

**DITHIANON**

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**EXPLANATION**

Dithianon is used as a multi-site protective fungicide which inhibits spore germination. It is used on a range of fruits and vegetables. Dithianon was considered for the first time by the present Meeting.

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

Four male and 4 female rats were administered once daily 25 mg <sup>14</sup>C-dithianon (dioxonaphtyl-labelled)/kg bw/day in dimethyl sulfoxide for 7 days. Faeces and urine were collected at daily intervals until 8 days after the last administration. After 24 h, males eliminated 24.3% via urine and 37.1% via faeces and females 22.9% and 32.2%, respectively. Seventy-two hours after the last treatment, 1.0% (males) and 1.8% (females) of the administered dose was still found in faeces and urine. Total recovery was 69.1% and 68.0% in the faeces and 27.5% and 28.3% in urine of males and females, respectively. The carcass contained 0.1% of the total administered radioactivity (Schlüter, 1985).

Radioactivity in blood was measured in 5 rats/sex over a period up to 6 days following a single oral dose of 25 mg <sup>14</sup>C-dithianon/kg bw. Plasma radioactivity levels 15 minutes after dosing were 0.07 mg/l (males) and 0.15 mg/l (females) increasing to peak levels of 1.2 mg/l (males) and 1.9 mg/l (females) 8 h after dosing. Thereafter the values decreased rapidly to 0.07 mg/l after 144 h for both sexes (Schlüter, 1985).

Rats (5/sex) were given a single oral dose of 25 mg <sup>14</sup>C-dithianon/kg bw/day for 7 days. Tissue distribution was measured over 5 periods up to 7 days after the last treatment. Twenty-four hours after the last treatment, most tissue

levels of radioactivity were below 0.01% of the administered dose except for liver (0.01-0.02%) and stomach and intestine (0.3-2.2%). After 7 days, all tissue residues were < 0.01% of the administered radioactivity (Schlüter, 1985).

One rat/sex was given a single oral dose of 50 mg <sup>14</sup>C-dithianon (dioxonaphthyl-labelled)/kg bw. After 5 days, a total of 95.8% and 101.3% was excreted by the male and female rat, respectively. Urinary excretion was almost complete within 48 h and accounted for 33.5%-30.3% of the total radioactivity. Faecal excretion was complete by 72 h and accounted for 62.1% and 70.9%. No radioactivity was detected in the expired air during the 48 h after dosing (Hawkins *et al.*, 1989).

Groups of rats (5/sex/group) were administered a single oral dose of 10 or 50 mg <sup>14</sup>C-dithianon. Total recovery was about 97% regardless of the dose. After 5 days most of the radioactivity was excreted in the faeces (about 65%) while 31% was recovered in urine. Elimination occurred predominantly during the first 24 h. Dithianon was absorbed to approximately the same extent at the low- and high-dose levels. Only a small proportion of the administered dose was recovered in tissues. Highest amounts after the low-dose administration were in kidneys (males 0.16 mg/kg and females 0.17 mg/kg), GIT (males and females 0.05 mg/kg) and whole-blood (males 0.04 mg/kg and females 0.05 mg/kg). After the administration of 50 mg the highest tissue residues were also found in kidneys (males 0.58 mg/kg and females: 0.66 mg/kg), GIT (males 0.15 mg/kg and females 0.23 mg/kg) and whole-blood (males 0.28 mg/kg and females 0.36 mg/kg) (Hawkins *et al.*, 1989).

The repeated administration of 14 daily non-labelled doses of 10 mg dithianon/kg bw/day followed by a single oral dose of 10 mg <sup>14</sup>C-dithianon/kg bw had no significant effect on the absorption, excretion and distribution of dithianon in rats (Hawkins *et al.*, 1989).

Two groups of 3 rats/sex with cannulated bile ducts received a single oral dose of 10 or 50 mg <sup>14</sup>C-dithianon/kg bw. Bile samples were collected at 3-h intervals and urine and faeces were collected at 24 h intervals. The rats were sacrificed 48 h after dosing. About 10 and 7% of the dose was recovered in the bile at the low and the high dose, respectively (Hawkins, 1989).

After a single oral administration of 10 or 50 mg <sup>14</sup>C-dithianon/kg bw to rats radioactivity in plasma was measured at various time intervals up to 240 h after dosing. Peak plasma levels were reached in 6 h (males 0.99 mg/l and 3.9 mg/l and females 0.76 mg/l and 3.81 mg/l at the low- and high-dose, respectively). At 240 h all concentrations were below the detection limit. The terminal half-lives were similar for both dose levels, 55.8 and 46.4 h for males and 56.8 and 56.7 h for females, at 10 and 50 mg/kg bw, respectively (Hawkins *et al.*, 1989).

## Hens

Groups of 5 laying hens were given capsules containing 0, 0.36 or 3.6 mg  $^{14}\text{C}$ -dithianon/day (purity 99.3%), orally for 5 consecutive days. These doses were equivalent to 3 or 30 ppm in the feed, respectively. Excreta and eggs were collected twice daily. Radioactivity was excreted very rapidly and at a constant rate. Irrespective of the dose, 4 days after the last administration, total recovery of  $^{14}\text{C}$  was about 94% of the dose with about 90% recovered in the excreta and 3-5% in the GIT contents. Detectable tissue residues were found in kidney and liver (0.03% and 0.02%, respectively). The  $^{14}\text{C}$  recovered in eggs comprised less than 0.01% of the total radioactive dose. The radioactivity in egg yolk was higher than in egg white and was maximal at sacrifice (0.005 mg/kg and 0.075 mg/kg at the low- and the high-dose, respectively) (Cheng, 1990a).

