

DITHIANON

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Explanation

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 multisite 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 (QA).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral administration

To obtain information on the absorption, distribution, rate and route of excretion and biotransformation of dithianon, studies were performed in adult male and female Sprague-Dawley rats (body weight 200 ± 50 g; 7–9 weeks old) using [^{14}C]dithianon and [$^{13}C/^{14}C$]dithianon (Figure 1).

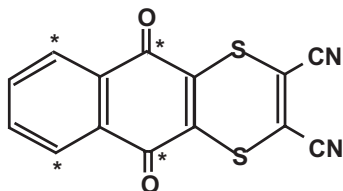
In a preliminary excretion study, two rats (one male and one female) were given a single oral dose of [^{14}C]dithianon at a dose of 200 mg/kg bw. As the animals suffered from severe diarrhoea, the study was terminated. Subsequently, another two rats were given a single oral dose of 50 mg/kg bw. Urine was collected at 8, 24, 48, 72, 96 and 120 hours. Faeces were collected at 24-hour intervals up to 120 hours. Expired air was monitored at 24-hour intervals up to 48 hours.

In the main study, oral doses of 10 mg/kg bw (low dose) and 50 mg/kg bw (high dose) were used; the 50 mg/kg bw dose was an effect level, the 10 mg/kg bw dose a no-effect level. Each animal in the test groups received approximately 370 kBq of [^{14}C]dithianon. The test substance was delivered in 1.0% (weight per volume [w/v]) sodium carboxymethylcellulose in water.

The test groups included in the blood kinetics, absorption, distribution, metabolism and excretion studies are shown in Table 1.

Total radioactive residues (TRR) in plasma, urine, cage washings and tissues were determined by liquid scintillation counting or combustion of aliquots to yield $^{14}CO_2$. Liquid scintillation counting was used to analyse trapped $^{14}CO_2$. The TRR in the carcasses were determined by digestion and liquid scintillation counting. The TRR in the faeces were determined by extraction and liquid scintillation counting of the faecal extracts. Thin-layer chromatography was used to characterize and identify extractable residue components.

In the preliminary study, it was found that faecal excretion was the predominant route (62.8% and 70.96% of the dose for males and females, respectively). No detectable radioactivity was found in the expired air.

Figure 1. Chemical structure of dithianon and position of radiolabel

* Indicates the position of ^{14}C and ^{13}C labels

Table 1. Test groups, number of animals, target dose rate and euthanasia time

Test groups	Radiolabel	Number of rats		Target dose (mg/kg bw)	Euthanasia at time post-dosing
		Male	Female		
Pilot excretion study (SOHD)	5, 6, 9, 10- ^{14}C	1	1	50	At least 120 h
SOLD	5, 6, 9, 10- ^{14}C	5	5	10	120 h
SOHD	5, 6, 9, 10- ^{14}C	5	5	50	120 h
MOLD	5, 6, 9, 10- ^{14}C	5	5	10	120 h after last dose
Pharmacokinetics (SOLD)	5, 6, 9, 10- ^{14}C	5	5	10	240 h
Pharmacokinetics (SOHD)	5, 6, 9, 10- ^{14}C	5	5	50	240 h
Tissue and organ distribution (SOLD)	5, 6, 9, 10- ^{14}C	5	5	10	One male and one female at 6, 24, 48, 96 and 168 h
Biliary excretion (SOLD)	5, 6, 9, 10- ^{14}C	3	3	10	48 h
Biliary excretion (SOHD)	5, 6, 9, 10- ^{14}C	3	3	50	48 h
Metabolite identification (SOHD)	5, 6, 9, 10- $^{13}\text{C}/^{14}\text{C}$	5	5	50	120 h

From Holmsen (2009)

MOLD, multiple oral low dose; SOHD, single oral high dose; SOLD, single oral low dose

In the main study, the actual doses administered were 9.34–11.82 mg/kg bw for the 10 mg/kg bw groups and 46.44–54.45 mg/kg bw for the 50 mg/kg bw groups.

Following administration of a single oral dose of [^{14}C]dithianon, ^{14}C residues were observed in blood within 15 minutes, and mean peak plasma levels of 0.813–0.992 parts per million (ppm) for the low-dose groups and 3.81–3.89 ppm for the high-dose groups occurred at 6 hours. The differences between males and females were insignificant. The radioactive residues in the blood declined quickly, with an elimination half-life of approximately 46–57 hours for the low- and high-dose groups. The pharmacokinetic parameters following administration of a single oral dose of [^{14}C]dithianon are summarized in [Table 2](#).

The extent of absorption of [^{14}C]dithianon by the rat following administration of single oral doses (low and high) was determined in a biliary excretion study. The percentage absorbed at 48 hours after dosing is shown in [Table 3](#). The amount of absorption (sum of the amounts in urine, bile, gastrointestinal tract tissues, carcass and liver) was 52% and 48% of the administered dose for male and female rats in

Table 2. Pharmacokinetic parameters following a single oral dose of [¹⁴C]dithianon

Pharmacokinetic parameter	Mean value (<i>n</i> = 5)			
	SOLD (10 mg/kg bw)		SOHD (50 mg/kg bw)	
	Male	Female	Male	Female
<i>T</i> _{max} (h)	6.0	6.0	6.0	6.0
<i>C</i> _{max} (g/g)	0.992	0.813	3.89	3.81
<i>T</i> _{½ elim} (h)	55.8	56.8	46.4	56.7
AUC (g·h/ml)	25.4	31.6	155.7	209.8
MRT (h)	NR	NR	NR	NR

From Holmsen (2009)

AUC, area under the blood concentration versus time curve calculated for the time interval between 0 and 240 h; *C*_{max}, maximum blood concentration; MRT, mean residence time; NR, not reported; SOHD, single oral high dose; SOLD, single oral low dose; *T*_{½ elim}, elimination half-life; *T*_{max}, time of maximum blood concentration

Table 3. Extent of absorption following a single oral dose of [¹⁴C]dithianon

Matrix	% of administered radioactive dose			
	SOLD (10 mg/kg bw)		SOHD (50 mg/kg bw)	
	Male	Female	Male	Female
Urine	31.38	30.10	33.02	23.54
Bile	11.59	9.52	7.21	7.49
Gastrointestinal tract	6.17	5.79	2.22	8.85
Carcass	2.36	2.64	1.11	6.53
Liver	0.05	0.03	0.02	0.03
Total absorption	51.55	48.08	43.58	46.44

From Holmsen (2009)

SOHD, single oral high dose; SOLD, single oral low dose

the low-dose bile group, respectively. For the high-dose bile group, the absorption amounted to 44% of the dose for male rats and 46% of the dose for female rats. The results indicate that a small portion (7–12%) of the dose eliminated in the faeces of intact animals was excreted via the bile. The remaining faecal excretion (50–60% of the dose) would appear to represent unabsorbed dithianon.

The distribution of the TRR in various tissues is presented in Tables 4 and 5. Results are reported for male and female rats at different time intervals following administration of 10 mg/kg bw. TRR distribution in tissues was examined at approximately 6, 24, 48, 96 and 168 hours post-dosing.

The TRR data from the low-dose groups showed that the highest ¹⁴C residues were initially present in the gastrointestinal tract, kidneys and plasma. The highest TRR levels were observed at 6 hours post-dosing in all tissues. At 168 hours, the TRR were highest in the kidneys (0.127–0.149 ppm), gastrointestinal tract (0.028–0.059 ppm) and blood (0.026–0.041 ppm). The TRR in the remaining tissues were less than or equal to 0.026 ppm or below the limits of detection at 168 hours. In summary, the tissue retention of orally administered dithianon was low. Higher levels of radioactivity were found in the kidney and gastrointestinal tract. The accumulation of radioactivity was observed mainly in the gastrointestinal tract, kidneys and liver.

The details of the excretion balance are shown in Table 6. Within 48 hours after dosing, more than 90% of the applied dose was excreted in urine and faeces, the majority of radioactivity being eliminated via faeces (64% and 68% of dose for females and males, respectively). In urine, 26–27% of

Table 4. Total radioactive residues in tissues of male rats given 10 mg/kg bw

Matrix	TRR (ppm)				
	6 h	24 h	48 h	96 h	168 h
Adrenal glands	0.290	< 0.398	< 0.178	< 0.105	< 0.027
Bone marrow	0.337	< 0.151	< 0.231	< 0.173	< 0.176
Brain	< 0.016	< 0.011	< 0.012	< 0.010	< 0.010
Eyes	0.087	0.045	< 0.023	0.020	< 0.017
Fat	0.067	< 0.040	< 0.028	< 0.026	< 0.031
Gastrointestinal tract	97.0	3.00	0.307	0.085	0.028
Heart	0.327	0.052	0.018	0.018	0.022
Kidneys	2.73	0.813	0.359	0.268	0.127
Liver	0.558	0.101	0.037	0.023	0.010
Lungs	0.241	0.097	0.038	0.030	0.023
Muscle	0.060	0.022	< 0.012	< 0.009	< 0.008
Pancreas	0.156	0.050	0.031	0.025	0.026
Plasma	0.757	0.175	0.056	0.033	0.010
Spleen	0.085	0.034	0.018	< 0.015	0.015
Testes	0.102	0.031	0.015	0.013	< 0.008
Thyroid gland	< LOD	< LOD	< LOD	< LOD	< LOD
Whole blood	0.549	0.154	0.067	0.048	0.026

From Holmsen (2009)

LOD, limit of detection

the dose was recovered after 48 hours. Total recovery of radioactivity (including cage wash) reached 96.1% and 98.8% of the administered dose in the females and males, respectively. No sex-specific differences in the route or rate of excretion were observed.

A summary of the distribution of the radioactivity after administration of a single oral dose of 10 or 50 mg/kg bw to rats is presented in Table 7.

The excretion of radioactivity during a 5-day period following administration of a 10 or 50 mg/kg bw single dose of [¹⁴C]dithianon and a 10 mg/kg bw repeated dose of dithianon is shown in Table 8. From this, it can be seen that the majority of the administered dose was excreted through the faeces (64.0–72.2%) and urine (26.7–31.4%).

After 14 daily oral doses of non-radioactive dithianon (10 mg/kg bw per day) followed by a single oral dose of [¹⁴C]dithianon (10 mg/kg bw), the pattern of excretion of radioactivity in both males and females 120 hours post-dosing was very similar to that obtained from animals receiving single oral doses of [¹⁴C]dithianon at both dose levels.

In conclusion, the biokinetics of dithianon were studied in rats following oral administration of ¹⁴C-labelled dithianon at nominal doses of 10 and 50 mg/kg bw. The material balance from the preliminary study showed that dithianon was not metabolized to volatile compounds, including carbon dioxide, as negligible amounts of radioactivity (< 0.05% of dose) were detected in the expired air from treated animals during the 5-day period following treatment at 50 mg/kg bw. Therefore, it was not necessary to collect carbon dioxide or volatile organics in the excretion–retention study.

Orally administered dithianon was about 40–50% absorbed in rats. There were no substantial dose- or sex-related differences in the absorption, elimination or distribution of radioactivity following oral administration of [¹⁴C]dithianon.

