to 36 mg/kg bw per day). A NOAEL in male mice was not observed. The LOAEL in male mice was 264 ppm (equivalent to 40 mg/kg bw per day), based on the increase in hepatocellular adenomas.

In a 78-week carcinogenicity study in rats, using time-weighted average dietary concentrations of 470 or 940 ppm (equivalent to 24 and 47 mg/kg bw per day) for males and of 380 or 760 ppm (equivalent to 19 and 38 mg/kg bw per day) for females, no treatment-related clinical signs were observed, and no neoplastic or non-neoplastic lesions were associated with dicofol treatment. The NOAEL in this study was 760 ppm (equivalent to 38 mg/kg bw per day), the highest dose tested.

In a 2-year study in rats, terminal body weights were decreased in both males and females at 250 ppm. Both male and female rats fed with dietary concentrations of dicofol of 50 and 250 ppm had decreased feed consumption, increased MFO activity and increased relative liver weight. Treatment-related microscopic changes in the liver, which included hepatocellular necrosis, and vacuolation in adrenal glands were also observed in animals of both sexes that received dicofol at 50 and 250 ppm. The NOAEL was 5 ppm (equal to 0.22 mg/kg bw per day), based on histopathological changes in the liver and adrenal gland at 50 ppm (equal to 2.2 mg/kg bw per day). No treatment-related tumours were observed in this study.

The Meeting concluded that dicofol causes liver tumours in male mice at doses associated with significant enzyme induction and liver hypertrophy, which are anticipated to exhibit a threshold response.

Dicofol gave a negative response in an adequate range of in vitro genotoxicity and in vivo chromosomal aberration tests.

The Meeting concluded that dicofol is unlikely to be genotoxic.

On the basis of the absence of genotoxicity, the absence of carcinogenic effects in rats and the expectation that the adenomas present in mice will exhibit a threshold, the Meeting concluded that dicofol is unlikely to pose a carcinogenic risk to humans at anticipated dietary exposure levels.

In a two-generation reproduction study in rats, $F_0$ females receiving 125 or 250 ppm showed reduced body weight gain and feed consumption. Treatment-related vacuolation was observed in the liver, ovaries and adrenal glands of $F_0$ and $F_1$ parental rats. Offspring toxicity was observed in $F_1$ and $F_2$ pups at 125 and 250 ppm. Viability was reduced in $F_1$ pups at 250 ppm and in $F_2$ pups at 125 and 250 ppm. The NOAEL for reproductive toxicity was 25 ppm (equal to 2.1 mg/kg bw per day), based on decreased viability at 125 ppm (equal to 10 mg/kg bw per day). The NOAEL for parental toxicity was 5 ppm (equal to 0.5 mg/kg bw per day), based on histopathological changes in the liver and ovaries at 25 ppm (equal to 2.1 mg/kg bw per day). The NOAEL for offspring toxicity was 25 ppm (equal to 2.1 mg/kg bw per day), based on decreased viability index and increased number of litters, with all offspring dying at 125 ppm (equal to 10 mg/kg bw per day).

In an enhanced one-generation study on reproduction in rats, a transient decrease in body weights, organ weight changes (liver, kidney and ovary) and histopathological changes in the liver were observed at the highest dose tested. No treatment-related effects on sperm parameters or other reproductive organs (estrous cycle, sexual maturation) were observed at doses up to 125 ppm. The NOAEL for parental toxicity was 25 ppm (equal to 1.7 mg/kg bw per day), based on the transient decrease in body weight, organ weight changes and histopathological findings in the liver seen at 125 ppm (equal to 8.7 mg/kg bw per day). The NOAEL for reproductive and offspring toxicity was 125 ppm (equal to 8.7 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, the maternal toxicity NOAEL was 2.5 mg/kg bw per day, based on a statistically significant reduction in maternal body weight gain, decreased feed consumption and feed efficiency and increased relative liver weight at 25 mg/kg bw per day. The increased incidence of salivation observed at 2.5 and 25 mg/kg bw was not confirmed in the range-finding developmental study or an acute neurotoxicity study. Therefore, this was not considered to be related to treatment. The NOAEL for embryotoxicity and teratogenicity was 25 mg/kg bw per day, the highest dose tested.

In a study of developmental toxicity in rabbits, the NOAEL was 4 mg/kg bw per day, based on abnormal faeces (dried, soft or liquid), decreased feed consumption, maternal weight loss, a significant increase in the incidences of abortion (4/19, control 1/18) and increased relative liver weight at 40 mg/kg bw per day. No treatment-related effects on fetal viability, average fetal body weights or external, soft tissue or skeletal examination were observed at doses up to 40 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 40 mg/kg bw per day, the highest dose tested.

The Meeting concluded that dicofol was not teratogenic.