## Goats

Two lactating goats were given  $^{14}\text{C}$ -dithianon in capsules orally for 5 consecutive days at a dose level of 6 mg/day or 60 mg/day. These dose levels were equivalent to 3 or 30 ppm in the feed, respectively. A control goat received placebo capsules. Body-weight, food consumption and clinical signs were recorded. Daily samples of urine, faeces and milk were collected. The goats were sacrificed 5 h after the last dose. Excretion was rapid and constant with the majority excreted in the faeces (50.2% to 53.7%) followed by the urine (27.9% and 24.2%). Less than 0.1% of the total radioactivity was recovered in the milk. Concentrations of radioactivity in milk, muscle and fat were less than 0.003 and 0.03 mg/kg for the low- and high-dosed animal. The levels in liver (0.02 and 0.17 mg/kg) and kidneys (0.06 and 0.49 mg/kg) were higher. Concentration in the bile was high, 0.3 and 2.9 mg/kg for the low- and the high-dose, respectively. This points to biliary involvement in the excretion also for the goat (Cheng, 1990b).

## Biotransformation

After 7 daily treatments of  $^{14}\text{C}$ -dithianon (dioxonaphtyl-labelled), urinary and faecal extracts were studied with thin-layer chromatography. There was no difference between males and females. The compound is quickly metabolized to a great number of polar compounds. None of these compounds exceeded 5% of the administered radioactivity. According to the authors this is due to the molecular structure of the substance, having different functional groups (CO, CN, S). Also cleavage of the molecule in various places is possible. It is assumed that very reactive products are formed which are immediately degraded further or react with endogeneous compounds in the animal to form polar products which are rapidly excreted. Less than 1% unchanged dithianon is found in the faeces (Schlüter, 1985).

In the study of Hawkins *et al.* (1989) extracts of urine and faeces were examined by TLC. More than 14 components were detected in the urine. The three major components had R<sub>f</sub> values of 0.98, 0.55 and 0.36. The two more polar compounds (R<sub>f</sub> 0.55 and 0.36) were hydrolyzed with β-glucuronidase or sulfatase giving rise to a higher proportion of the component with R<sub>f</sub> value of 0.98. In the faeces the major components had the same chromatographic properties as those found in urine extracts. No attempt was made to characterize any of the metabolites.

### **Toxicological studies**

#### **Acute toxicity studies**

The acute toxicity of dithianon to several animal species is given in Table 1. Signs of toxicity following oral administration included sedation, dyspnoea, abnormal appearance, changes in gait and body posture, emaciation and diarrhoea.

The acute oral toxicity in Wistar rats of the formulation Delan Liquid (25% dithianon) was 1325 mg/kg bw (Sommer & Frohberg, 1968a).

#### **Short-term toxicity studies**

##### **Mice**

In a range-finding study, groups of 6 Crl:CD-1(ICR)BR mice/sex were fed diets containing 0, 100, 500 or 1000 ppm dithianon (purity 92%) for 4 weeks. Observation included clinical signs, body-weight, food consumption, haematology and clinical chemistry. Macroscopical examinations were performed on all mice, but weight and histopathology of liver and kidney were examined in female mice only. Body-weight gain was decreased at the highest dose. Hb, PCV and RBC counts were slightly lower in male mice at 1000 ppm and in female mice at 500 and 1000 ppm. T<sub>3</sub> and T<sub>4</sub> concentrations were lower in treated mice (dose-related in females). Increased relative liver and kidney weight were observed in mid- and high-dose females. A dose-related increased deposition of pigment in Kupffer cells was seen in 4/6 female livers at 500 ppm and in 6/6 female livers at 1000 ppm (Brown, 1987).

**Table 1. Acute toxicity of dithianon**

Species	Strain	Sex	Route	LD <sub>50</sub> mg/kg bw	LC <sub>50</sub> mg/m <sup>3</sup>	Reference
Mouse <sup>1</sup>	JCL:ICR	M	oral	492		Sakamoto <i>et al.</i> (1975)
		F		528		
		M	i.p.	100		
		F		77		
		M&F	dermal	>3200		
		M&F	s.c.	>3200		
Mouse	NMRI	M&F	i.p.	49		Sommer & Frohberg (1969)
Rat <sup>2</sup>	KFM-Han Wistar	M	oral	720		Ullmann (1987a)
		F		678		
Rat <sup>3</sup>	KFM-HAN wistar	M&F	inhal. (4-hr exp.)		2089	Ullmann (1984)
Rat <sup>2</sup>	KFM-Han wistar	M&F	dermal (24-hr exp.)	>2000		Ullmann (1986a)

<sup>1</sup> the purity of dithianon was 95%

<sup>2</sup> the purity of dithianon was 92%

<sup>3</sup> technical dithianon of 94.7% purity was used.

## Rats

Groups of 10 rats/sex were fed diets containing 0, 30 or 180 ppm dithianon (purity 92%) equal to 0, 2.5 and 14.6 mg/kg bw/day for males and 0, 2.97 and 16.3 mg/kg bw/day for females, respectively, for 90 days. The high-dose group received 1080 ppm (equal to 86.7 mg/kg bw/day for males and 99.5 mg/kg bw/day for females) and consisted of 20 rats/sex of which 10/sex were kept after the 90-day dosing for a 4-week recovery period. Observations included clinical signs, body-weight, food consumption, haematology, clinical chemistry, urinalysis, ophthalmoscopy, auditory acuity and dentition, macroscopy, organ weight and histopathology. Body-weight gain was decreased in high-dose males and females; Hb and Ht and the number of erythrocytes was significantly decreased and the number of reticulocytes was increased. Male rats at all dose levels showed significantly decreased T<sub>3</sub> levels (not clearly dose-related) and at the highest dose decreased T<sub>4</sub> levels. TSH was slightly decreased in males at low- and mid-dose and increased at the highest dose. Blood urea was increased in high-dose males (statistically significant) and females. Chloride was decreased in females at 1080 ppm as was total protein. Albumin and  $\alpha_1$ -globulin was increased in males (statistically significant) and females. Urinalysis showed that, in males at 1080 ppm, specific gravity was decreased. Absolute and relative liver and kidney weight and relative adrenal weight were slightly increased in both sexes at 108 ppm. In females a tendency to an increased

absolute and relative kidney weight was seen at 180 ppm. This is considered to be an effect because the kidney in the female rat is the target organ. After the recovery period, females still exhibited a slightly depressed erythrocyte count and an increased kidney weight and in males  $T_4$  levels were still depressed, all at the highest dose. No histopathological lesions were found. The NOAEL in this study was 30 ppm, equal to 2.5 mg/kg bw/day, based on increased kidney weight in females (Leuschner & Neumann, 1987).

