5.11 DITHIANON (180)

TOXICOLOGY

Dithianon ($C_{14}H_4N_2O_2S_2$) is the International Organization for Standardization (ISO)–approved name for 5,10-dihydro-5,10-dioxonaphtho[2,3-b]-1,4-dithiine-2,3-dicarbonitrile (International Union of Pure and Applied Chemistry [IUPAC]), with Chemical Abstracts Service (CAS) No. 3347-22-6. Dithianon is used on a range of fruits and vegetables as a multi-site contact fungicide that inhibits spore germination.

Dithianon was evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1992, when an acceptable daily intake (ADI) of 0–0.01 mg/kg body weight (bw) was established. It is being reviewed at the present Meeting as part of the periodic re-evaluation programme of the Codex Committee on Pesticide Residues (CCPR). All the pivotal studies met the basic requirement of the relevant guideline and contained certificates of compliance with good laboratory practice (GLP) or quality assurance.

Biochemical aspects

At tested doses of 10 and 50 mg/kg bw, orally administered dithianon was about 40–50% absorbed in rats, with a time to maximum concentration in blood of approximately 6 h. There were no substantial dose- or sex-related differences in the absorption, elimination or distribution of radioactivity following oral administration of [^{14}C]dithianon. The majority of the administered dose was recovered in faeces (64.0–72.2%) and urine (26.7–31.4%).

The material balance from a preliminary study showed that dithianon was not metabolized to volatile compounds, including carbon dioxide. The elimination half-life was between 46 and 57 h. There was no bioaccumulation of dithianon in tissues. The parent compound was extensively metabolized by the following key transformation steps: oxidation of the sulfur atoms, cleavage of the dithiine ring, reduction of the 1,4-naphthoquinone moiety and further glucuronidation, as well as substitution of the carbonitrile moieties by amino and carboxy groups. The only metabolite in rat urine at a level greater than 2% was M216F020 (glucuronic acid conjugate of 1,4-dihydroxynaphthalene). All other identified metabolites were present in insignificant amounts. The metabolic pathways were similar in male and female rats.

Toxicological data

Dithianon technical has moderate acute toxicity in rats (oral median lethal dose [LD_{50}] approximately 300 mg/kg bw). The dermal LD_{50} in rats was greater than 2000 mg/kg bw. Dithianon is slightly to moderately toxic by acute inhalation in rats, with a median lethal concentration (LC_{50}) between 0.31 and 2.1 mg/L, depending on particle size. Dithianon is non-irritating to rabbit skin but is a severe eye irritant. It was found to be a skin sensitizer (guinea-pig maximization test).

Short-term oral toxicity studies were conducted in mice, rats and dogs. These studies indicate that the kidney is the main target organ. A 4-week (range-finding) study in mice with dithianon administered in the diet resulted in slight anaemia and haemosiderin deposition in the liver of females at 500 ppm, with a no-observed-adverse-effect level (NOAEL) of 100 ppm (equivalent to 15 mg/kg bw per day). A 90-day rat oral toxicity study revealed slight anaemia (both sexes) as well as histopathological findings of renal tubular epithelial cell degeneration and regenerative hyperplasia (females only) at 1080 ppm, with a NOAEL of 180 ppm (equal to 14.6 mg/kg bw per day).

Studies in dogs included a 90-day dietary study and a 1-year dietary study. In the 90-day study, the doses used were 0, 40, 200 and 1000 ppm (equal to 0, 0.63, 2.95 and 12.6 mg/kg bw per day). In the 1-year study, the same dietary doses were used, but the compound intakes were 0, 1.6, 7.3 and 37.1 mg/kg bw per day. In the 90-day study, the NOAEL was 2.95 mg/kg bw per day, based on

decreases in body weight (females), decreased body weight gain (males), decreased food consumption, increases in alkaline phosphatase activity and increased kidney weights (males and females) and increased thromboplastin time (females) at 12.6 mg/kg bw per day. Oral administration of dithianon for 1 year resulted in slight anaemia, liver impairment and effects on kidney and thyroid. The NOAEL for dithianon fed to dogs in their diets for 1 year was 200 ppm (equal to 7.3 mg/kg bw per day), based on increases in kidney and liver weights in both sexes at 1000 ppm (equal to 37.1 mg/kg bw per day).