Table 5. Total radioactive residues in tissues of female rats given 10 mg/kg bw

Matrix	TRR (ppm)				
	6 h	24 h	48 h	96 h	168 h
Adrenal glands	< 0.257	0.183	< 0.009	< 0.155	< 0.073
Bone marrow	< 0.523	0.255	< 0.275	< 0.357	< 0.229
Brain	0.018	0.016	< 0.010	< 0.010	< 0.008
Eyes	0.057	0.066	< 0.020	0.016	< 0.021
Fat	< 0.034	< 0.032	< 0.025	< 0.023	< 0.019
Gastrointestinal tract	110	11.8	1.03	0.271	0.059
Heart	0.164	0.074	0.031	0.020	< 0.011
Kidneys	2.01	0.757	0.438	0.219	0.149
Liver	0.585	0.133	0.048	0.025	0.012
Lungs	0.325	0.116	0.051	0.030	0.017
Muscle	0.054	0.030	0.012	< 0.009	< 0.010
Ovaries	0.549	0.269	0.117	< 0.085	< 0.070
Pancreas	0.135	0.098	0.025	0.024	< 0.012
Plasma	0.754	0.239	0.096	0.035	< 0.011
Spleen	0.108	0.058	0.029	0.027	0.019
Thyroid gland	0.805	< LOD	< LOD	< LOD	< LOD
Uterus	0.256	0.116	0.036	0.019	< 0.015
Whole blood	0.519	0.225	0.114	0.066	0.041

From Holmsen (2009)

LOD, limit of detection

Table 6. Excretion of radioactivity in urine and faeces after oral administration in rats (50 mg/kg bw)

Matrix	Time interval (h)	Excretion of radioactivity (% of dose)	
		Male	Female
Urine	0–24	18.62	21.09
	24–48	7.36	6.29
	48–72	0.61	0.55
	72–96	0.33	0.16
	Total	26.92	28.09
Faeces	0–24	31.80	30.36
	24–48	36.14	34.02
	48–72	2.43	2.79
	72–96	0.27	0.34
	Total	70.64	67.51
Cage wash	—	1.22	0.46
Total		98.78	96.06

From Bross & Seiferlein (2009)

Table 7. Tissue distribution at maximum plasma level ($T_{max} = 6$ hours)

Matrix	% of dose			
	10 mg/kg bw		50 mg/kg bw	
	Male	Female	Male	Female
Liver	0.304	0.267	0.111	0.137
Kidney	0.425	0.353	0.120	0.105
Bone marrow	0.000 11	0.000 13	0.000 05	0.000 06
Plasma	0.132	0.061	0.027	0.042
Total	0.861	0.681	0.258	0.284

From Bross & Seiferlein (2009)

Table 8. Mean excretion and retention of radioactivity by male and female rats after a single oral dose of [^{14} C]dithianon at a dose level of 10 or 50 mg/kg bw and after 14 daily doses of dithianon followed by a single oral dose of [^{14} C]dithianon at a dose level of 10 mg/kg bw per day

Dose regime	Mean % excretion and retention of radioactivity					
	Single (10 mg/kg bw)		Single (50 mg/kg bw)		Repeated (10 mg/kg bw)	
	Male	Female	Male	Female	Male	Female
Tissues	0.08	0.20	0.11	0.18	0.17	0.15
Urine (0–120 h)	31.04	31.43	29. 93	31.28	30.80	26.66
Cage washing	0.11	0.16	0.60	0.68	0.27	0.37
Faeces (0–120 h)	65.98	64.03	66.67	65.44	67.04	72.15
Total recovery	97.21	95.82	97.30	97.57	98.28	99.33

From Hawkins et al. (1988)

The majority of the administered dose was recovered in faeces (64.0–72.2%) and urine (26.7–31.4%).

In the low-dose bile group, approximately 10% of the radioactivity was eliminated in the bile and 30% was eliminated in the urine, indicating that at least 40% of the applied dose was absorbed. In the high-dose bile group, a comparable percentage of the dose was absorbed. Of the absorbed dose, urinary excretion predominated over biliary excretion. There was no accumulation of radioactivity in tissues and, except for the gastrointestinal tract and kidneys, very little residue remained in the tissues at 168 hours post-dosing. The sex difference was not significant overall. Following oral administration, dithianon was absorbed quickly into the blood and reached a maximum concentration (C_{max}) at 6 hours in both the low-dose and high-dose groups. C_{max} was 0.813–0.992 g/g in the low-dose group and 3.81–3.89 g/g in the high-dose group. The elimination half-life ($T_{1/2 \text{ elim}}$) was between 55.8 and 56.8 hours (low dose) and 46.4 and 56.7 hours (high dose). The area under the curve (AUC) values were 25.4–31.6 g·h/ml for the low dose and 155.7–209.8 g·h/ml for the high dose. The label was mostly excreted as polar metabolites and glucuronic acid conjugates in bile and urine, indicating an extensive metabolism of the parent compound. Residues in faeces consisted of polar metabolites, but there were negligible amounts of parent compound.

The studies described above (Hawkins et al., 1988; Hawkins & Elsom, 1993; Bross & Seiferlein, 2009; Holmsen, 2009) were conducted in compliance with GLP of the United States Environmental Protection Agency (USEPA), Japan, the Organisation for Economic Co-operation and Development (OECD), the European Commission (EC) and/or the United Kingdom. A QA statement was attached.

Table 9. Percentage of dithianon absorbed, remaining in the treated skin and removed from the site of application following a single topical application of [^{14}C]dithianon aqueous paste

Application rate (mg/cm ²)	Absorbed ^a		Treated skin		Skin swabs/gauze wash		Total
	%	mg equivalent ^b	%	mg equivalent ^b	%	mg equivalent ^b	%
0.16	0.32	0.006	3.14	0.06	95.6	1.83	99.1
1.66	0.27	0.054	1.91	0.38	101.0	20.10	103.2

From Bounds (1998)

^a Urine, faeces, cage wash, carcass, blood and untreated skin.

^b Calculated from the group mean achieved dose.

(b) Dermal application

The extent of absorption and excretion of radiolabelled [^{14}C]dithianon (AC 37114) up to 168 hours following a single topical application as an aqueous paste at two concentrations to male rats was determined. The low dose was approximately 0.16 mg/cm² [^{14}C]dithianon (equal to 8.0 mg/kg bw), and the high dose was approximately 1.7 mg/cm² [^{14}C]dithianon (equal to 79 mg/kg bw). The preparation was applied for 8 hours under semiocclusion with a stainless steel gauze held in place over the silicone saddle by surgical tape and vet-wrap bandage, and then the application site was washed with 1% Tween 80 in distilled water to remove any unabsorbed material.

The majority of the applied radioactivity (group mean > 95%) was recovered in the skin swabs and gauze washes for both the low- and high-dose preparations. Very little of the dose was absorbed (approximately 0.3%), and only a small proportion of the dose remained in the treated skin at the end of the experiment (3% and 2% for the low-dose and high-dose groups, respectively) for both dose preparations. Table 9 summarizes the findings.

In conclusion, [^{14}C]dithianon applied as an aqueous paste at two dose levels had a very low potential for dermal absorption in the rat. Only approximately 0.3% of the applied dose was absorbed.

The study was conducted in compliance with GLP, and a QA statement was attached (Bounds, 1998).

1.2 Biotransformation

The objective of this study was to investigate the metabolites of dithianon in excreta, selected tissues and plasma from male and female Sprague-Dawley (CrI:CD(SD)) rats after a single oral dose of 10 mg/kg bw (one low-dose group) or 50 mg/kg bw (two high-dose groups). In order to achieve the required specific activity, ^{14}C -labelled dithianon (96.1% radiochemical purity labelled in the 5 and 10 positions) was mixed with non-radiolabelled compound (^{12}C and ^{13}C) at a ratio of 1:1. Animals were dosed by gavage using a 0.5% aqueous solution of carboxymethylcellulose containing 1% Cremophor. The purity and the isotope ratio were checked by high-performance liquid chromatography (HPLC) and HPLC with tandem mass spectrometry (MS/MS) prior to dosing. The study was conducted between 12 January 2007 and 28 April 2009.

As dithianon is subject to extensive metabolic degradation, identification of its metabolites is challenging and has not been successfully achieved in previous investigations. Urine and faecal samples of one high-dose group were used for isolation and identification of metabolites. In one low-dose group and the other high-dose group, liver and kidney tissues as well as plasma of male and female rats were analysed for metabolites at maximum plasma levels of radioactivity.

More than 14 metabolites were detected in urine of rats given [^{14}C]dithianon as a single oral low dose, a single oral high dose and multiple oral low doses. However, identification of dithianon

Table 10. Summary of identified metabolites in urine, faeces, liver, kidney and plasma of rats

Metabolite designation	Urine	Faeces	Liver	Kidney	Plasma
Dithianon (M216F000)					
M216F001		x	x		
M216F002	x			x	x
M216F003	x				
M216F004		x	x		
M216F005		x			
M216F006	x				
M216F007		x			
M216F008	x	x	x	x	x
M216F009	x	x			
M216F011	x	x			
M216F012	x	x	x	x	x
M216F013	x				
M216F014	x				
M216F015	x	x	x	x	x
M216F016	x	x			
M216F017		x	x		
M216F018	x				
M216F019	x			x	
M216F020	x			x	x
M216F022	x			x	x
M216F023	x				
M216F024	x				
M216F025	x				
M216F026	x			x	
M216F027		x	x		

From Bross & Seiferlein (2009)

metabolites was not possible due to their detection in minor quantities. The sum of the detected metabolites approached about 25–30% of the dose. The patterns of metabolites detected in rat urine samples were very similar with respect to dose level or duration of administration.

The structures and codes of all identified metabolites in urine, faeces, liver, kidney and plasma are summarized in Table 10.

The HPLC chromatograms of urine of the first 48 hours were dominated by one major peak, representing 0.9–10% of the administered dose. This metabolite was identified as M216F020 (glucuronic acid conjugate of 1,4-dihydroxynaphthalene) and is the only one present in amounts above 2% of the dose. Besides this metabolite, the patterns indicated the presence of many other metabolites (up to 16 further peaks), which were all insignificant in quantitative terms, as none of them amounted to more than 2% of the dose, with most of them accounting for less than 1%. Among these, the following metabolites were identified: M216F006 (accounting for 0.30–1.69% of the dose), M216F022 (0.71–1.29%), M216F012/M216F019 (0.28–1.11%), M216F003 (0.21–0.49%) and M216F025 (0.36–0.50%).

Table 11. Composition of radioactivity in rat urine following a 50 mg/kg bw oral dose

Metabolite identity / designation	Time interval (h)	% of dose in urine	
		Male	Female
Identified			
M216F025	0–24	0.50	0.49
	24–48	0.38	0.36
M216F006	0–24	1.28	1.69
	24–48	0.42	0.30
M216F020	0–24	10.06	9.00
	24–48	2.86	0.92
M216F022	0–24	1.09	1.29
	24–48	0.71	0.76
M216F012/M216F019	0–24	0.52	1.11
	24–48	0.28	0.41
M216F026	0–24	0.96	n.d.
	24–48	0.20	n.d.
M216F008	0–24	n.d.	1.08
	24–48	n.d.	0.39
M216F003	0–24	n.d.	0.49
	24–48	0.21	0.38
M216F015	0–24	0.20	n.d.
	24–48	n.d.	n.d.
M216F002	0–24	n.d.	0.35
	24–48	n.d.	0.12
<i>Total identified</i>	0–48	19.67	19.14
Characterized by HPLC^a			
Polar substances (0–20 min)	0–24	2.92	n.d.
	24–48	0.86	0.12
Semipolar substances (20–40 min)	0–24	1.09	5.32
	24–48	1.44	2.53
Non-polar substances (> 40 min)	0–24	n.d.	0.27
	24–48	n.d.	n.d.
<i>Total characterized</i>	0–48	6.31	8.24
Total identified and characterized	0–48	25.98	27.38

From Bross & Seiferlein (2009)

n.d., not detected

^a Characterized by HPLC, meaning sum of all peaks in the respective retention time area from the chromatograms; each of them comprised not more than 1.3% of the dose, with most of them being below 1% of the dose.

The composition of radioactivity in urine samples in the 50 mg/kg bw group is given in Table 11. Only the time intervals 0–24 hours and 24–48 hours were considered, as the total excreted dose in all later sampling intervals was below 1%.

The acetonitrile extracts of faeces of the first 48 hours show a multitude of metabolites with up to 30 peaks. The patterns were dominated by five peaks, four of which could be identified. These were M216F012, accounting for 0.37–0.55% of the dose (sum of both tautomeric forms), M216F015,

accounting for 0.28–0.78% of the dose, and M216F027, which occurred in two isomeric forms that together represent between 0.99% and 1.95% of the dose. The unidentified peak, which was non-polar (retention time [t_R] = 60 minutes), accounted for 0.20–0.47% of the dose. Besides these five more dominant metabolites, many further peaks were present in the chromatograms; however, all of them were less significant in quantitative terms. Among these, the following metabolites were identified: M216F007 (accounting for 0.04–0.14% of the dose), M216F016 (0.06–0.13%), M216F004 (0.13–0.35%), M216F008 (0.09–0.18%), M216F001 (0.07–0.14%) and M216F017 (0.18–0.30%). The unchanged parent compound was not detected in the acetonitrile extracts.