In an acute neurotoxicity study, the NOAEL was 15 mg/kg bw per day. At 75 and 350 mg/kg bw per day, reduced body weights and feed consumption were observed in both male and female rats; an increased number of rats in these groups had urine-stained or faecal-stained fur. Male and female

rats given the 350 mg/kg bw per day dose had ataxia, other signs of sensorimotor dysfunction and decreased motor activity within the week after treatment. However, these effects were not evident 2 weeks after administration. The neurohistological evaluation of rats in the 350 mg/kg bw per day dose group did not reveal any pathology related to the test substance.

The NOAEL from a 90-day neurotoxicity study was 5 ppm (equivalent to 0.2 mg/kg bw per day), based on affected parameters in the functional observational battery, altered absolute and relative organ weights and reduced body weight, feed consumption values and motor activity at 100 ppm (equal to 6.7 mg/kg bw per day). The neurohistological examination of the 500 ppm group rats did not reveal any pathology related to the test substance.

Reports of cases of acute poisoning indicate that dicofol causes signs and symptoms such as nausea, dizziness, vomiting, confusion and lethargy. The symptoms resolved within 3 weeks. Epidemiological studies on children exposed to dicofol, among other chemicals, were inconclusive.

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

## Toxicological evaluation

After evaluation of new information and re-evaluation of previous data, the Meeting confirmed the ADI of 0–0.002 mg/kg bw derived from the NOAEL in the 2-year toxicity and carcinogenicity study in rats of 0.22 mg/kg bw per day, based on histopathological changes in the liver and adrenal gland. A safety factor of 100 was applied. The ADI is supported by the NOAEL of 0.2 mg/kg bw per day from the 90-day neurotoxicity study in rats. There is a margin of 20 000 between the maximum ADI and the LOAEL for liver adenomas in the male mouse.

An acute reference dose (ARfD) of 0.2 mg/kg bw was established on the basis of the NOAEL of 15 mg/kg bw in the acute neurotoxicity study in rats, based on decreased body weight and decreased feed intake at 75 mg/kg bw. This ARfD was supported by the NOAEL of 15 mg/kg bw in a single-dose oral toxicity study in rats, based on decreased feed intake and hypertrophy of adrenal zona fasciculata at 75 mg/kg bw. Although these effects were mild, they were observed in two studies, and therefore 75 mg/kg bw was considered a marginal LOAEL. A safety factor of 100 was applied.

An addendum to the toxicological monograph was prepared.

### *Levels relevant to risk assessment*

| Species | Study | Effect | NOAEL | LOAEL |
|---|---|---|---|---|
| Mouse | Thirteen-week study of toxicity[a] | Toxicity | 125 ppm, equal to 18 mg/kg bw per day | 250 ppm, equal to 38 mg/kg bw per day |
| | Seventy-eight-week study of toxicity and carcinogenicity[a] | Toxicity | 122 ppm, equivalent to 18 mg/kg bw per day | 243 ppm, equivalent to 36 mg/kg bw per day |
| | | Carcinogenicity[b] | — | 264 ppm, equivalent to 40 mg/kg bw per |

| Species | Study | Effect | NOAEL | LOAEL |
|---|---|---|---|---|
| | | | | day[c] |
| Rat | Thirteen-week study of toxicity[a] | Toxicity | 100 ppm, equal to 6.5 mg/kg bw per day | 500 ppm, equal to 32 mg/kg/bw |
| | Two-year studies of toxicity and carcinogenicity[a,d] | Toxicity | 5 ppm, equal to 0.22 mg/kg bw per day | 50 ppm, equal to 2.2 mg/kg bw per day |
| | | Carcinogenicity | 250 ppm, equal to 14 mg/kg bw per day[e] | — |
| | Single oral dose toxicity[f] | Toxicity | 15 mg/kg bw per day | 75 mg/kg bw per day |
| | Two-generation study of reproductive toxicity[a] | Parental toxicity | 5 ppm, equal to 0.5 mg/kg bw per day | 25 ppm, equal to 2.1 mg/kg bw per day |
| | | Reproductive toxicity | 25 ppm, equal to 2.1 mg/kg bw per day | 125, equal to 10 mg/kg bw per day |
| | | Offspring toxicity | 25 ppm, equal to 2.1 mg/kg bw per day | 125 ppm equal to 10 mg/kg bw per day |
| | One-generation study of reproduction[a] | Parental toxicity | 25 ppm, equal to 1.7 mg/kg bw per day | 125 ppm, equal to 8.7 mg/kg bw per day |
| | | Reproductive toxicity | 125 ppm, equal to 8.7 mg/kg bw per day[e] | — |
| | | Offspring toxicity | 125 ppm, equal to 8.7 mg/kg bw per day[e] | — |
| | Developmental toxicity[f] | Maternal toxicity | 2.5 mg/kg bw per day | 25 mg/kg bw per day |
| | | Embryo and fetal toxicity | 25 mg/kg bw per day[e] | — |
| | Acute neurotoxicity[f] | Neurotoxicity | 15 mg/kg bw per day | 75 mg/kg bw per day |
| | Ninety-day neurotoxicity study[a] | Neurotoxicity | 5 ppm, equivalent to 0.2 mg/kg bw per day | 100 ppm, equivalent to 6.7 mg/kg bw per day |
| Rabbit | Developmental toxicity[f] | Maternal toxicity | 4 mg/kg bw per day | 40 mg/kg bw per day |
| | | Embryo and fetal toxicity | 40 mg/kg bw per day[e] | — |
| Dog | Thirteen-week and 1-year studies of toxicity[a,d] | Toxicity | 30 ppm, equal to 0.82 mg/kg bw per day | 100 ppm, equal to 3.3 mg/kg bw per day |