Long-term toxicity and carcinogenicity studies were undertaken in mice (80 weeks) and rats (104 weeks). In male mice at 500 ppm in the diet, there was an association between the observed kidney damage (chronic nephropathy) and an increased incidence of early mortality, indicating that the maximum tolerated dose (MTD) was exceeded at 500 ppm. No increases in tumour incidence were noted in any of the treatment groups. The NOAEL for chronic toxicity in mice was 20 ppm (equivalent to 3 mg/kg bw per day), based on increased absolute and relative kidney weights for males and females and an exacerbation of spontaneous chronic nephropathy in females at 100 ppm. Dithianon was not carcinogenic in mice at a dietary concentration of 500 ppm, the highest dose tested. In the rat study, the NOAEL for chronic toxicity was 20 ppm (equivalent to 1 mg/kg bw per day), based on histopathological kidney lesions (females) at 120 ppm. Increased incidences of renal tubule adenomas and carcinomas in kidney were observed in female rats at 600 ppm (equivalent to 30 mg/kg bw per day). The highest dietary concentration (600 ppm) also resulted in a consistently significant lower body weight (19.8–20.5%) in female rats compared with controls at weeks 72, 84 and 104. Moreover, only the highest dose (i.e., 600 ppm) in female rats demonstrated severe glomerulonephropathy with sclerosis. The tumours in rat kidneys were associated with severe nephrotoxicity in proximal tubular cells. This dose exceeded the MTD in females.

In a 7-day dietary study in rats, hydropic degeneration of the proximal tubular epithelial cells was seen in both males and females receiving 600 ppm (equivalent to 60 mg/kg bw per day) or 1080 ppm (equivalent to 108 mg/kg bw per day) at 4 and 7 days, with significantly greater incidence and severity in females. In females at day 7, the newly regenerated tubular epithelial cells with signs of hydropic degeneration demonstrated the susceptibility of renal tubules to damage. In contrast, males showed no evidence of further degeneration, suggesting adaptation to the toxic effects by day 7. Electron microscopy suggested that the mitochondria in the proximal tubular cells were the target. The NOAEL for this study was 120 ppm (equivalent to 12 mg/kg bw per day) in females.

These findings were further substantiated by a 28-day dietary renal turnover study in female rats. Continuous labelling techniques using bromodeoxyuridine (BrdU) showed an increase in tubular cell turnover at 600 ppm (equivalent to 60 mg/kg bw per day), consistent with the histopathological results. The NOAEL for the repeated cellular degenerative/regenerative responses was 120 ppm (equivalent to 12 mg/kg bw per day). It appears that persistent cellular damage to proximal tubular epithelial cells triggers a regenerative response in basophilic tubules, which is the basis for the development of proliferative lesions following long-term (2-year) dietary exposure of female rats to 600 ppm. Neither the degenerative/regenerative (cell turnover) responses nor renal tumours (in the carcinogenicity study) were observed at concentrations of 20 or 120 ppm. The high susceptibility of female rats to renal effects (proximal tubular degeneration, regeneration and tumours) might be related to the involvement of cyclooxygenase-2 (COX-2), but experimental evidence for this as an explanation for the sex difference is not available.

Dithianon was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. The majority of results were negative, including in vivo studies. There were some positive results in vitro that occurred only at cytotoxic doses.

The Meeting concluded that dithianon is unlikely to be genotoxic in vivo.

In view of the lack of in vivo genotoxicity, the lack of any tumorigenic response in mice and male rats and the fact that the kidney tumours in female rats occurred only at doses that were cytotoxic, the Meeting concluded that dithianon is unlikely to pose a carcinogenic risk at human dietary exposure levels.

In a multigeneration study in rats, the NOAEL for fertility and reproductive functions was 600 ppm (equal to 27.6 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 200 ppm (equal to 9 mg/kg bw per day), based on reductions in food consumption and body weight gain in parental animals of both generations at 600 ppm.

Developmental toxicity studies have been carried out in rats and rabbits. In a rat developmental study, the NOAEL for maternal toxicity and embryo and fetal toxicity was 20 mg/kg bw per day, based on decreases in body weight gain and food consumption in the dams and increased number of resorptions and a subsequent reduction in the mean number of fetuses per dam at 50 mg/kg bw per day and above. In a rabbit developmental toxicity study, the NOAEL for maternal toxicity was 10 mg/kg bw per day, based on reductions in body weight gain and food consumption at 25 mg/kg bw per day. The NOAEL for developmental effects was 25 mg/kg bw per day, based on an increased incidence of post-implantation loss resulting from an increase in abortions and resorptions and a subsequent reduction in the mean number of fetuses per doe at 40 mg/kg bw per day. There were no developmental effects in the absence of maternal toxicity.