Kidney slides of 10 control female rats and of 20 rats at 1080 ppm (including 10 from the recovery experiment) were re-evaluated. The incidence of hydropic degeneration and tubular hyperplasia was increased in treated rats at termination of the treatment period (Grasso, 1991a).

Groups of rats (5/sex/group) were exposed to 0.11, 0.31 or 1.07 g dithianon/m<sup>3</sup>, 6 h/day, 5 days/week for 2 weeks (head/nose exposure). One of the mid-dose groups was kept for a recovery period of 14 days. Additionally, 2 groups of 5 rats/sex were exposed to filtered air and the vehicle alone, respectively. No dose-related effects were observed on clinical signs, body-weight, food consumption, haematology, blood chemistry, urinalysis, organ weight, macroscopy or histopathology. The NOAEL in this study was  $\geq 1.07$  g/m<sup>3</sup> (Bhide, undated).

Groups of Wistar rats (9/sex/group) were exposed to an aerosol of 0 or 33.6 mg/m<sup>3</sup> Delan WP (equal to 26 mg/m<sup>3</sup> dithianon), 4 h/day, 6 days/week for a total of 13 exposures (head/nose exposure). No effects were observed on clinical signs, body-weight, food consumption, haematology, clinical chemistry, macroscopy or histopathology. The NOAEL in this study was  $\geq 33.6$  mg/m<sup>3</sup> Delan WP, equal to 26 mg/m<sup>3</sup> dithianon (Sommer & Frohberg, 1971; Spicer, 1971a).

In a 5-day dermal range-finding study, 2000 mg dithianon/kg bw/day was administered to 5 Crl:CD(SD)BR rats/sex for 6 h/day on 5 consecutive days. Brown staining was observed at the application site and on surrounding hair with dermal erythema or fissuring. Body-weight and food consumption were slightly reduced in treated male rats (Lackenby, 1987).

In a dermal limit test, suspensions of dithianon (purity 92%) in PEG 200 were applied to the skin of Crl:CD(SD)BR rats (5/sex/group) at doses of 0 or 1000 mg/kg bw/day, 6 h/day on 22 consecutive days. Mortality, food consumption and blood biochemistry showed no dose-related effects. Body-weight gain was decreased in treated males. In treated females, Hb, RBC and PCV were slightly lower and MCV was slightly higher. Adrenal and kidney weights were increased in female rats. At histopathology slight irritation was observed in the treated skin. The incidence of basophilic tubules was increased in the kidneys of treated rats; in some of the females, the lesion was associated with the

presence of occasional mitotic figures and eosinophilic material in the tubular lumen (Brown, 1989).

Because of the effects seen at 1000 mg/kg bw/day, an additional study was carried out according to the same protocol with 5 rats/sex/group administered 0, 40 or 200 mg dithianon/kg bw/day. At both dose levels the same haematological changes were observed not only in females but also in some treated males. Body weight was decreased and adrenal, kidney and liver weights were increased in treated rats. Skin damage (moderate to marked acanthosis, slight to moderate hyperkeratosis, leucocyte infiltration in the stratum corneum and the dermis and occasionally ulceration) was observed in all treated rats. Most of the systemic effects may be secondary, due to stress caused by the marked local irritation. A NOAEL was not determined in this study (Brown, 1989).

### Dogs

In a 4-week range-finding study, one beagle dog/sex was fed 1000 ppm dithianon (purity 92%) for 14 days. After 7 days off-dose, they were treated for 7 days at 60 ppm. One dog/sex, used as control dog for the initial 15 days, was given 1000 ppm dithianon in the diet from days 15-21 and 1500 ppm from days 21-28. Food consumption and body-weight were markedly reduced at 1500 ppm, while at 1000 ppm only slight changes in food consumption were observed (Pickersgill, 1987).

Dithianon (purity 92%) was fed in the diet to groups of 4 beagle dogs/sex at dose levels of 0, 40, 200 or 1000 ppm (equal to 0, 0.6, 3.0 or 12.6 mg/kg bw/day for males and 0, 0.7, 3.0 or 12.6 mg/kg bw/day for females, respectively) for 90 days. No dose-related effects were observed on mortality, clinical signs, haematology, urinalysis, eye examination, hearing, dentition, macroscopy or histopathology. Food consumption was decreased in females at 1000 ppm. Body-weight gain was lower at the highest dose. A marked increase in ALP was observed in both sexes after 6 and 13 weeks at 1000 ppm. The absolute and relative weights of liver, spleen and kidneys were increased in all high-dose dogs and absolute and relative thymus weight was decreased at the highest dose. No histopathological findings were observed. The NOAEL in this study was 200 ppm, equal to 3 mg/kg bw/day, based on increased organ weights at the high dose (Neumann & Leuschner, 1989).

Groups of beagle dogs (4/sex/group) were fed diets containing 0, 40, 200 or 1000 ppm dithianon (purity 92%) for 52 weeks. Observations included clinical signs, food consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, macroscopy, organ weight and histopathology. During the first 13 weeks, food consumption was decreased in high-dose males. Male body-weight was lower during the whole study period. Hb, RBC and PCV were decreased in all high-dose dogs and MCHC was decreased in high-dose females

throughout the study. At termination, platelet count was increased in high-dose males. In 3/4 high-dose males and 3/4 high-dose females erythrocytes in which deposition of cholesterol had occurred were present. At the highest dose, ALP activity was increased and BUN and creatinine were decreased. The incidence of blood in urine was increased in females at the mid- and high-dose level at week 26 and in high-dose females at termination. Absolute and relative liver, kidney and thyroid weights (spleen not measured) were increased at 1000 ppm. In females a tendency to an increased liver weight was observed at 200 ppm. The incidence of hepatocellular hypertrophy in the liver was increased in females at 200 and in both males and females at 1000 ppm. In the same groups there was also an increase in tubular pigment in the kidney. Intranuclear inclusions and foci of pigmented histiocytes in the liver were observed in most high-dose dogs. The NOAEL in this study was 40 ppm, equivalent to 1 mg/kg bw/day, based on increased liver weight and histopathological changes at 200 ppm (Clay, 1991).