[a] Dietary administration.

[b] Male liver adenomas only.

[c] Lowest dose tested.

[d] Two or more studies combined.

[e] Highest dose tested.

[f] Gavage administration.

*Estimate of acceptable daily intake for humans*

0–0.002 mg/kg bw

*Estimate of acute reference dose*

0.2 mg/kg bw

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

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

***Critical end-points for setting guidance values for exposure to dicofol***

| | |
|---|---|
| *Absorption, distribution, excretion and metabolism in mammals* | |
| Rate and extent of oral absorption | Extensively absorbed from the gastrointestinal tract within 24 h |
| Distribution | Adipose tissue > adrenal gland > thyroid > liver > whole blood |
| Potential for accumulation | Slightly, in fat |
| Rate and extent of excretion | Majority excreted within 96 h, primarily in faeces |
| Metabolism in animals | Metabolism involved dechlorination and oxidation of the ethanol moiety and hydroxylation of the aromatic rings |
| Toxicologically significant compounds (animals, plants and the environment) | Dicofol |
| *Acute toxicity* | |
| Rat, $LD_{50}$, oral | 578 mg/kg bw (purity 94–96%) |
| Rat, $LD_{50}$, dermal | > 5000 mg/kg bw |
| Rat, $LC_{50}$, inhalation | > 5.0 mg/L |
| Rabbit, skin irritation | Slight to moderately irritating |
| Rabbit, eye irritation | Slight to moderately irritating |
| Guinea-pig, skin sensitization (Buehler test) | Slight to moderate sensitizer |
| *Short-term studies of toxicity* | |
| Target/critical effect | Decreased body weight; reduced cortisol response (dogs) |
| Lowest relevant oral NOAEL | 0.82 mg/kg bw per day (dogs) |
| Lowest relevant dermal NOAEL | 3 mg/kg bw per day (90-day study in dogs) |
| Lowest relevant inhalation NOAEC | Not available |

| | |
|---|---|
| *Genotoxicity* | |
| | Not genotoxic |
| *Long-term studies of toxicity and carcinogenicity* | |
| Target/critical effect | Decreased body weight, hepatocellular necrosis, increased ALT and AP |
| Lowest relevant NOAEL | 0.22 mg/kg bw per day (2-year toxicity/carcinogenicity study in rats) |
| Carcinogenicity | Unlikely to pose a carcinogenic risk to humans at anticipated dietary exposure levels |
| *Reproductive toxicity* | |
| Reproduction target/critical effect | Decreased viability index (rats) |
| Lowest relevant reproductive NOAEL | 2.1 mg/kg bw per day |
| Developmental target/critical effect | None |
| Lowest relevant developmental NOAEL | 40 mg/kg bw per day (highest dose tested) |
| *Neurotoxicity/delayed neurotoxicity* | |
| Acute neurotoxicity target/critical effect | Ataxia, decreased motor activity at systemically toxic dose |
| Lowest relevant acute neurotoxicity NOAEL | 15 mg/kg bw |
| Subchronic neurotoxicity target/critical effect | Decreased motor activity at systemically toxic doses |
| Lowest relevant subchronic neurotoxicity NOAEL | 0.2 mg/kg bw per day (90-day neurotoxicity study) |
| *Other toxicological studies* | |
| | No data |
| *Medical data* | |
| | Reversible neurological effects and nonspecific symptoms after acute poisoning |

***Summary***

| | Value | Study | Safety factor |
|---|---|---|---|
| ADI | 0–0.002 mg/kg bw | Chronic toxicity/carcinogenicity study in rats supported by the 90-day neurotoxicity study in rats | 100 |
| ARfD | 0.2 mg/kg bw | Acute neurotoxicity study in rats | 100 |