The investigation of water, ammonia and microwave extracts as well as of the protease supernatants with two different HPLC systems characterized the released radioactivity as being composed of a multitude of metabolites with a broad spectrum of polarity, all present only in insignificant amounts. None of the patterns showed any sex-related differences. The composition of radioactivity in faecal samples is summarized in Table 12. Only the time intervals 0–24 hours and 24–48 hours were considered, as the total excreted dose in all later sampling intervals was below 3.2%. Total identified metabolites in faeces accounted for 5.8% of the dose in females and 7.3% in males. The total degree of identification and characterization amounted to 56% and 54% of the dose in males and females, respectively.

A summary of the composition of radioactivity in liver, kidney and plasma is presented in Table 13 for the 10 mg/kg bw dose group and in Table 14 for the 50 mg/kg bw dose group.

In conclusion, dithianon was rapidly and extensively metabolized to a very large number of mainly polar metabolites in rats. There were no fundamental sex differences in metabolism. As a result of its different functional groups (CO, CN, S), the molecule is exposed to chemical and enzymatic attacks at various sites of the molecule, leading to many reactive primary products that undergo further transformation by reacting with endogenous compounds (e.g. glucosidation, sulfonylation, acetylation, coupling to amino acids). A key step in its metabolic degradation is the oxidation of the sulfur atoms, leading to a cleavage of the dithiine ring and, after further reactions, resulting in a 1,4-naphthoquinone moiety that bears various substituents, such as carboxy groups, hydroxyl groups (M216F024, in this case already conjugated with glucuronic acid), amino acids (M216F016, bearing a glycine moiety) or amino groups (M216F012) and sulfonate groups (M216F005) or both (M216F007) at the quinone part of the ring system. The amino groups can be further acetylated (M216F003, M216F011).

Many identified metabolites show a reduction of the 1,4-naphthoquinone moiety to a 1,4-dihydroxynaphthalene ring that is still bearing the above-mentioned substituents (M216F002, M216F006, M216F013, M216F014, M216F018). One hydroxyl group of this dihydroxynaphthalene ring is further conjugated with glucuronic acid, leading, for example, to the metabolites M216F020, M216F022, M216F023 or M216F025. M216F020 represents the glucuronide of the 1,4-dihydroxynaphthalene ring without any further substituents and was found to be the only metabolite of quantitative significance (occurring in amounts > 5% of the dose in urine).

Furthermore, opening of the dithiine ring and transformation into a thiazine ring (M216F004, M216F008 with an additional carboxy group) were observed, as well as further conjugation with glycine (M216F019). Another metabolite after cleavage of the dithiine ring is M216F027, a copper(II) complex in which two dimercapto-naphthoquinone units bind to one copper ion, which was found to be a main metabolite of faeces. However, it cannot be excluded that this metabolite represents an artefact generated during sample workup. Whereas the vast majority of the identified metabolites show the cleavage of the dithiine ring, demonstrating the importance of this degradation route for dithianon, some metabolites still maintaining the intact three-ring system (i.e. without cleavage of the dithiine ring) were identified. Metabolite M216F026 represents a glucuronide of the intact parent compound having a glucuronic acid unit at the 1,4-dihydroxynaphthalene moiety, which was yielded by prior reduction of the naphthoquinone system. The substitution of the carbonitrile groups in the

Table 12. Composition of radioactivity in faeces of the 50 mg/kg bw dose group

Metabolite identity / designation	Time interval (h)	% of dose in faeces	
		Male	Female
Identified (in acetonitrile extracts)			
M216F007	0–24	0.14	0.07
	24–48	0.04	0.04
M216F016	0–24	0.13	n.d.
	24–48	n.d.	0.06
M216F012 (sum of both tautomers)	0–24	0.47	0.37
	24–48	0.55	0.45
M216F004	0–24	0.33	0.13
	24–48	0.32	0.35
M216F008	0–24	0.18	0.16
	24–48	0.09	0.13
M216F001	0–24	0.14	0.07
	24–48	0.08	n.d.
M216F017	0–24	0.28	0.18
	24–48	0.18	0.30
M216F015	0–24	0.69	0.28
	24–48	0.78	0.38
M216F027 (sum of both isomers)	0–24	0.99	1.12
	24–48	1.95	1.73
Total identified	0–48	7.34	5.82
Characterized by HPLC (in acetonitrile extracts) ^a			
Polar substances (0–20 min)	0–24	0.09	n.d.
	24–48	n.d.	n.d.
Semipolar substances (20–40 min)	0–24	0.44	0.36
	24–48	0.37	0.15
Non-polar substances (> 40 min)	0–24	1.46	2.14
	24–48	1.27	1.70
Characterized by extraction			
Water extracts	0–24	9.89	9.57
	24–48	10.61	8.89
Ammonia extracts	0–24	6.94	7.30
	24–48	9.06	9.54
Protease supernatants	0–24	1.51	1.29
	24–48	1.71	1.61
Microwave extracts	0–24	2.59	2.58
	24–48	2.80	3.43
Total characterized	0–48	48.74	48.56
Total identified and characterized	0–48	56.08	54.38
Final residue (RRR)	0–48	7.52	8.49

From Bross & Seiferlein (2009)

n.d., not detected; RRR, residual radioactive residue

^a Characterized by HPLC means sum of all peaks (all of them being below 0.5% dose) in the respective retention time area from the patterns of acetonitrile extracts.

Table 13. Composition of radioactivity in tissues and plasma of the 10 mg/kg bw dose group

Metabolite identity / designation	% of dose					
	Liver		Kidney		Plasma	
	Male	Female	Male	Female	Male	Female
Identified (in acetonitrile extracts)						
M216F012/M216F019	0.006	0.004	0.006	n.d.	0.005	n.d.
M216F004	0.011	0.007	n.d.	n.d.	n.d.	n.d.
M216F008	n.d.	0.005	0.004	0.008	0.010	0.001
M216F001	0.009	0.006	n.d.	n.d.	n.d.	n.d.
M216F017	n.d.	0.003	n.d.	n.d.	n.d.	n.d.
M216F015	0.013	0.011	0.004	0.006	n.d.	0.004
M216F027	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M216F020	n.d.	n.d.	0.059	0.020	0.034	n.d.
M216F022	n.d.	n.d.	0.009	n.d.	0.013	n.d.
M216F026	n.d.	n.d.	0.010	0.003	n.d.	n.d.
M216F002	n.d.	n.d.	0.004	0.003	n.d.	n.d.
<i>Total identified</i>	0.039	0.036	0.096	0.040	0.062	0.005
Characterized by HPLC^a						
Acetonitrile extracts (sum of further peaks)	0.080	0.073	0.026	0.050	0.030	0.028
Characterized by extraction						
Water extracts	0.106	0.049	0.171	0.115	0.029	0.020
<i>Total characterized</i>	0.186	0.122	0.197	0.165	0.059	0.048
Total identified and characterized	0.225	0.158	0.293	0.205	0.121	0.053
Final residue (RRR)	0.003	0.002	0.006	0.004	0.000	0.000

From Bross & Seiferlein (2009)

n.d., not detected; RRR, residual radioactive residue

^a Characterized by HPLC means sum of all further peaks that were not identified in the patterns of acetonitrile extract.

intact parent molecule by carboxy and amino moieties (M216F015, M216F017) was observed as a further metabolic conversion of dithianon.

The main transformation steps observed are oxidation of the sulfur atom, cleavage of the dithiane ring, resulting in a naphthoquinone moiety (bearing various substituents at the quinone part of the ring system), and reduction of the naphthoquinone ring to a 1,4-dihydroxynaphthalene moiety, with further glucuronidation and substitution of the carbonitrile groups by amino and carboxy units in the intact parent molecule. The metabolic pathway is shown in [Figure 2](#).

The studies described above in this section (Schlueter & Memmesheimer, 1994a,b; Bross & Schreiner, 2007; Bross & Seiferlein, 2009) were conducted in compliance with GLP according to guidelines of the USEPA, Japan, the OECD, the EC and/or the United Kingdom. A QA statement was attached.

2. Toxicological studies

2.1 Acute toxicity

The results of the studies on the acute toxicity of dithianon are presented in [Table 15](#). All the studies were conducted according to OECD, EC and USEPA guidelines and complied with GLP.

Table 14. Composition of radioactivity in tissues and plasma of the 50 mg/kg bw dose group

Metabolite identity / designation	% of dose					
	Liver		Kidney		Plasma	
	Male	Female	Male	Female	Male	Female
Identified (in acetonitrile extracts)						
M216F012/M216F019	0.002	n.d.	n.d.	n.d.	n.d.	0.001
M216F004	0.003	0.003	n.d.	n.d.	n.d.	n.d.
M216F008	0.004	0.002	n.d.	0.002	0.001	0.001
M216F001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M216F017	n.d.	0.002	n.d.	n.d.	n.d.	n.d.
M216F015	0.005	0.004	n.d.	0.001	0.002	0.002
M216F027 (sum of both isomers)	n.d.	0.003	n.d.	n.d.	n.d.	n.d.
M216F020	n.d.	n.d.	0.021	0.014	n.d.	n.d.
M216F022	n.d.	n.d.	0.004	0.004	n.d.	n.d.
M216F026	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M216F002	n.d.	n.d.	n.d.	0.003	0.001	n.d.
<i>Total identified</i>	0.014	0.014	0.025	0.024	0.004	0.004
Characterized by HPLC^a						
Acetonitrile extracts (sum of further peaks)	0.027	0.035	0.005	0.005	0.011	0.019
Characterized by extraction						
Water extracts	0.039	0.048	0.045	0.040	0.006	0.013
<i>Total characterized</i>	0.066	0.083	0.050	0.045	0.017	0.032
Total identified and characterized	0.080	0.097	0.075	0.069	0.021	0.036
Final residue (RRR)	0.001	0.001	0.001	0.002	0.000	0.000

From Bross & Seiferlein (2009)

n.d., not detected; RRR, residual radioactive residue

^a Characterized by HPLC means sum of all further peaks that were not identified in the patterns of acetonitrile extract.

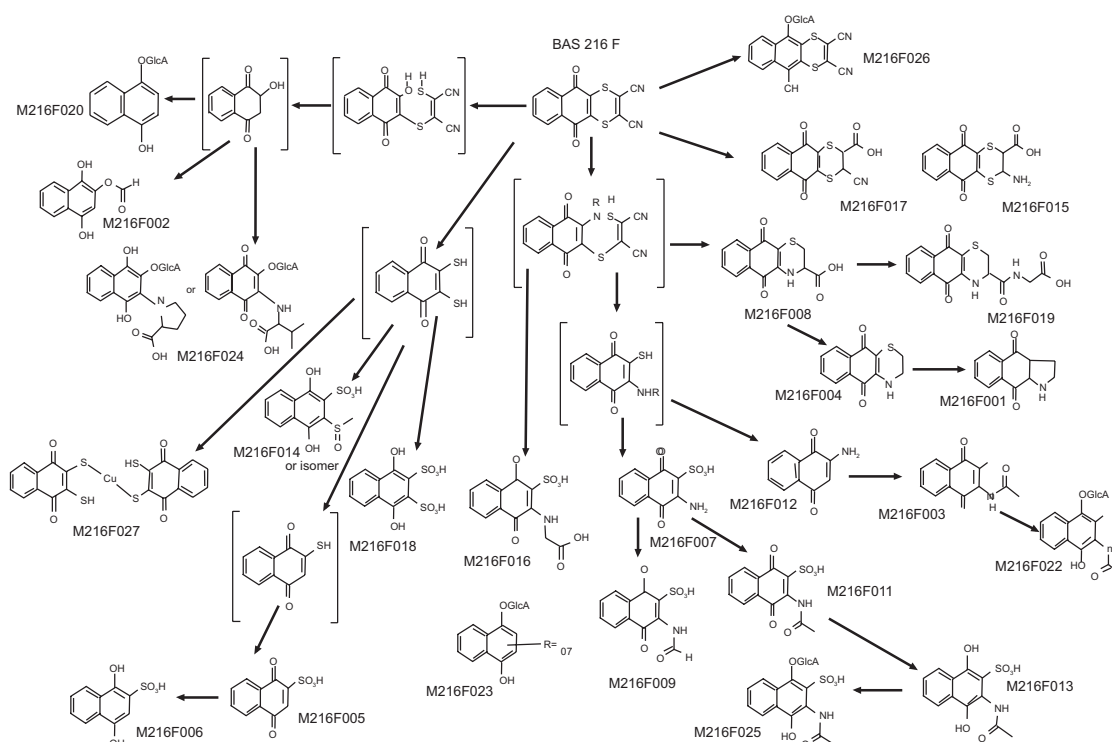
2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 4-week range-finding study performed to determine the dose levels for a subsequent 80-week study, dithianon technical (92% active ingredient) was fed to four groups of Crl:CD-1(ICR) BR strain mice (six of each sex per group) at a dietary concentration of 0, 100, 500 or 1000 ppm (equivalent to 0, 15, 75 and 150 mg/kg bw per day for both sexes) for 28 consecutive days. The mice were 41 days old, and body weights were between 23 and 30 g (males) and 20 and 24.1 g (females). Feed consumption, body weight and substance intake were determined. The animals were examined regularly for signs of toxicity or mortality. Haematology and thyroid hormone levels were determined after 4 weeks on test. Surviving animals were subjected to gross pathological assessment, and liver and kidneys were weighed (females), followed by histopathological examination of the liver and kidney (females).