Groups of 4 beagle dogs/sex were administered 0, 40, 400 or 1000 ppm dithianon (purity 95.3%) in the diet for 2 years. No effects were observed on clinical signs, mortality, body-weight, urinalysis or ophthalmoscopy. Food consumption was slightly depressed in high-dose dogs during the first 3 months of the study. At the highest dose level, Hb, PCV and RBC were decreased throughout the study and platelet counts tended to increase. At 1000 ppm, activity of ALP and ASAT were increased, as were levels of total protein and  $\beta$ -globulin. At the end of the study, a decrease in glucose 6-phosphatase activity in the liver was seen in all female dogs and 1 male dog at 1000 ppm. Absolute and relative liver, kidney, pituitary, pancreas, thyroid weights were increased in dogs at 1000 ppm. Liver weight was also increased at 400 ppm. An irregular appearance of the exterior and cut surface of the liver was seen at 1000 ppm. At histopathology liver changes, consisting of hepatocyte enlargement associated with the presence of Kupffer cells which contained brown material, were observed at 1000 and sometimes at 400 ppm, more marked in females than in males. In the kidney tubular brown pigmentation was increased at 1000 ppm. The NOAEL in this study was 40 ppm, equivalent to 1 mg/kg bw/day, based on increased liver weight and histopathological changes at 400 ppm (Noel *et al.*, 1969)

Additionally, stains for iron were used on all liver sections and bone marrow smears and sections were examined. The incidence and degree of iron accumulation was increased at 1000 ppm, particularly in females (Spicer, 1971b).

## Long-term toxicity/carcinogenicity studies

### Mice

Groups of Crl:CD-1 (ICR)BR mice (51/sex/group) were fed concentrations of 0, 20, 100 or 500 ppm dithianon (purity 92%) in the diet for 80 weeks. Observations included clinical signs, mortality body-weight, food and water consumption, haematology (differential white cell count), macroscopy, organ weights (brain, liver, thyroids, kidneys and testes of 20 mice/sex/group) and histopathology (about 40 tissues from control and high-dose mice and from mice that died or were killed in extremis, and tissues of lungs, liver and kidneys of all mice in the mid- and low-dose groups).

Mortality was increased in high-dose males during the second part of the study. The incidence of fur staining in mice was increased at 500 ppm. Male terminal body-weight was lower in high-dose group. Relative kidney weight was increased in mice at 100 and 500 ppm and relative brain weight was decreased in high-dose males. Histopathological kidney changes were observed in males at 500 ppm and in females at 100 and 500 ppm. Tumour incidence was not enhanced. The NOAEL in this study was 20 ppm, equal to 2.9 mg/kg bw/day, based on increased kidney weight and an increased incidence of chronic nephrosis (Brown, 1990).

### Rats

Groups of Charles River CD rats (35/sex/group) were fed diets containing 0, 20, 200 or 1000 ppm dithianon (purity 95%) for 2 years. High-dose rats received 500 ppm dithianon in their feed during the first 4 weeks of the study. An interim kill was performed on 5 rats/sex/group at weeks 26 and 52 for clinical and pathological examinations. The remaining rats were sacrificed after 104 weeks except for the high-dose group in which the surviving males and females were killed at week 88 and 96, respectively, due to declining general condition. Observations included mortality, clinical signs, body-weight, food and water consumption, food efficiency, haematology, blood chemistry, urinalysis, macroscopy, organ weight and histopathology. Mid- and high-dose rats showed yellow discoloration of the fur during the first part of the study. Body-weight gain and food consumption were reduced in high-dose rats and in females at 200 ppm and water consumption was increased at the highest dose. PCV and Hb were decreased in rats at 1000 ppm throughout the study and PCV was also decreased at week 78 and 103 in rats at 200 ppm. At the high-dose level, protein levels were increased in males and females and polyurea and proteinuria were occasionally found in this group towards the end of the study. Relative liver and kidney weight were increased in males and females at 1000 ppm and at 200 ppm in females. Relative thyroid weight was increased in high-dose males. No dose-related histopathological findings were observed. The NOAEL in this study was 20 ppm, equivalent to 1 mg/kg bw/day, based on

effects on body-weight, red blood cells and liver and kidney at 200 ppm and higher (Wheldon *et al.*, 1969; Spicer & Benson, 1971).

Groups of Crl:CD(SD)BR rats (50/sex/group) were fed diets containing 0, 20, 120 or 600 ppm dithianion (purity 92%) for 104 weeks. Observations included mortality, clinical signs, body-weight, food and water consumption, ophthalmoscopy, macroscopy, organ weight (adrenals, liver, ovaries, brain, kidneys and testes) and histopathology (about 40 tissues from control and high-dose rats and from rats that died or were killed in extremis, and tissues of lungs, liver and kidneys of all mid- and low-dose rats). Satellite groups (20/sex/group) were kept according to the same protocol but observations also included haematology, clinical chemistry (including thyroid hormone assays), urinalysis and thyroid weight. The incidence of fur staining and rough haircoat was increased in high-dose females. At high-dose, body-weight gain and food consumption were decreased in males up to week 24 and in females during the whole study. At 600 ppm and occasionally at 120 ppm, Hb, RBC, PCV and MCV were decreased in both sexes.  $T_3$  levels were decreased at the highest dose. At 120 and 600 ppm, glucose, BUN and  $\gamma$ -GT were increased in both sexes. Throughout the study cholesterol levels were decreased at the highest dose. No clear effects were observed in urinalysis. Relative kidney and liver weights were increased in both sexes at 600 ppm. The incidence of treatment-related kidney lesions (glomerulonephropathy and tubular nephrosis) was increased in both sexes at 600 ppm. In the kidneys of females at this dose level, an increased incidence of proliferative tubules, adenomas and carcinomas was observed. In females at 120 ppm, glomerulonephropathy and tubular nephrosis showed an increased incidence. The NOAEL in this study was 20 ppm, equivalent to 1 mg/kg bw/day, based on non-neoplastic kidney lesions observed at 120 ppm (Brown, 1991).