The Meeting concluded that dithianon did not cause developmental toxicity at doses that were not toxic to the dams and that it was not teratogenic.

In a 4-week neurotoxicity study in rats, a NOAEL of 15 mg/kg bw per day was identified based on clinical observations of smeared anogenital region with urine and dark discoloured urine at 30 mg/kg bw per day. There were no signs of neurotoxicity in any other study.

Skin and eye irritation have been repeatedly observed in dithianon-exposed workers. In operators spraying dithianon-containing products, erythema, swelling, itching, blistering and peeling of the skin have been reported.

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

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.01 mg/kg bw for dithianon based on a NOAEL of 1 mg/kg bw per day for histopathological kidney lesions in females at 6 mg/kg bw per day in a 2-year toxicity study of rats and using a 100-fold safety factor.

The Meeting established an acute reference dose (ARfD) of 0.1 mg/kg bw for dithianon, taking into account a NOAEL of 12 mg/kg bw and using a safety factor of 100. The NOAEL was based on a mechanistic study in which nephrotoxicity was assessed in rats following 4 and 7 days of dosing. At these time points, a dietary intake of 60 mg/kg bw per day of dithianon induced repeated cellular degenerative/regenerative responses in kidney tubular cells of female rats.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighty-week study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equivalent to 3 mg/kg bw per day	100 ppm, equivalent to 15 mg/kg bw per day
		Carcinogenicity	500 ppm, equivalent to 75 mg/kg bw per day[b]	—
Rat	Seven-day study of toxicity[a]	Nephrotoxicity	120 ppm, equivalent to 12 mg/kg bw per day	600 ppm, equivalent to 60 mg/kg bw per day
	Ninety-day study of	Toxicity	180 ppm, equal to 14.6	1080 ppm, equal to 86.7

Species	Study	Effect	NOAEL	LOAEL
	toxicity[a]		mg/kg bw per day	mg/kg bw per day
	Twenty-four-month study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equivalent to 1 mg/kg bw per day	120 ppm, equivalent to 6 mg/kg bw per day
		Carcinogenicity	120 ppm, equivalent to 6 mg/kg bw per day	600 ppm, equivalent to 30 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Offspring	600 ppm, equal to 27.6 mg/kg bw per day[b]	—
		Parental toxicity	200 ppm, equal to 9 mg/kg bw per day	600 ppm, equal to 27.6 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	10 mg/kg bw per day	25 mg/kg bw per day
		Embryo and fetal toxicity	25 mg/kg bw per day	40 mg/kg bw per day
Dog	Twelve-month study of toxicity[a]	Toxicity	200 ppm, equal to 7.3 mg/kg bw per day	1000 ppm, equal to 37.1 mg/kg bw per day

[a] Dietary administration.

[b] Highest dose tested.

[c] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.1 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 dithianon

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	T_{max} approximately 6 h, 42–52% absorbed
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Excreted in faeces (64.0–72.2%) and urine (26.7–31.4%)
	Half-life 46–57 h
Metabolism in animals	Extensive
Toxicologically significant compounds in animals, plants and the environment	Dithianon
Acute toxicity	
Rat, LD_{50}, oral	300 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw

Rat, LC_{50}, inhalation	0.31 mg/L
Rabbit, dermal irritation	Non-irritant
Rabbit, ocular irritation	Severely irritant
Guinea-pig, dermal sensitization	Sensitizer (Magnusson and Kligman test)
Short-term studies of toxicity	
Target/critical effect	Kidney, tubular damage
Lowest relevant oral NOAEL	12 mg/kg bw per day (7-day study in rats)
	7.3 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney, tubular damage
Lowest relevant NOAEL	1 mg/kg bw per day (24-month study in rats)
Carcinogenicity	Only in kidneys of female rats at cytotoxic doses
Genotoxicity	
	Not genotoxic in vivo
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	27.6 mg/kg bw per day, the highest dose tested, in rats
Developmental target/critical effect	Increase in post-implantation loss in the presence of maternal toxicity; not teratogenic
Lowest relevant developmental NOAEL	25 mg/kg bw per day in rabbits
Neurotoxicity/delayed neurotoxicity	
	Not neurotoxic
Other toxicological studies	
	Mechanistic studies on kidney toxicity
Medical data	
	Local skin and eye irritation effects in exposed plant workers and operators

Summary

	Value	**Study**	**Safety factor**
ADI	0–0.01 mg/kg bw	Two-year dietary toxicity study in rats	100
ARfD	0.1 mg/kg bw	Four/seven-day nephrotoxicity study in rats	100