No mortalities and no treatment-related clinical signs were observed during the study. Body weights in the 1000 ppm group (males and females) were consistently lower than in the controls throughout the study period, with overall decreased body weight gains of approximately 35% for

Figure 2. Metabolic pathway for dithianon in rats

[BAS 216 F is dithianon]

From Bross & Seiferlein (2009)

Table 15. Acute toxicity of dithianon

Species	Strain	Sex	Route	Batch No., purity (%)	LD ₅₀ (mg/kg bw)	LC ₅₀ (mg/l)	Results	Reference
Rat	Wistar	F	Oral	DT2003-2035, 96.9	~300	—	a	Gamer & Leibold (2005b)
Rat	Wistar	M & F	Oral	Charge 15C/86, 92 ± 0.6	720 (M) 678 (F) 702 (M & F)	—	b	Ullmann (1987a)
Rat	Wistar	M & F	Dermal	DT2003-2035, 96.9	> 2000	—	c	Gamer & Leibold (2005c)
Rat	Wistar	M & F	Dermal	15C/86, 92 ± 0.6	> 2000	—	c	Ullmann (1986a, 1993)
Rat	Wistar	M & F	Inhalation, 4 h (dust exposure, nose only)	DT 2003-2035, 96.9	—	M, 0.31 F, 0.58 Combined, 0.33 (MMAD ranged from 2.0 to 3.2 µm)	—	Gamer & Leibold (2005a)

Table 15 (continued)

Species	Strain	Sex	Route	Batch No., purity (%)	LD ₅₀ (mg/kg bw)	LC ₅₀ (mg/l)	Results	Reference
Rat	Wistar	M & F	Inhalation, 4 h (aerosol exposure, nose only)	30/84, 94.7	—	M, 1.8 F, 2.4 Combined, 2.1 (MMAD ranged from 3 to 7 µm)	—	Ullmann (1984a)
Rabbit	New Zealand White	M & F	Acute dermal irritation / corrosivity	DT2003-2035, 96.9	—	—	Non-irritant	Remmele & Leibold (2005a)
Rabbit	New Zealand White	M & F	Primary skin irritation (4 h occlusive application)	15C/86, 92 ± 0.6	—	—	Non-irritant	Ullmann (1986b)
Rabbit	New Zealand White	M & F	Acute eye irritation	DT2003-2035, 96.9	—	—	Severe eye irritation potential	Remmele & Leibold (2005b)
Rabbit	New Zealand White	M & F	Primary eye irritation	15C/86, 92 ± 0.6	—	—	Irreversibly irritating to the rabbit eye (not rinsed)	Ullmann (1987b)
Guinea-pig	HsdPoc: DH	F	Skin sensitization effects (guinea-pig maximization)	DT2003-2035, 96.9	—	—	Has a sensitizing effect on the skin of the guinea-pig	Gamer & Leibold (2005d)
Guinea-pig	Dunkin-Hartley	M & F	Delayed hypersensitivity in the albino guinea-pig (guinea-pig maximization)	30/84, 94.7	—	—	Not considered to be a skin sensitizer after challenge or rechallenge	Ullmann (1984b)

F, female; LC₅₀, median lethal concentration; LD₅₀, median lethal dose; M, male; MMAD, mass median aerodynamic diameter

^a Three dose levels were administered (50, 300 and 500 mg/kg bw). Clinical observations in the 500 and 300 mg/kg bw groups revealed impaired and poor general state, dyspnoea, staggering, piloerection, smeared fur and diarrhoea; findings were observed from hour 1 until the end of study day 6 after administration. Clinical observations in the 50 mg/kg bw groups revealed impaired general state, dyspnoea and diarrhoea; findings were observed from hour 1 after administration until the end of study day 1. The mean body weights of the surviving animals of the administration groups increased throughout the study period. During necropsy, findings in the animals that died comprised few or several black erosions/ulcers in the glandular stomach and slight red discoloration of contents of the small intestine (500 mg/kg bw, two females; 300 mg/kg bw, two females). No macroscopic pathological abnormalities were noted in one animal of the 500 mg/kg bw group that died and in the surviving animals examined at the end of the observation period (300 mg/kg bw, four females; 50 mg/kg bw, six females).

^b Signs of toxicity, which were first observed at 1 or 2 h after dosing in all groups, included sedation and ruffled fur. Other clinical observations, which were seen at 400 mg/kg bw and above, included curved body position, dyspnoea and diarrhoea.

^c No systemic clinical observations were noted in the animals. At the application site, very slight or well-defined erythema and a distinct brown discoloured application area were observed on days 1 and 2.

both males and females, compared with controls, at study termination. Feed consumption was decreased in the 1000 ppm group (males and females) only during the first week of treatment, compared with controls. There were no effects on feed consumption or body weights in the other treatment groups.

In the 1000 ppm group (males and females) and in the 500 ppm group (females only), haemoglobin concentrations were lower than in the controls, indicating a slight anaemia for these animals. There were no other treatment-related effects on haematology. However, females of the two higher dose groups showed a dose-related decrease in plasma triiodothyronine (T_3) and thyroxine (T_4) levels, whereas treated males from the same groups showed a dose-related decrease in plasma T_4 levels only. No other effects on thyroid hormone parameters were observed. A protocol amendment was added to measure liver and kidney weights in females, but not in males. The reason for the amendment is not specified in the study report. However, it may be related to the reductions in haemoglobin that occurred at two dose levels in the females and were more pronounced than those that occurred at only one dose level in males. There were no treatment-related effects on organ weights in females. Microscopic examination of the liver and kidney of females revealed an increased incidence of iron deposition in Kupffer cells of the liver (haemosiderin) in six of six females in the 1000 ppm group and in four of six females in the 500 ppm group. Haemosiderin deposition was slightly increased in severity at 1000 ppm compared with 500 ppm. It was concluded that 500 ppm (75 mg/kg bw) should be selected as the high dose for an 18-month study, because it was associated with slight toxicity that would probably not alter the normal lifespan of animals. The no-observed-adverse-effect level (NOAEL) was 100 ppm (equivalent to 15 mg/kg bw per day), based on slight anaemia and histopathological effects in the livers of females at 500 ppm.

The study was conducted prior to the implementation of GLP, but a QA statement was attached (Brown, 1987).

Rats

In a preliminary range-finding study, dithianon technical (batch/purity not reported) was fed to four groups of Sprague-Dawley rats (four of each sex per group) at a dietary concentration of 0, 315, 1250 or 5000 ppm (equivalent to 0, 30, 119 and 476 mg/kg bw per day for both sexes) for 28 consecutive days. Feed consumption and body weight were determined. The animals were examined regularly for mortality or signs of toxicity. After 4 weeks on test, surviving animals were subjected to gross pathological assessment, followed by histopathological examinations of the gastrointestinal tract. According to protocol, haematology and clinical chemistry parameters and organ weights were not measured. Animals in the 5000 ppm group exhibited a loss of body weight and markedly reduced feed consumption. Consequently, at 5000 ppm, seven of eight animals died during weeks 3 and 4. There was a decrease in overall body weight gain of 31% for males and 46% for females in the 1250 ppm group compared with the controls, with a resultant decrease in mean absolute body weight at study termination. There was a reduction in feed consumption in the 1250 ppm group (males and females) during the first half of the study, compared with controls, but feed consumption in this group recovered to near control levels during the second half of the study. There were no effects on body weights or feed consumption in the 315 ppm group. Macroscopic examination showed empty gastrointestinal tracts and lung congestion for animals in the 5000 ppm group that died during the study. At terminal necropsy, animals in the 1250 ppm group showed intestines containing liquid orange-coloured contents. There were no other abnormalities in any of the groups. Microscopic examination of the selected tissues revealed no treatment-related histopathological effects in any of the groups. The NOAEL for dithianon was 315 ppm (equivalent to 30 mg/kg bw per day), based on decreased overall body weight gains and decreased feed consumption for males and females at 1250 ppm. Based on these results, the highest dietary concentration of 1000 ppm was selected for use in a 2-year dietary toxicity study in order to avoid undue anorexia.

The study was pre-GLP, and no QA statement was attached (Wheldon & Frohberg, 1966).

Dithianon technical (purity 92%; batch No. 15C/86) was fed to four groups of Sprague-Dawley rats (10 of each sex per group) for 90 days at a dietary concentration of 0, 30, 180 or 1080 ppm (equal

to 0, 2.5, 14.6 and 86.7 mg/kg bw per day for males and 0, 3.0, 16.3 and 99.5 mg/kg bw per day for females). Another group of 10 animals of each sex was assigned to a 4-week recovery period. This dose schedule was selected on the basis of a 14-day pilot study performed with dithianon dietary concentrations of 180, 1080, 1440 and 2160 ppm. At 1440 ppm, the body weights were decreased by 5% in the males and 18.6% in the females, and at 2160 ppm, by 31.8% in the males and 25.8% in the females, after 2 test weeks. In view of this, the highest dose selected was 1080 ppm in the 90-day study. Test substance/diet mixtures were freshly prepared each day. The age of male and female rats at the start of the study was 32–33 days and 34–35 days, respectively. Body weights at study initiation were 91–124 g (males) and 89–124 g (females). Feed consumption, body weight and substance intake were determined weekly. Ophthalmoscopy was performed prior to treatment and at 13 weeks. The animals were examined for mortality or signs of toxicity at least once a day. During week 13 on test, the animals were subjected to a comprehensive clinical examination that included urinalysis, clinicochemical and haematological examinations. All animals were subjected to gross pathological assessment, followed by histopathological examinations.

No treatment-related mortalities occurred during the treatment period. No overt signs of toxicity were observed that could be attributed to administration of the test material. Feed consumption values for males and females in all treated groups were comparable to those of controls during the treatment period. There were no treatment-related effects on body weight in the 30 or 180 ppm dose groups. Mean body weights at study termination were statistically significantly lower in males and females receiving 1080 ppm, and overall body weight gains were decreased by 14% in males and 11% in females, compared with controls.

An evaluation of haematological parameters showed that in animals of both sexes receiving 1080 ppm, erythrocyte count, packed cell volume and haemoglobin concentration were slightly but statistically significantly reduced, and the reticulocyte count was slightly but statistically significantly increased, compared with controls. Other haematological parameters were within normal limits. An evaluation of clinical chemistry parameters showed slight increases in blood urea nitrogen in males and females (statistically significant in males only) at 1080 ppm. Increases in total protein and albumin levels in males and decreases in T_4 levels in males and in chloride levels in females were also noted at 1080 ppm. T_3 levels were statistically significantly decreased in the males at all tested doses. The decrease in T_4 levels was not considered to be toxicologically significant because of the absence of any corresponding pathological findings or thyroid gland weight changes. All animals showed normal urinary status following 90 days of treatment. For the 1080 ppm group, absolute and relative (to body weight) kidney, liver and adrenal gland weights were elevated in males and females compared with controls, whereas absolute heart weight was statistically significantly decreased in females in comparison with controls. Gross necropsy, ophthalmoscopy and microscopic evaluation performed by the original study pathologist revealed no treatment-related findings after the 90-day exposure. However, an independent microscopic re-evaluation of the kidney tissue sections from females in the 1080 ppm group (Grasso, 1991a) revealed tubular epithelial cell damage (hydropic degeneration) in 4 of 10 animals and tubular epithelial cell hyperplasia (tubular hyperplasia) in 2 of 10 animals, compared with 0 of 10 control animals for both findings.