Grasso (1991b) re-evaluated the kidney sections from female rats in the Brown, 1991 study. Similar changes were reported as in the original evaluation, including proliferative tubular changes (see Table 2). The incidence of kidney lesions was increased at 120 and 600 ppm. A comparison of the kidney tumours found by Brown (1991) and Grasso (1991b) is given in Table 3. The re-evaluation reduced the number of adenomas, but the number of adenocarcinomas remained the same. In neither evaluation were tumours seen at doses below 600 ppm.

**Table 2. Incidence of proliferative and some non-proliferative lesions in kidneys of female rats in a long-term/carcinogenicity study (according to Grasso, 1991b)**

	Dose level (ppm)			
	0	20	120	600
Basophilic tubules	24	24	34	30
Eosinophilic inclusions	0	2	3	9
Atypical hyperplasia	0	0	4	10
Proliferative tubules	0	0	0	6
Adenoma	0	0	0	7
Adenocarcinoma	0	0	0	2

**Table 3. Tumour incidence in female rats in a long-term/carcinogenicity study according to Brown (1991) and a re-evaluation by Grasso (1991b)**

Pathologist		Dose level (ppm)			
		0	20	120	600
Brown	adenoma	0	0	0	10*
	carcinoma	0	0	0	2*
Grasso	adenoma	0	0	0	7
	carcinoma	0	0	0	2

\* one animal had an adenoma and a carcinoma therefore 11 animals had tumours.

### Reproduction studies

Groups of 10 male and 20 female rats received 0, 20, 200 or 500 ppm dithianon (purity not specified) in the diet for 100 days before the initial mating. The treatment was continued throughout 3 generations of 2 litters each. Observations were made on general condition and behaviour, food consumption ( $F_0$  generation only) and body-weight. Conception rate, gestation time, litter

size, pup mortality, litter and mean pup weights at birth as well as at weaning (day 21) were studied. Autopsy, organ weight and histopathological examinations of selected organs were performed on all rats of the F<sub>3b</sub> generation. Grooming was impaired over the 3 generations at 500 ppm and, in the F<sub>2b</sub> generation, at 200 ppm. At the highest dose, body-weight gain was depressed in all generations and in the 200 ppm of the F<sub>2b</sub> generation. Litter size at birth was not affected but throughout the study entire litters tended to be lost completely before weaning so litter size at weaning was generally lower in treated groups than in control groups (none dose-related). In the F<sub>1b</sub> matings (both litters) the incidence of litter loss was increased at 500 ppm. Over the three generations a tendency to a lower mean pup weight at birth as well as at weaning was observed at 500 ppm. Pup mortality was very variable in both treated and control groups but at the highest dose pup mortality seemed to be increased in the F<sub>1a</sub>, F<sub>2a</sub> and F<sub>2b</sub> litters. Liver and kidney weights of F<sub>3b</sub> pups were increased in males and females at 500 ppm. Kidney weight was also increased in 200 ppm males as was adrenal weight in 500 ppm males. The NOAEL was 20 ppm, equivalent to 1 mg/kg bw/day, on the basis of decreased body-weight gain and increased kidney weight (Palmer & Readshaw, 1969).

Groups of 28 Crl:CD(SD)BR rats/sex were fed diets containing 0, 35, 200 or 600 ppm dithianon (purity 91.6%). After 100 days of treatment, animals (F<sub>0</sub> generation) were mated to start a 2-generation (1 litter/generation) study. F<sub>1</sub> parents (24/sex/group) selected from F<sub>1a</sub> offspring were mated after 100 days to produce one litter. During the premating and mating period, body-weight gain and food consumption were decreased in F<sub>0</sub> and F<sub>1</sub> males and females at 600 ppm and in F<sub>0</sub> females during gestation at the highest dose. No treatment-related effects were observed on clinical signs, mating and fertility indices in any of the parenteral rats, gestational length, pup weight, sex ratio and pup viability, physical and functional development and macroscopy of both parents and pups. Histopathology and weights of liver and kidneys were not available. The NOAEL in this study was 200 ppm, equivalent to 10 mg/kg bw/day, based on decreased body-weight gain and food consumption at 600 ppm (Osterburg, 1991).

### **Special studies on embryotoxicity and/or teratogenicity**

#### **Mice**

Groups of 24 pregnant NMRI mice were orally administered 0, 3.3, 10, 30, or 90 mg dithianon/kg bw/day suspended in MHEC from days 6-15 of gestation. The dams were observed for clinical signs, food consumption and body-weight and were killed at day 19 of gestation. The number of implantations, resorptions, live and dead fetuses were recorded. Fetuses were weighed, sexed and examined for external and skeletal malformations. At 90 mg/kg bw/day, all dams died, 4 by day 7, and the last dam at day 15 of gestation. At autopsy pale parenchymatous organs were observed and the

intestine was filled with haemorrhagic fluid. Body-weight was decreased at 10 and 30 mg/kg bw/day and food consumption was decreased at 30 mg/kg bw/day during treatment. Fetal and placental weights were decreased at 30 mg/kg bw/day. Delayed ossification was observed at 10 and 30 mg/kg bw/day. No malformations were observed. The NOAEL in this study for maternal and embryo-fetal toxicity was 3.3 mg/kg bw/day (Leuschner, 1976a).

### Rats

In a preliminary study, groups of 8 mated Crl:CD(SD)BR rats were administered by gastric intubation 0, 20, 40 or 70 mg dithianon/kg bw/day suspended in CMC from days 6-15 post-coitum. At the end of the study, another 2 groups of 8 mated rats were treated according to the same protocol with 0 or 100 mg dithianon/kg bw/day. The dams were sacrificed on day 20 of gestation and the fetuses were removed and examined for external/visceral malformations. Excessive urination and increased water consumption was observed at 100 mg/kg bw/day. Maternal body-weight was decreased during days 6-9 of gestation and body-weight gain was depressed during treatment at 70 and 100 mg/kg bw/day. At 100 mg/kg bw/day, post-implantation loss was increased and mean fetal weight was reduced. The incidence of external malformations was not increased. The NOAEL for maternal toxicity was 40 mg/kg bw/day and 70 mg/kg bw/day for embryotoxicity (Müller, 1989a).