In conclusion, the NOAEL in rats fed dithianon for 90 days was 180 ppm (equivalent to 14.6 mg/kg bw per day), based on decreased overall body weight gains, increased absolute and relative kidney, liver and adrenal gland weights, and changes in haematological and clinical chemistry parameters in males and females, as well as histopathological findings of renal tubular epithelial cell degeneration and regenerative hyperplasia (females only), at 1080 ppm.

This GLP study was conducted according to OECD and USEPA test guidelines (Leuschner, 1987, 1990a,b).

Dogs

Dithianon technical (purity $92 \pm 0.6\%$; batch No. 15 c/86) was fed to four groups of Beagle dogs (four of each sex per group) for 90 days at a dietary concentration of 0, 40, 200 or 1000 ppm (equal to 0, 0.63, 2.95 and 12.6 mg/kg bw per day, respectively, for males and 0, 0.67, 3.0 and 12.6 mg/kg bw per day, respectively, for females). The test substance/diet mixtures were prepared daily. At the beginning of the experiment, the dogs were 8 months (male) and 12 months (females) of age. Initial body weights were 9.2–11.6 kg (males) and 7.8–10.4 kg (females). Feed consumption and general health status of the animals were determined daily, and body weights were measured once a week. Clinical chemistry and haematological examinations were carried out once before treatment began and during weeks 6 and 13. Urinalyses were carried out before treatment and during weeks 5 and 12. Ophthalmological examinations were carried out before dosing commenced and during week 13 of treatment. All animals were subjected to complete gross and histopathological examinations, and selected organs were weighed.

All animals survived up to study termination, and no overt signs of toxicity were observed that could be attributed to administration of the test material. For animals in the 1000 ppm group, feed consumption and body weight were slightly, but consistently, reduced throughout the study, compared with controls. Overall body weight gains were decreased during the treatment period for males and females at 1000 ppm. By study termination, the mean body weight gain in male and female controls was 1.1 kg (11% increase), whereas the mean body weight gain in 1000 ppm males was 0.1 kg (1% increase). The 1000 ppm females lost approximately 0.35 kg (4%) by study termination. There were no treatment-related effects on feed consumption, body weight or body weight gain in the other dose groups. There were no treatment-related effects on haematological or urinalysis parameters. Analysis of clinical chemistry parameters showed that alkaline phosphatase activity was statistically significantly elevated in the 1000 ppm group in both males and females, compared with controls. In the 1000 ppm group, absolute and relative kidney weights were slightly and generally statistically significantly increased in both males and females. However, as there were no macroscopic or microscopic changes in this organ that were attributable to treatment and because there were no corresponding blood or urinary clinical chemistry changes, slight increases in kidney weight were difficult to interpret as to their clinical significance. Three addenda were submitted with this study (Leuschner, 1989, 1990c, 1991). None of the addenda changed the interpretation of the study, except one addendum noted that there was a statistically significant increase in thromboplastin time for females at 1000 ppm.

It was concluded that the subchronic (90-day) NOAEL for dithianon technical in dogs was 200 ppm (equal to 2.95 mg/kg bw per day), based on a decrease in body weight (females only), decreased body weight gain (males) and decreased feed consumption, increases in alkaline phosphatase activity and kidney weights for males and females and an increase in thromboplastin time for females at 1000 ppm. The study was conducted in compliance with GLP regulations of the United States Food and Drug Administration, the OECD and Japan. A QA statement was attached (Neuwann, 1987).

Four groups of Beagle dogs (four of each sex per group) were fed dithianon technical (purity 92%; batch No. 15C/86) at a dietary concentration of 0, 40, 200 or 1000 ppm (equal to 0, 1.6, 7.3 and 37.1 mg/kg bw per day, respectively, for males and 0, 1.6, 7.9 and 37.5 mg/kg bw per day, respectively, for females) for 52 consecutive weeks. The age of the dogs at study commencement was 5–7 months, including an acclimatization period of 5 weeks. At the start of treatment, the males weighed 5.6–8.8 kg and females 5.45–8.6 kg. Diets were prepared once per week. Feed consumption for all groups, including the controls, was approximately 3-fold greater than in the 90-day study in dogs. Thus, dogs in the 52-week study received higher doses compared with dogs in the 90-day study. The differences in feed consumption between the 52-week and the 90-day studies may be related to the use of different diets. Feed consumption and the state of health of the animals were determined daily,

and body weights were measured once a week. Haematological examinations as well as urinalyses were carried out once before treatment began and during study weeks 13, 26 and 52. Clinical chemistry examinations were carried out once before treatment began and during study weeks 26 and 52. Ophthalmological examinations were carried out before the beginning of treatment and during study weeks 13 and 52. All animals were subjected to complete gross and histopathological examinations, and selected organs were weighed.

All animals survived to study termination, and there were no clinical observations that were considered to be related to treatment. Body weight gains for males and females at 1000 ppm were reduced by 20% and 15%, respectively, compared with controls, at week 13 of the study. Overall body weight gains for treatment weeks 1–52 were decreased by 20% in the males at 1000 ppm, compared with the controls. Females in the 1000 ppm group gained more weight than the controls during weeks 26–52 of treatment (Tables 16 and 17), resulting in no effect on overall body weight gain at study termination. In the 1000 ppm group, there was a statistically significant decrease in feed consumption in males (13%) between weeks 1 and 13 of treatment. There were slight decreases in feed consumption of approximately 7% during the remainder of the treatment period in males and females at 1000 ppm. There were no treatment-related effects on body weight gain or feed consumption in the other treatment groups. No treatment-related ocular lesions were observed.

Haematological evaluations (Table 18) revealed a mild anaemia at the 1000 ppm concentration in both sexes. This was characterized by slight reductions in haemoglobin concentration, red blood cell count and packed cell volume, which were apparent by week 13 of treatment and reached statistical significance by week 26, compared with the controls. The reductions in red blood cell parameters were still present at week 52. At week 52, the 1000 ppm treatment level showed immature red blood cells in three of four males and in three of four females, and the mean platelet counts and white blood cell counts were slightly elevated (males only). In contrast, anaemia was not observed at the 1000 ppm level in the 90-day feeding study in dogs. The anaemia observed in males and females at 1000 ppm at week 13 in the 52-week study may be due to the approximately 3-fold greater feed consumption in the 52-week study, which resulted in a higher dose, compared with the 90-day study.

Clinical chemistry findings (Table 19) consisted of statistically significant increases in alkaline phosphatase activity at 1000 ppm in both sexes at weeks 26 and 52. There were no treatment-related effects noted in the urinalyses.

Organ weight data (Table 20) showed increases in absolute and relative liver, kidney and thyroid gland weights for males and females in the 1000 ppm group, compared with controls. For the liver and kidneys, these increases were statistically significant, except for the absolute organ weights in males. For the thyroid glands, only the increase in relative organ weight in females was statistically significant. Increases in thyroid gland weights, noted in Table 20 for 1000 ppm males and females, may be related to hepatic enzyme induction.

There were no treatment-related macroscopic findings at necropsy. Microscopically, hepatocellular hypertrophy was observed in the 200 ppm (females only) and 1000 ppm groups (males and females) (Table 21). In the absence of any degenerative changes in hepatocytes, this was considered to reflect possible enzyme induction. For males and females at 1000 ppm, there were occasional foci of pigmented histiocytes (Kupffer cells) in the sinusoids, which stained positively for iron. In addition, a slight increase in brown pigment in the proximal tubular epithelial cells in the kidney was observed in the 200 ppm (females only) (although there is no statistically significant variation in weight of kidney) and 1000 ppm groups (males and females).

In view of the above, the NOAEL for dithianon fed to dogs in their diet for 52 weeks 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.

The study was conducted in accordance with GLP standards as per the USEPA. A QA statement was attached (Clay, 1991).

Table 16. Mean body weights of dogs after 13, 26 and 52 weeks of dithianon administration in the diet

Dietary concentration (ppm)	Mean body weight (kg)					
	Males			Females		
	Week 13	Week 26	Week 52	Week 13	Week 26	Week 52
0	10.01	10.41	11.05	8.73	9.36	9.33
40	9.56	9.94	10.30	8.16	8.95	9.38
200	10.40	11.19	11.30	8.28	8.55	8.73
1000	9.26	9.64	10.16	8.34	8.79	9.59

From Clay (1991)

Table 17. Body weight gain in dogs over selected intervals of dithianon administration

Dietary concentration (ppm)	Mean body weight gain (kg)			
	Weeks 1–13	Weeks 13–26	Weeks 26–52	Weeks 1–52
Males				
0	2.14	0.40	0.64	3.18
40	2.18	0.38	0.36	2.91
200	2.79	0.79	0.11	3.69
1000	1.71	0.38	0.53	2.61
Females				
0	1.84	0.64	–0.04	2.44
40	1.76	0.79	0.43	2.98
200	1.60	0.28	0.18	2.05
1000	1.58	0.45	0.80	2.83

From Clay (1991)

*(b) Dermal application**Rats*

In a 21-day dermal toxicity study in rats, dithianon technical (purity 92%; batch No. 15C/86) was suspended in polyethylene glycol 200 and applied dermally to Sprague-Dawley rats (five of each sex per group) at a dose of 40, 200 or 1000 mg/kg bw per day for 6 hours per day for 22 consecutive days. Control rats (10 of each sex per dose) received daily dermal doses of the vehicle alone. A dose volume of 4 ml/kg bw was applied to shaven skin, covered by a gauze patch held in place by an occlusive wrap of tin foil and an elastic bandage. At the end of each treatment period, test sites were wiped with moist gauze and dried. Animals were about 8–10 weeks old at the time of initiation of treatment; males weighed 286.9–359 g, and females, 186–223.9 g. Skin reactions were recorded daily. Feed consumption and body weight were determined weekly. The state of health was checked at least daily. Clinicochemical and haematological examinations of samples taken on day 23 were carried out. At necropsy, all animals were assessed by gross pathological examination, and selected organs were weighed. Histopathological examinations were also performed on all animals.

There were no mortalities during the study, and daily dermal exposure to dithianon at doses up to 1000 mg/kg bw per day did not cause any behavioural changes or clinical signs of toxicity. Animals receiving dithianon showed signs of skin irritation at the application site from about day 6. These signs

Table 18. Changes in haematological parameters after dietary administration of dithianon to dogs for 52 weeks

Dietary concentration (ppm)	Mean haematology values											
	Males						Females					
	Hb (g/dl)	RBC (10 ⁶ /mm ³)	PCV (%)	MCHC (g/dl)	L (%)	N (%)	Hb (g/dl)	RBC (10 ⁶ /mm ³)	PCV (%)	MCHC (g/dl)	L (%)	N (%)
Week 26												
0	15.8	6.68	45.8	34.5	3.93	6.23	16.6	6.82	47.7	34.9	3.60	6.68
40	16.2	6.66	46.7	34.6	3.88	6.28	15.8	6.40	45.3	34.7	5.18*	7.73
200	15.9	6.60	46.4	34.4	4.35	7.43	15.8	6.42	45.5	34.7	3.95	7.40
1000	13.9*	5.86*	41.0*	33.9*	4.20	7.83	14.1**	5.88**	41.8**	33.7*	5.18*	10.08**
Week 52												
0	16.5	7.41	48.9	33.8	3.10	5.58	17.9	7.64	52.2	34.4	3.63	7.03
40	17.04	7.38	50.2	33.9	3.45	7.38*	16.5	7.03	48.8	33.8	3.83	7.38
200	17.4	7.39	51.0	34.0	3.60	6.98	16.8	7.17*	49.2	34.2	3.30	7.00
1000	15.0	6.64*	45.4	33.1	4.25DR*	8.83*	14.3	6.23	43.5*	32.9*	4.20	8.10

From Clay (1991)

Hb, haemoglobin; L, lymphocytes; MCHC, mean corpuscular haemoglobin concentration; N, neutrophils; PCV, packed cell volume; RBC, red blood cells; * $P < 0.05$; ** $P < 0.01$; DR, significant using the dose-response test**Table 19. Changes in clinical chemistry parameters after dietary administration of dithianon to dogs for 52 weeks**

	Dietary concentration (ppm)							
	0		40		200		1000	
	M	F	M	F	M	F	M	F
Week 26								
AP (IU/l)	121	158	102	115	166	154	200DR*	338*
BUN (mg/dl)	16	18	13	17	14	15	12*	11***
Creatinine (mg/dl)	1.0	1.0	0.9	0.9	0.9	0.9	0.8**	0.8***
Total protein (g/dl)	5.3	5.5	5.4	5.6	5.8*	5.7	5.7	5.7
Albumin (g/dl)	3.4	3.5	3.4	3.5	3.6	3.6	3.6	3.6
Week 52								
AP (IU/l)	94	147	89	77	98	113	186	265*
BUN (mg/dl)	15	16	11	15	15	14	11	11**
Creatinine (mg/dl)	1.0	1.0	0.9**	1.0	0.9	0.9*	0.8***	0.8***
Total protein (g/dl)	5.9	5.8	5.9	6.1	6.3	5.8	6.3	6.1
Albumin (g/dl)	3.0	3.1	2.9	3.1	3.3	3.0	3.2DR*	3.1

From Clay (1991)

AP, alkaline phosphatase; BUN, blood urea nitrogen; F, female; IU, international units; M, male; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; DR, significant using the dose-response test

Table 20. Mean organ weight after dietary administration of dithianon to dogs for 52 weeks

	Dietary concentration (ppm)							
	0		40		200		1000	
	M	F	M	F	M	F	M	F
Body weight (g)	10 738	9200	10 075	9088	11 125	8638	10 025	9413
Adrenal (left)								
Absolute weight (g)	0.549	0.668	0.615	0.612	0.530	0.585	0.588	0.608
Relative (to body) weight (%)	0.0051	0.0073	0.0061	0.0067	0.0048	0.0069	0.0059	0.0068
Adrenal (right)								
Absolute weight (g)	0.620	0.689	0.616	0.649	0.503	0.600	0.588	0.601
Relative (to body) weight (%)	0.0058	0.0075	0.0061	0.0071	0.0045	0.0071	0.0059	0.0066
Kidney (left)								
Absolute weight (g)	24.934	20.844	27.418	20.865	25.750	22.017	30.752	28.301*
Relative (to body) weight (%)	0.2319	0.2268	0.2720	0.2295	0.2330	0.2539	0.3079***	0.3067**
Kidney (right)								
Absolute weight (g)	24.259	20.486	26.314	21.533	25.552	21.449	30.586	25.936DR**
Relative (to body) weight (%)	0.2254	0.2234	0.2615	0.2372	0.2309	0.2480	0.3061***	0.2805**
Liver								
Absolute weight (g)	315.1	268.7	309.3	311.3	326.2	322.6	443.3	452.0DR*
Relative (to body) weight (%)	2.93	2.93	3.08	3.42	2.95	3.73	4.38*	4.81*
Testes (left)								
Absolute weight (g)	10.529	—	10.202	—	9.851	—	10.351	—
Relative (to body) weight (%)	0.983	—	0.1015	—	0.0885	—	0.1029	—
Testes (right)								
Absolute weight (g)	10.773	—	9.946	—	9.543	—	10.39	—
Relative (to body) weight (%)	0.1007	—	0.989	—	0.0857	—	0.1006	—
Ovary (left)								
Absolute weight (g)	—	0.569	—	0.805	—	0.438	—	0.916
Relative (to body) weight (%)	—	0.0060	—	0.0088	—	0.0050	—	0.0110
Ovary (right)								
Absolute weight (g)	—	0.456	—	0.631	—	0.438	—	0.601
Relative (to body) weight (%)	—	0.0049	—	0.0070	—	0.0040	—	0.0073
Thyroid (left)								
Absolute weight (g)	0.407	0.350	0.420	0.417	0.385	0.346	0.491	0.511
Relative (to body) weight (%)	0.0038	0.0039	0.0041	0.0045	0.0035	0.0040	0.0049	0.0054DR*
Thyroid (right)								
Absolute weight (g)	0.424	0.351	0.378	0.347	0.388	0.363	0.463	0.481
Relative (to body) weight (%)	0.0040	0.0038	0.0037	0.0038	0.0035	0.0043	0.0047	0.0051DR*

From Clay (1991)

F, female; M, male; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; DR, significant using the dose–response test

include erythema, oedema, atonia, fissuring and desquamation. At terminal sacrifice (day 23), body weight gain was reduced by 47% for males (only) receiving 1000 mg/kg bw per day, compared with controls. For females (only) at 1000 mg/kg bw per day, haematological parameters revealed a slight

Table 21. Histopathological data in dogs after administration of dithianon for 52 weeks

	Incidence of finding							
	Dietary concentration (ppm)							
	0		40		200		1000	
	M	F	M	F	M	F	M	F
<i>Number of animals examined</i>	4	4	4	4	4	4	4	4
Liver								
Hepatocyte pigment	4	4	4	4	4	4	4	4
Histiocyte pigment	0	0	0	0	0	0	3	3
Hepatocellular hypertrophy	0	0	0	0	0	4	4	4
Intracellular inclusions	0	0	0	0	0	0	0	1
Kidney								
Mineralization	4	3	4	4	4	4	4	4
Pigment	4	1	3	1	4	3	4	4
Basophilic tubules	0	0	0	0	0	0	1	0
Focal nephropathy	0	0	1	0	0	0	0	1
Hyaline droplets	0	0	0	0	1	0	0	0
Testis								
Atrophy	1	—	0	—	0	—	2	—
Orchitis	0	—	0	—	0	—	1	—
Thyroid								
Leukocyte foci	2	0	1	0	0	0	0	0
Cyst	2	0	0	1	1	0	2	0
Lymphocytic thyroiditis	0	0	0	1	0	0	1	2
Parathyroid								
Cyst	1	0	2	2	1	2	0	2

From Clay (1991)

F, female; M, male

anaemia, as indicated by slight decreases in red blood cell count, haemoglobin concentration and packed cell volume, compared with controls. For males and females, no treatment-related effects were noted for feed consumption, clinical chemistry and urinalysis parameters, absolute and relative (to body weight) organ weights or gross necropsy findings. For females (only) at 1000 mg/kg bw per day, histopathology revealed slight nephrotoxicity, as indicated by increased incidences of regenerative basophilic tubules of moderate severity with occasional mitotic figures and luminal eosinophilic material.

In conclusion, the NOAEL in this 21-day dermal toxicity study was 200 mg/kg bw per day, based on decreased body weight change for males and on slight anaemia and histopathological effects in the kidneys of females at 1000 mg/kg bw per day (the highest dose tested).

The study was conducted prior to implementation of GLP. A QA statement was attached (Brown, 1989).

(c) *Exposure by inhalation*

Rats

In a 14-day inhalation study, three groups of Wistar rats (five of each sex per group) were exposed via nose-only inhalation to dithianon technical (purity not reported) (administered as an

aerosol in water) for 6 hours per day, 5 days per week, for 2 weeks (total of 10 exposures) at a concentration of 110, 310 or 1070 mg/m³ (0.110, 0.310 or 1.070 mg/l) (gravimetric). Additionally, one control group of five rats of each sex was exposed to filtered air, and another control group of five rats of each sex was exposed to water (vehicle). An intermediate-dose recovery group of five rats of each sex was exposed to dithianon technical at a concentration of 310 mg/m³ (0.310 mg/l) for 6 hours per day, 5 days per week, for 2 weeks and then allowed a 2-week recovery period. Rats were about 7 weeks old at the start of the experiment.

Feed consumption was measured daily, whereas body weights were measured prior to exposure, every fourth day and on the day of sacrifice. The animals were examined individually before and during the exposures and also following exposure each day. Animals in the recovery group were further examined daily during the observation period of 14 days to determine their general health, behaviour and condition. After 2 weeks on test, the animals were subjected to a comprehensive clinical examination, which included urinalysis, clinicochemical and haematological examinations. All animals were subjected to gross pathological assessment, including organ weights of selected tissues, followed by histopathological examinations. The mass median aerodynamic diameters (MMADs) for all concentrations of dithianon technical tested were obtained during the second and fourth hours of each exposure. The MMADs for the 110, 310 and 1070 mg/m³ groups were 3.1, 3.5 and 4.1 µm, respectively. These particle size data indicate that respirable concentrations of the test material were administered to the test animals, thereby validating the delivery method used in this study.

Results from this study showed neither mortality nor treatment-related clinical signs of toxicity and no treatment-related effects on feed consumption, body weight or organ weights. Additionally, no treatment-related effects on haematological, clinical chemistry or urinalysis parameters and no treatment-related macroscopic or microscopic findings were noted in this study.

Based on these results, the no-observed-adverse-effect concentration (NOAEC) from this study is 1070 mg/m³ (1.07 mg/l) (gravimetric), the highest concentration tested. In addition, based on route-to-route extrapolation, the NOAEC of 1070 mg/m³ (1.07 mg/l) from the 14-day inhalation toxicity study in rats can be converted to a systemic NOAEL using the following equation:

$$\text{NOAEC (mg/l)} \times (\% \text{ inhalation absorption}) \times \text{breathing rate (l/h)} \times \text{exposure time (h/day)} / \text{average body weight of the rat during the treatment period (kg)} = \text{NOAEL (mg/kg bw per day)}$$

Assuming a 100% inhalation absorption value, a breathing rate for the Wistar rat of 8.46 litres per hour, an exposure time of 6 hours per day and an average body weight for the Wistar rat from the 14-day inhalation toxicity study of 0.0865 kg, a systemic NOAEL of 628 mg/kg bw per day is obtained:

$$(1.07 \text{ mg/l}) \times (1) \times (8.46 \text{ l/h}) \times (6 \text{ h/day}) / 0.0865 \text{ kg} = 627.9 \text{ mg/kg bw per day}$$

The study was conducted prior to implementation of GLP. A QA statement was not attached (Bhide, 1986).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

A chronic dietary study was conducted to evaluate the effect of dithianon on the incidence and morphology of tumours following administration to mice for 80 weeks. Groups of CrI:CD-1(ICR)BR strain mice (51 of each sex per group) received dithianon technical (purity 92%; batch No. 15C/86) in the diet at a concentration of 0, 20, 100 or 500 ppm (equivalent to 0, 3, 15 and 75 mg/kg bw per day) for 80 consecutive weeks. Diets were prepared weekly. At the start of the study, the animals were 46 days old and weighed 20.8–34 g (males) and 19.5–26.8 g (females). Feed consumption and body weight were determined once a week for the first 16 weeks and at 4-week intervals thereafter. Water

consumption was determined daily during weeks 23–26. The animals were examined for mortality twice a day and for signs of toxicity once a day; moreover, comprehensive clinical examinations of the animals were performed once a week. Blood smears were prepared from all mice killed during the study and from all surviving mice at weeks 52 and 78 and at terminal sacrifice (week 80). At the end of the treatment period, all surviving animals were sacrificed and subjected to gross pathological assessment, and selected organs were weighed. Histopathological examinations were performed on all control and high-dose animals and on animals that died during the study. In addition, lungs, liver and kidneys and any macroscopically abnormal tissues were examined from surviving low- and intermediate-dose animals.

There was a slight increase in mortality during the last 25 weeks of the study in the males receiving 500 ppm, compared with the controls. There was no effect on mortality in females. Survival rates at study termination in the animals receiving 0, 20, 100 and 500 ppm were 73%, 75%, 71% and 63%, respectively, for males and 80%, 86%, 71% and 82%, respectively, for females.

Females in the 500 ppm group had an increased incidence of fur staining, compared with the controls. There were no treatment-related effects on body weight gain, feed consumption or water consumption in males or females. Absolute and relative kidney weights were increased in a concentration-related manner in the 100 and 500 ppm groups in males and females, and these increases were generally statistically significant.