Groups of 25-32 mated female Crl:CD(SD)BR rats were dosed orally by gavage with 0, 20, 70 or 100 mg dithianon/kg bw/day (purity 91.6%) suspended in CMC from days 6-15 of gestation. Another 2 groups of 25 mated female rats received 0 or 50 mg dithianon/kg bw/day and were treated according to the same protocol. On day 20 the dams were sacrificed and the fetuses were removed and examined. Observations included mortality, clinical signs, body-weight, food consumption, number of corpora lutea, number and position of implantations, early and late resorptions, and live and dead fetuses. Fetuses were weighed, sexed and examined for external, skeletal or visceral abnormalities. At 100 mg/kg bw/day, 5/25 dams died and at 70 mg/kg bw/day, 1/32 dams died. Body-weight gain and food consumption were dose-relatedly reduced at 50, 70 and 100 mg/kg bw/day. Dams at 70 and 100 mg/kg bw/day revealed an increased incidence of abnormal findings in the stomach and large intestines at necropsy. Post-implantation loss and intra-uterine deaths were markedly and dose-relatedly increased at 50, 70 and 100 mg/kg bw/day. Mean fetal weight was reduced at the highest dose. No teratogenic effects were observed. The NOAEL for maternal and embryo-fetal toxicity was 20 mg/kg bw/day (Müller, 1991).

### Rabbits

Groups of 12 pregnant New Zealand white rabbits were orally dosed by gavage with 0, 3.3, 10, 30 or 90 mg dithianon/kg bw/day suspended in MHEC

from days 6-18 of pregnancy. The dams were killed on day 29 of pregnancy and fetuses were removed and weighed, sexed and examined for external and skeletal malformations. At the highest dose, all dams died prematurely between days 8 and 12 of pregnancy. At autopsy their parenchymatous organs appeared pale and the intestine was filled with fluid. In dams at 30 mg/kg bw/day food consumption was reduced during treatment and body-weight was decreased. The resorption rate and the post-implantation loss were increased at 30 mg/kg bw/day and placental weight was decreased. Slightly retarded ossification was observed in fetuses at 30 mg/kg bw/day. The NOAEL in this study for maternal and embryo-fetal toxicity is 10 mg/kg bw/day (Leuschner, 1976b).

In a preliminary study, groups of 8-9 mated female New Zealand white rabbits received 0, 10, 20 or 40 mg dithianon/kg bw/day (purity 91.6%) by oral gavage from days 6-18 of gestation. The dams were sacrificed on day 28 and the ovaries and uteri were removed and examined for number of corpora lutea, live and dead fetuses, early and late resorptions. Fetuses were weighed, sexed and externally examined. At the highest dose, two abortions were observed and one female showed 100% intra-uterine deaths at necropsy. During treatment, food and water consumption were reduced and body-weight was decreased from days 6-9 post-coitum at 40 mg/kg bw/day. The mean number of early resorptions and the post-implantation loss was increased at the highest dose. No external malformations were observed. The NOAEL for maternal and embryo-fetal toxicity is 20 mg/kg bw/day (Müller, 1989b).

Groups of 20 mated New Zealand white rabbits were orally administered by gavage 0, 10, 25, or 40 mg dithianon/kg bw/day (purity 91.6%) in CMC from days 6-18 post-coitum. On day 28, the dams were sacrificed and fetuses were delivered by caesarean section. The number of corpora lutea, early and late resorptions and live and dead fetuses were recorded. The fetuses were weighed, sexed and examined for external, visceral or skeletal abnormalities. Mortality of dams was 0, 4, 3 and 4 in the control, low-, mid- and high-dose group, respectively. Three high-dose dams aborted; at necropsy no relevant findings were observed. Body-weight gain and food consumption were decreased at 25 and 40 mg/kg bw/day during treatment. At 10 mg/kg bw/day food consumption was also slightly decreased. An increased pre- and post-implantation loss was seen and the number of fetuses was reduced at the highest dose. In this study there were no indications for structural malformations associated with treatment. The NOAEL for maternal toxicity was 10 mg/kg bw/day and 25 mg/kg bw/day for embryo-fetal toxicity (Müller, 1990).

### **Special studies on genotoxicity**

A number of genotoxicity tests had been carried out with dithianon. The results are summarized in Table 3. The only positive effect found was in the chromosomal aberration test *in vitro*. However, an *in vivo* test having the same endpoint, was negative.