An evaluation of haematological parameters showed no treatment-related effects on differential white blood cell count. According to protocol, erythrocyte parameters were not measured. Microscopic examination revealed an exacerbation of spontaneous chronic nephropathy in males at 500 ppm and in females at 100 and 500 ppm. For males at 500 ppm, 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. There were no unusual tumours noted in any of the treatment groups. The incidence and spectrum of the spontaneous background tumours observed were comparable among all groups.

In conclusion, the NOAEL for chronic toxicity was 20 ppm (approximately 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 for females at 100 ppm. Dithianon was not carcinogenic to mice at a dietary concentration of 500 ppm, which exceeded the MTD. Thus, the NOAEL for carcinogenicity in this study was 500 ppm (equivalent to 75 mg/kg bw per day).

This study was conducted in compliance with GLP, and a QA statement was attached (Brown, 1990).

Rats

In a 2-year carcinogenicity study, Sprague-Dawley (CrI:CD(SD)BR) rats (70 of each sex per group) received diets containing dithianon technical (purity 92%; batch No. 15C/86) at a concentration of 0, 20, 120 or 600 ppm (equivalent to 0, 1, 6 and 30 mg/kg bw per day) for a period of 104 weeks. Satellite groups of animals (20 of each sex per dose) were used in the chronic toxicity evaluations. At the initiation of the study, the animals were 46–47 days old and weighed 162.7–254.8 g (males) and 135.9–199.2 g (females). Feed consumption and body weight were determined once a week for the first 16 weeks and at 4-week intervals thereafter. Water consumption was determined daily for the satellite animals during weeks 44–47. The animals were examined for mortality and overt toxicity once a day, and comprehensive clinical examinations were performed once a week. Ophthalmological examinations were carried out prior to the start of the study and at week 102. Urinalyses, clinicochemical and haematological examinations were carried out during weeks 13, 26, 52, 78 and 103 using 20 animals of each sex per group when possible. All animals were subjected to gross pathological assessment, and selected organs were weighed. Histopathological examinations were performed on all control and high-dose animals; animals that died during the study; lungs, liver,

kidneys and thyroid glands and any macroscopically abnormal tissues from low- and intermediate-dose animals; and tissues from low- and intermediate-dose animals for any treatment-related changes that were noted in the high-dose group.

Survival rates at study termination for the animals receiving 0, 20, 120 and 600 ppm were 37%, 43%, 29% and 29%, respectively, for males and 34%, 54%, 51% and 62%, respectively, for females. Clinical observations showed a progressive increase in the incidence of fur staining and rough hair-coat during the course of the study in 600 ppm females. Body weight gain and feed consumption were slightly decreased in 600 ppm males during the initial months of the study. The overall body weight gain depression was approximately 12% for males receiving 600 ppm. In females receiving 600 ppm, body weight gain and feed consumption were decreased throughout the duration of the study; overall body weight gain was depressed 25–32%, compared with controls, beginning at week 13. This deviation indicated that the high dose level of 600 ppm exceeds the MTD for female rats (Tables 22 and 23).

Haematological evaluations (Table 24) consistently showed slight decreases in haemoglobin, red blood cell counts and packed cell volume in animals receiving 600 ppm. Clinicochemical determinations (Table 25) revealed consistent increases in γ -glutamyl transpeptidase in 600 ppm males towards the end of the study. Blood urea nitrogen values were increased consistently in 600 ppm males and females beginning at week 52 as well as at week 13 for 600 ppm males. In addition, inorganic phosphorus concentration was increased in 600 ppm males at termination, compared with controls. There was a consistently decreased cholesterol concentration in animals receiving 600 ppm. At termination of the study, an assay of thyroid hormones (thyroid stimulating hormone, T_4 and T_3) showed a decreased T_3 level, but not a decreased T_4 level, in the 600 ppm male group. Urinalysis revealed an increase in epithelial cells on examination of urinary sediments in 600 ppm females at week 26 and an increased protein content in 600 ppm males and females during the second half of the study. Organ weight analysis (Table 26) showed that absolute and relative kidney weights and relative liver weights of 600 ppm animals were increased.

At terminal necropsy, a higher incidence of gross kidney lesions (pallor, cysts, masses and an irregular surface) was noted in the 600 ppm animals. Histopathological examination confirmed that the kidney was the target organ for the toxicity of dithianon. The treatment-related renal effects consisted of an exacerbation of a chronic and progressive glomerulonephropathy and tubular nephrosis, which occur spontaneously in rats. At the 600 ppm level, predominantly in males (16/69), the effects had progressed to end-stage kidneys with extensive cortical destruction. Although no statistically significant treatment-related effects on survival were evident in male rats, the incidence of morbidity and mortality associated with renal and urogenital tract lesions (especially in males with end-stage kidneys) was increased at 600 ppm. In fact, half of the cases of end-stage kidneys in the 600 ppm males were considered to have been the primary cause of death. In addition, increased incidences of parathyroid hyperplasia, urogenital tract infections, aortic mineralization and arteritis in the testes and pancreas were observed at 600 ppm in males, which were probably secondary to the chronic renal effects (Table 27).

Data on the incidence of neoplastic changes in rats after chronic administration of dithianon are provided in Table 28. The incidence of renal adenoma and carcinoma was increased in high-dose females when compared with the respective control group.

For female rats, an independent review (Grasso, 1991b) of all female kidney sections from the rat carcinogenicity/chronic toxicity study was conducted. This evaluation showed that the high concentration level (600 ppm) in the rat carcinogenicity study induced tissue necrosis (increased incidences of severe glomerulonephropathy [9/70 versus 0/70 in controls], glomerular sclerosis [21/70 versus 4/70 in controls] and fibrosis [23/70 versus 3/70 in controls]) (Table 29). This re-evaluation also revealed an increase in the incidence of chronic progressive nephropathy in 120 ppm and 600 ppm females.

Table 22. Mean body weight in rats after 72, 84 and 104 weeks of dithianon administration

Dietary concentration (ppm)	Mean body weight (g)					
	Males			Females		
	Week 72	Week 84	Week 104	Week 72	Week 84	Week 104
0	681.4	695.4	647.1	422.7	457.6	463.9
20	688.2	705.3	676.2	428.1	454.8	458.8
120	689.5	705.1	692.0	434.9	459.1	475.6
600	655.5	648.7	589.5	338.6	364.0	371.9

From Brown (1991)

Table 23. Statistically significant differences in body weight gain of rats after dithianon administration

Dietary concentration (ppm)	Sex	Mean body weight (g)				
		Weeks 0–12	Weeks 12–24	Weeks 24–36	Weeks 36–48	Weeks 48–60
120	Males	** +	—	—	—	—
600	Males	* +	—	—	—	—
120	Females	*	**	—	—	—
600	Females	***	***	***	***	**

From Brown (1991)

+ Increasing with dose; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ **Table 24. Changes in haematological parameters after chronic administration of dithianon to rats**

Parameter	Dietary concentration (ppm)							
	0		20		120		600	
	M	F	M	F	M	F	M	F
Week 52								
MCV (fl)	55.5	58.3	54.7	58.7	54.0*	58.6	51.9***	57.7
MCH (pg)	18.2	19.4	18.1	19.4	17.9	19.6	17.6	19.3
MCHC (g/dl)	32.8	33.3	33.1	33.1	33.1*	33.4	34.0***	33.4
RBC ($10^6/\text{cm}^3$)	8.24	7.28	8.11	7.29	8.17	7.10	8.23	6.96**
Hb (g/dl)	15.0	14.1	14.7	14.1	14.6	13.9	14.5	13.4***
PCV (%)	45.7	42.4	44.3	42.8	44.1*	41.6	42.6***	40.1***
Week 103								
MCV (fl)	58.6	63.1	57.3	60.8**	58.1	61.2*	57.3	60.5**
MCH (pg)	19.6	21.4	19.2	20.7	19.5	20.9	19.2	20.6
MCHC (g/dl)	33.4	33.8	33.5	34.0	33.6	34.2	33.5	34.1
RBC ($10^6/\text{cm}^3$)	7.63	7.10	7.58	7.13	7.68	7.02	7.00	7.01
Hb (g/dl)	14.9	15.1	14.5	14.7	15.0	14.7	13.3**	14.5
PCV (%)	44.7	44.6	43.3	44.3	44.5	42.9	39.7**	42.3

From Brown (1991)

F, female; Hb, haemoglobin; M, male; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cells; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 25. Changes in clinical chemistry parameters after chronic administration of dithianon to rats

Parameter	Dietary concentration (ppm)							
	0		20		120		600	
	M	F	M	F	M	F	M	F
Week 52								
GGT (IU/l)	3	3	3	2	6	4	7	3
BUN (mg/dl)	13	16	13	17	12	16	14**	20**
Cholesterol (mg/dl)	128	143	135	134	135	136	97**	90***
AP (IU/l)	110	56	118	52	147*	51	109	52
P (mg/dl)	4.7	3.5	4.9	3.9	5.0	3.7	5.0	4.1
Glucose (mg/dl)	76	109	95	118	101**	125	117***	110
CPK (IU/l)	652	279	542	360	360	222	270*	270
Week 103								
GGT (IU/l)	6	5	7	5	7	4	14**	8
BUN (mg/dl)	16	14	15	14	16	15	19**	19*
Cholesterol (mg/dl)	90	90	109	105	106*	91	82	75*
AP (IU/l)	89	48	105	41	131	51	101	67
P (mg/dl)	4.5	4.6	4.6	4.0**	4.7*	4.7	6.0***	4.9
Glucose (mg/dl)	72	74	91*	88*	89*	88*	105***	88*
CPK (IU/l)	520	569	563	491	240**	562	178**	327
Week 105								
T ₃ (ng/dl)	55.2	58.6	54.8	62.3	46.4	63.6	36.6**	49.1
T ₄ (g/dl)	2.2	1.6	2.0	1.6	2.5	1.8	2.1	1.4
TSH (ng/ml)	3.1	2.2	3.1	1.9	2.1	2.2	4.1	2.3

From Brown (1991)

AP, alkaline phosphatase; BUN, blood urea nitrogen; CPK, creatine phosphokinase; GGT, γ -glutamyl transferase; IU, international units; P, phosphorus; T₃, triiodothyronine; T₄, thyroxine; TSH, thyroid stimulating hormone; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

The severity of the chronic progressive nephropathy was also increased in a concentration-related manner at 120 and 600 ppm. Furthermore, there was evidence of a secondary, proliferative syndrome, which was limited to the females receiving 600 ppm. The proliferative findings consisted of a late manifestation of chronic tubular hyperplasia (atypical hyperplasia), preneoplastic lesions (proliferative tubules), benign tumours (tubular adenomas) and malignant tumours (adenocarcinoma) (Table 30).

The NOAEL for the late proliferative process in which the atypical hyperplasia progresses to proliferative tubules and in turn to tubular adenomas and adenocarcinomas in female rats was 120 ppm, compared with 600 ppm (highest dose tested) in male rats. Thus, for female rats, there was a steep dose-response relationship for the induction of neoplastic lesions. There were no other treatment-related tendencies observed in the type, distribution or incidence of other tumours.

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. The NOAEL for carcinogenicity was 120 ppm (equivalent to 6 mg/kg bw per day), based on increased incidences of preneoplastic lesions (proliferative tubules), benign tumours (tubular adenomas) and malignant tumours (adenocarcinomas) in females at 600 ppm (equivalent to 30 mg/kg bw per day).