**Table 3. Results of genotoxicity assays on dithianon**

Test system	Test object	Concentration of dithianon	Purity	Results	Reference
<i>In vitro</i>					
Ames test	<i>S. typhimurium</i> TA1535, TA1537 TA1538, TA98 TA100	1-333.3 µg/pl in DMSO <sup>a</sup>	91.6%	negative <sup>c</sup>	Müller & Miltenburger, (1986)
		33.3-3333.3 µg/pl in DMSO <sup>b</sup>		negative <sup>c</sup>	
Ames test	<i>S. typhimurium</i> TA1535, TA100 TA1538, TA98 TA1537	0.1-5 µg/pl <sup>d</sup>	95%	negative <sup>c</sup>	Shirasu <i>et al.</i> (1977)
Ames test	<i>S. typhimurium</i> TA 1537, TA98 TA 1538; TA1535  TA100	0.3-6.6 µg/pl <sup>a</sup> 10-2000 µg/pl <sup>b</sup>	91.6%	negative <sup>c</sup> negative <sup>c</sup>	Timm (1987)
		0.1-20 µg/pl <sup>a</sup> 10-2000 µg/pl <sup>b</sup>		negative <sup>c</sup> negative <sup>c</sup>	
		1.0-333.3 µg/pl <sup>a</sup> 10-3333.3 µg/pl <sup>b</sup>		negative <sup>c</sup> negative <sup>c</sup>	
Bacterial mutation assay	<i>E. coli</i> WP2 <u>hcr</u>	0.1-5µg/pl <sup>d</sup>	95%	negative <sup>c</sup>	Shirasu <i>et al.</i> (1977)
Rec assay	<i>B. subtilis</i> H17, M45	0.1-5 µg/pl <sup>d</sup>	95%	negative <sup>c</sup>	Shirasu <i>et al.</i> (1977)
Chromosome aberration assay	Chinese hamster V79 cells	25-600 ng/ml in DMSO <sup>a</sup>	91.6%	positive <sup>c</sup>	Heidemann (1988)
		500-5000 ng/ml in DMSO <sup>b</sup>		positive <sup>c</sup>	
CHO/HGPRT mutation assay	Chinese hamster V 79 cells	20-200 ng/ml in DMSO <sup>a</sup> 60-600 ng/ml in DMSO <sup>b</sup>	94.7%	negative <sup>c</sup> negative <sup>c</sup>	Miltenburger (1984)
UDS assay	Rat hepatocytes	0.1-20 µg/ml in 1% DMSO	91.6%	negative <sup>c</sup>	Timm (1986)
<i>In vivo</i>					
Micro nucleus test and in CMC	NMRI (SPF) mice	1, 10 or 100 mg/kg orally	94.6%	negative <sup>c</sup>	Schultze Schencking, M. & Unkelbach, H. (1984)
Host-mediated assay	<i>S. typhimurium</i> G46 in male ICR mice	100-400 mg/kg	95%	negative <sup>c</sup>	Shirasu <i>et al.</i> (1977)
Chromosomal aberration assay	Wistar rat bone marrow	22.3, 106 and 393.5 mg/kg bw in PEG 400	91.6%	negative <sup>c</sup>	Volkner (1990)

<sup>a</sup> without metabolic activation

<sup>b</sup> with metabolic activation

<sup>c</sup> positive control(s) yielded expected positive results.

<sup>d</sup> with and without metabolic activation

### **Special studies on nephrotoxicity**

Groups of CrL:CD(SD)BR rats (15/sex/group) were administered 0, 120, 600 or 1080 ppm dithianon (purity 90%) in the diet for 7 days. Interim kills were performed on 5 rats/sex/group at days 2 and 4, the remaining animals were sacrificed at day 7. Food consumption was measured during treatment and body and kidney weights were recorded. Kidney and adrenal tissues as well as tissues from organs showing visible abnormalities were histopathologically examined. Sections of the cortex of the right kidney of all rats were examined by electron microscopy. Kidney samples of the control and high-dose groups after day 2 were not evaluated because of a technical problem. At both interim kills and at final sacrifice, body-weight was decreased at high dose and absolute and relative kidney weight were increased at all dose levels in a dose-related way. The incidence of pale kidneys was increased at the highest dose. After 7 days, kidneys showed hydropic degeneration of the proximal tubular cells and basophilic tubules at 600 and 1080 ppm. In high-dose females the lesions were more severe and persistent. To a lesser extent these effects were already found after 4 days. Basophilic tubules represent tubular degeneration and reflect the extensive loss of proximal tubular cells through hydropic degeneration. From electron microscopical examination it appeared that the mitochondriae were especially involved. They were affected in a dose-related manner at most time points or doses investigated (in females even after day 2 at 120 ppm). The mitochondria were swollen and had lost their cristae. The lesions were most severe in female rats treated with 1080 ppm. In addition, the proximal cells in female rats also showed inclusions of osmophilic material within the lysosomes. This suggests that lipid peroxidation had taken place (Price, 1991).

A hypothesis was proposed that because dithianon shows a toxic effect to the kidneys, the increase in tumour incidence at a high toxic dose is most probably due to a non-genotoxic mechanism. The toxic action may be due to accumulation of a toxic glutathion conjugate in the kidneys, similar to the mechanism of action for hexachloro 1:3-butadiene.

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

A dose of 500 mg dithianon (purity 91-94%) moistened with distilled water was applied to the shaven intact and abraded skin of New Zealand white rabbits for 24 h. No skin irritation up to 7 days after application was observed (Sommer & Frohberg, 1968b).

Dithianon (purity 92%) was tested for irritation to shaven intact skin areas of 3 male and 3 female New Zealand white rabbits under occlusive conditions. No edema or erythema were observed 24, 48 and 72 h after a 4-h exposure (Ullmann, 1986b).

Delan liquid (formulation with 25% dithianon) produced marked erythema and occasionally edema in 5 male and 5 female New Zealand white

rabbits when applied under occlusive conditions to the shaven intact and abraded skin for 24 hours. The effects were still present 72 h after application (Sommer & Frohberg, 1968a).

Nine New Zealand white rabbits were given doses of 100 mg dithianon (purity 91-94%) into the conjunctival sac of the left eye. The eyes of 6/9 rabbits were washed, 3/9 after 2 seconds and the remaining 3/9 after 4 seconds. In the unwashed eyes redness, swelling and secretion of the conjunctiva, swelling of the iris, iridic injection and distinct turbidity of the whole cornea were observed. The pupils did not react to light. After 2 weeks, the conjunctival irritation was still apparent (slight) in 1 rabbit and the corneal changes were not reversible in 1/3 rabbits and only partly in the other 2/9. When the eyes of the rabbits were washed no signs of irritation were observed (Sommer & Frohberg, 1968b).

Two New Zealand white rabbits received a single installation of 0.1 ml Delan liquid into the conjunctival sac of the left eye. Slight erythema, swelling and secretion of the conjunctiva developed in both eyes after 24 h. After a week, the conjunctiva still showed slight erythema and swelling (Sommer & Frohberg, 1968a).

Application of 100 mg dithianon (purity 92%) into the left eyes of 3 male and 3 female New Zealand white rabbits caused corneal opacity, iritis and inflammation of the conjunctivae. Severe corneal opacity was still observed in 2/6 rabbit by day 21 (Ullmann, 1987b).

### **Special studies on skin sensitization**

Dithianon (purity 94.7%) had slight sensitizing properties when tested in Dunkin-Hartley albino guinea-pigs by the maximization test (Ullmann, 1985). This weak sensitization was also observed when dithianon (purity 91.6%) was tested by the open epicutaneous test (OET) on Dunkin-Hartley guinea-pigs (Ullmann & Kups, 1989a). After a re-challenge with 3 different dithianon impurities (D13, D14 or D25) in the OET with dithianon-sensitized guinea-pigs at least one impurity gave a positive result (Ullmann *et al.*, 1990). Pure dithianon (99.2%) failed to produce sensitization when also tested in the open epicutaneous test (Ullmann & Kups, 1989b).

Dithianon was tested in albino guinea-pigs for photoallergenic properties. In one test, the observed weak sensitizing potential was enhanced by simultaneous UV-exposure (Heusener, 1986). This slight photo-allergenic effect was not established by two additional tests carried out with either dithianon technical (purity 91.6%) or dithianon pure (purity 99.6%) (Ullmann *et al.*, 1989d, 1989e).

## COMMENTS

After oral administration to rats, goats and hens, dithianon was rapidly absorbed, distributed and excreted. Five days after its oral administration to rats almost all of the administered radioactivity had been eliminated, 62-70% via faeces and 30-34% via urine. Only a small proportion of the dose was recovered in tissue, with highest levels in kidneys, the gastrointestinal tract and whole blood. Up to 10% had been excreted in the bile after 48 h. Dithianon was quickly metabolized to a number of polar metabolites, which have defied identification due to their lability. Less than 1% unchanged dithianon was found in the faeces.

The acute oral toxicity of dithianon was moderate in mice and rats. WHO has classified dithianon as slightly hazardous (WHO, 1992).

Short-term toxicity studies with mice, rats and dogs indicated that the kidney is the primary target organ. Increased kidney weight, hydropic degeneration of the proximal tubular cells and basophilic tubules were demonstrated in the rat.

In a 90-day study in rats, dithianon was administered at dietary concentrations of 0, 30, 180, or 1080 ppm. At high concentrations effects on red blood cells, an increase in liver and kidney weight and histopathological changes in the kidney were noted. The NOAEL was 30 ppm, equal to 2.5 mg/kg bw/day for males and 3 mg/kg bw/day for females based on increased kidney weight.

Three studies with dogs were reviewed. In a 90-day study, dithianon was fed at dietary concentrations of 0, 40, 200, or 1000 ppm. The NOAEL was 200 ppm, equal to 3 mg/kg bw/day based on increased organ weights in the high-dose group. In both the 52-week (0, 40, 200 or 1000 ppm) and 2-year (0, 40, 400 or 1000 ppm) studies dithianon caused effects on red blood cells increased liver and kidney weights, hepatocellular hypertrophy and tubular pigmentation in the kidney. In each study the NOAEL was 40 ppm, equivalent to 1 mg/kg bw/day, based on increased liver weight and histopathological changes at 200 ppm and 400 ppm, respectively.

In an 80-week long-term feeding study in mice at dietary concentrations of 0, 20, 100, or 500 ppm, the NOAEL was 20 ppm, equal to 2.9 mg/kg bw/day, based on increased kidney weight and an increased incidence of chronic nephrosis. Two 2-year rat studies at dietary concentrations of 0, 20, 200, or 1000 ppm and 0, 20, 120, or 600 ppm, respectively, were conducted. In the first study, only effects on body-weight, red blood cells and liver and kidney weight were observed at 200 ppm and higher, equivalent to 10 mg/kg bw/day. In the second study these effects were confirmed together with a number of histopathological renal changes, especially in females at 600 ppm. At this level

kidney adenomas and adenocarcinomas were also found. The NOAEL was 20 ppm, equivalent to 1 mg/kg bw/day, based on non-neoplastic kidney lesions observed at 120 ppm. The Meeting concluded that dithianon induced kidney tumours in female rats at 600 ppm. It has been hypothesized that tumour induction is secondary to other renal changes seen in rats.

Two reproduction studies in rats were reviewed. In the first study at dietary concentrations of 0, 20, 200, or 500 ppm dithianon, the body-weight gain of parents and pups was decreased, pup mortality was increased and liver and kidney weight were increased. The NOAEL was 20 ppm, equivalent to 1 mg/kg bw/day based on decreased body-weight gain and increased kidney weight. In the second study (dietary concentrations of 0, 35, 200, or 600 ppm) the NOAEL was 200 ppm, equivalent to 10 mg/kg bw/day based on decreased body-weight gain and food consumption at 600 ppm.

Teratogenicity studies were conducted with mice, rats, and rabbits. In mice, maternal toxicity and delayed ossification were observed. The NOAEL for both effects was 3.3 mg/kg bw/day. In rats, maternal toxicity, post-implantation loss and reduced fetal weight were observed at doses of 50-100 mg/kg bw/day. The NOAEL was 20 mg/kg bw/day. In a study in rabbits, 30 mg/kg bw/day caused maternal toxicity, post-implantation loss and retarded ossification. The NOAEL was 10 mg/kg bw/day. In a second study in rabbits, maternal toxicity was observed at 25 and 40 mg/kg bw/day but not at 10 mg/kg bw/day. The NOAEL for fetotoxicity was 25 mg/kg bw/day. Teratogenic effects were not observed in any of the studies.

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

The Meeting concluded, after consideration of the long-term toxicity studies and the genotoxicity data, that dithianon did not pose a carcinogenic hazard for humans.

An ADI was allocated, based on NOAELs in two-year studies in rats and dogs, using a 100-fold safety factor.

## TOXICOLOGY EVALUATION

### Level causing no toxicological effect

Mouse: 20 ppm in the diet, equal to 2.9 mg/kg bw/day (80-week study)

Rat: 20 ppm in the diet, equivalent to 1 mg/kg bw/day (2-year studies)

Dog: 40 ppm in the diet, equivalent to 1 mg/kg bw/day (one and two-year studies).

#### **Estimate of acceptable daily intake for humans**

0-0.01 mg/kg bw

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

1. Characterization of the metabolites of dithianon in mammals and plants.
2. Clarification of the mechanism of nephrotoxicity and induction of kidney tumours.
3. Observations in humans.

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