Importantly, the proliferative process in female rats demonstrates a progression of hyperplastic and preneoplastic lesions to neoplasia following long-term exposure to the compound at an

Table 26. Variations in organ weights after chronic administration of dithianon to rats

	Dietary concentration (ppm)							
	0		20		120		600	
	M	F	M	F	M	F	M	F
Body weight (g)	621.9	453.0	605.0	455.0	671.4	415.3	545.2**	321.4***
Liver								
Absolute weight (g)	14.139	10.539	13.726	10.177	14.698	9.502	15.130	10.316
Relative (to body) weight (%)	2.2740	2.3459	2.2800	2.2458	2.2260	2.2927	2.8233**	3.2884**
Kidney (left)								
Absolute weight (g)	1.724	1.199	1.703	1.182	1.849	1.169	1.994	1.413
Relative (to body) weight (%)	0.2769	0.2679	0.2828	0.2672	0.2851	0.2880	0.3741***	0.4544***
Kidney (right)								
Absolute weight (g)	1.870	1.225	1.697	1.211	1.848	1.219	2.030	1.603
Relative (to body) weight (%)	0.3009	0.2737	0.2815	0.2737	0.2843	0.2981	0.3791***	0.5052***
Adrenal (left)								
Absolute weight (g)	0.037	0.049	0.039	0.039	0.082	0.035	0.041	0.037
Relative (to body) weight (%)	0.0060	0.0112	0.0064	0.0086	0.0109	0.0085	0.0079	0.0119
Adrenal (right)								
Absolute weight (g)	0.032	0.065	0.047	0.038	0.035	0.030	0.037	0.043
Relative (to body) weight (%)	0.0051	0.0147	0.0077	0.0084	0.0053	0.0072	0.0070	0.133
Brain								
Absolute weight (g)	2.252	2.016	2.213	2.009	2.231	1.999	2.247	1.984
Relative (to body) weight (%)	0.3625	0.4453	0.3670	0.4561	0.3441	0.4934	0.4234*	0.6301***
Testis (right)								
Absolute weight (g)	2.014	—	1.531	—	1.989	—	1.607	—
Relative (to body) weight (%)	0.325	—	0.2512	—	0.3135	—	0.2869	—
Testis (left)								
Absolute weight (g)	2.005	—	1.629	—	1.784	—	2.232	—
Relative (to body) weight (%)	0.3215	—	0.2673	—	0.2833	—	0.3933	—
Ovary (right)								
Absolute weight (g)	—	0.037	—	0.095	—	0.043	—	0.034
Relative (to body) weight (%)	—	0.082	—	0.0208	—	0.0103	—	0.0102
Ovary (left)								
Absolute weight (g)	—	0.070	—	0.066	—	0.037	—	0.106
Relative (to body) weight (%)	—	0.154	—	0.138	—	0.089	—	0.0335
Thyroid (left)								
Absolute weight (g)	0.022	0.016	0.021	0.016	0.017	0.015	0.025	0.014
Relative (to body) weight (%)	0.0035	0.0039	0.0030	0.0039	0.0028	0.0033	0.0041	0.0042
Thyroid (right)								
Absolute weight (g)	0.038	0.014	0.021	0.015	0.019	0.016	0.028	0.014
Relative (to body) weight (%)	0.0054	0.0032	0.0030	0.0034	0.0031	0.0034	0.0046	0.0045

From Brown (1991)

F, female; M, male; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 27. Non-neoplastic findings in rats after chronic administration of dithianon

	Dietary concentration (ppm)							
	0		20		120		600	
	M	F	M	F	M	F	M	F
Kidney and urinary tract								
Renal lesion as cause of demise	0/44	0/46	0/40	0/33	0/50	0/34	11/50	0/28
Urogenital tract lesion as cause of demise	2/44	1/46	0/40	0/33	0/50	1/34	6/50	0/28
Aorta								
Mineralization	1/70	0/69	0/39	0/33	0/50	0/34	6/70	0/70
Seminal vesicle								
Vesiculitis	3/68	—	2/43	—	2/48	—	11/69	—
Parathyroid								
Hyperplasia	0/66	0/69	0/65	0/66	0/64	0/64	8/65	0/64
Prostate								
Prostatitis	6/70	—	5/40	—	6/49	—	14/69	—
Testis								
Atrophy	16/70	—	13/47	—	6/54	—	23/70	—
Arteritis	2/70	—	1/47	—	3/54	—	17/70	—
Leydig cell hyperplasia	2/70	—	4/47	—	2/54	—	6/70	—
Pancreas								
Arteritis	2/69	0/67	0/39	0/33	0/50	1/32	8/69	1/69
Fatty atrophy	1/69	2/67	0/39	2/33	1/50	0/32	5/69	1/69
Kidney								
Glomerulonephropathy	19/70	18/70	25/68	25/68	23/70	33/70	25/69	40/69
Tubular nephrosis	0/70	0/70	0/68	0/68	0/70	3/70	31/69	29/69
Proliferative tubules	0/70	0/70	0/68	0/68	0/70	3/70	0/69	9/69
End-stage kidney	0/70	0/70	0/68	0/68	0/70	0/70	16/69	2/69
Cyst formation	2/70	3/70	1/68	0/68	1/70	0/70	11/69	3/69

From Brown (1991)

F, female; M, male

excessively toxic dose (600 ppm), which exceeded the MTD. Moreover, the histological morphology depicting the cell of origin (proximal tubular epithelial cell) is common to all of the proliferative lesions (i.e. atypical hyperplasia, proliferative tubules, tubular adenoma and adenocarcinoma). Moreover, it appears likely that a secondary mechanism involving repeated episodes of nephrotoxicity in female Sprague-Dawley rats eventually leads to the induction of kidney tumours following long-term (2-year) dietary exposure to dithianon at a concentration exceeding the MTD. The results from the two short-term mechanistic studies cited in [section 2.6](#) and the 90-day subchronic and 2-year carcinogenicity rat studies support the weight of evidence that the increased incidence of kidney tumours in female rats occurs at an excessively toxic dose and is a secondary proliferative response to this toxicity. It was considered that this mechanism is not relevant to humans at potential levels of exposure to dithianon.

The study complied with GLP requirements, and a QA statement was attached (Brown, 1991; Grasso, 1991b).

Table 28. Neoplasms (benign and malignant) that occurred in rats after chronic administration of dithianon

	Incidence of neoplasms							
	Dietary concentration (ppm)							
	0		20		120		600	
	M	F	M	F	M	F	M	F
Kidney								
B-adenoma	0/70	0/70	1/68	0/68	0/70	0/70	0/69	10/69
M-carcinoma	0/70	0/70	1/68	0/68	0/70	0/70	0/69	2/69
M-sarcoma	0/70	1/70	0/68	0/68	0/70	0/70	0/69	0/69
B-lipomatous tumour	0/70	0/70	2/68	0/68	0/70	0/70	1/69	0/69
N-metastatic tumour	0/70	2/70	1/68	1/68	1/70	0/70	2/69	0/69
Skin								
Squamous cyst	6/70	1/70	7/58	1/56	4/62	0/51	11/70	1/70
B-papilloma	5/0	1/70	10/58	2/56	3/62	0/51	13/70	0/70
Seminal vesicle								
N-metastatic tumour	0/68	—	0/43	—	0/48	—	1/69	—
Testis								
B-Leydig cell tumour	7/70	—	5/47	—	3/54	—	7/70	—
Prostate								
N-metastatic tumour	0/70	—	0/40	—	0/49	—	1/69	—
Parathyroid								
B-adenoma	1/66	1/69	0/65	0/66	1/64	0/64	1/65	0/64
Pancreas								
B-islet cell adenoma	6/69	1/67	2/39	1/33	3/50	2/32	1/69	2/69

From Brown (1991)

B, benign; F, female; M, male; malignant; N, lymph node

Another independent review of all kidney slides from the 2-year carcinogenicity study in rats was performed by Dr Gordon Hard, a pathology expert with special expertise in nephrotoxicity, to get a more detailed picture of the type of histopathological changes induced in the rat kidney after 2 years of dietary treatment. The aim of the review was to provide a more detailed histological description based on in-depth microscopic examination and to attempt to define a mode of action underlying the observed increase in tumour incidence occurring only in the high-dose female rats. Kidneys from all animals in the control, low-dose, mid-dose and high-dose female groups and control and high-dose male groups were examined. Kidneys from a major portion of the mid-dose males were also evaluated, as well as those from the two renal tumour-bearing low-dose males.

In the review of the histopathological kidney slides, findings in females were in accordance with the findings in the original study with regard to tumour incidence. A single adenoma was observed in 10 of 69 high-dose females. A renal tubule carcinoma was diagnosed in two females of the high-dose group, both of which also had an adenoma. In one animal, a small proliferative lesion was borderline between atypical tubule hyperplasia and incipient adenoma, and a single focus of atypical tubule hyperplasia was observed in a further two animals. The renal tubule tumours appeared to show a similar morphological pattern of attempted tubular differentiation within lobular clusters, suggesting a common mode of initiation and subsequent pathogenesis. Furthermore, foci of atypical tubule

Table 29. Incidence of kidney lesions in female rats after chronic administration of dithianon

	Incidence (number of lesions observed)			
	Dietary concentration (ppm)			
	0	20	120	600
<i>No. of tissues examined</i>	70	70	70	70
Atypical hyperplasia	0	0	4	10
Proliferative tubules	0	0	0	6
Adenoma	0	0	0	7
Adenocarcinoma	0	0	0	2
Transitional cell carcinoma	0	0	0	1
Eosinophilic inclusions	0	2	3	9
Glomerular sclerosis	4	6	5	21
Glomerular fibrosis	3	5	3	23
Glomerulonephropathy				
- Minimal	42	42	41	24
- Mild	16	10	15	20
- Moderate	7	9	7	10
- Severe	0	1	0	9

From Grasso (1991b)

Table 30. Incidence of proliferative lesions in the kidneys of female rats (re-evaluation of female kidney sections)

	Incidence (number of lesions observed)			
	Dietary concentration (ppm)			
	0	20	120	600
<i>No. of tissues examined</i>	70	70	70	70
Microscopic observations				
Basophilic tubules ^a	24	24	34	39
Atypical hyperplasia	0	0	4	10
Proliferative tubules	0	0	0	6
Adenoma	0	0	0	7
Adenocarcinoma	0	0	0	2

From Grasso (1991b)

^a Associated with chronic progressive nephropathy.

hyperplasia and adenomas of small size were located within the outer stripe of outer medulla, which conformed with the site of compound-related tubule change. Dithianon exposure in the high dose group females was associated with nephrosis localized to the proximal part of the straight segment or P3 of the proximal tubule, mainly in the outer part of the outer stripe of outer medulla and in the medullary rays. Changes consisted of scattered cells with enlarged nuclei (usually with hypertrophied nucleoli), most being 2–3 times larger than normal, but with a few that were very large and represented karyomegaly (4 times larger or more). Although the nuclear change was relatively frequent, it was not scattered diffusely through all tubules of the outer stripe of outer medulla; instead, several

enlarged nuclei were present together in specific tubules, but not in neighbouring P3 tubules. In addition, examination of the P3 tubules revealed the presence of occasional nuclear fragments within condensed hyaline cytoplasm in the P3 lumen, representing degenerate cells, and an occasional P3 cell undergoing mitosis. In comparison with the control females, chronic progressive nephropathy was exacerbated in the high-dose females, with a mode of severity at grade 3, but (in contrast to the high-dose males) with only one animal having an end-stage kidney. In the mid-dose females, similar renal damage was not observed. In accordance with the original study report, no renal tubule tumours were present in this group. Contrary to the re-evaluation of Grasso (1991b), no foci of atypical tubule hyperplasia were observed in the mid-dose females. In low-dose females, kidney slides were not different from those of the control animals.

In male rats, the major finding in the high-dose animals was exacerbated chronic progressive nephropathy. The direct treatment-related effect was limited to the outer stripe of outer medulla, as in the females. The majority of high-dose males had either patent (open) P3 tubules as a variation from normal or obviously dilated P3 tubules. In a few cases, there were narrow tracts of strongly dilated tubules (representing P3 tubules) with more flattened epithelial lining and sometimes modest thickening of the basement membrane running through the outer stripe of outer medulla. In addition, there was a scatter of cells with minimal to mild nuclear enlargement in the P3 tubules of most high-dose males. The extent of nuclear enlargement in male kidney was limited and not as prominent as in the high-dose females. Nuclei of the dimensions constituting karyomegaly were not seen. In rats with end-stage kidney, some of the tubules of the outer stripe of outer medulla were widely dilated or cystic. In mid-dose males, no treatment-related findings were noted. In low-dose males, two animals had a renal tubule tumour, one with an adenoma and one with a carcinoma. Both of these tumours had a distinctive appearance of amphophilic/vacuolar and lobular morphology (unlike the high-dose female tumours). This distinctive phenotype recently has been identified as a spontaneous neoplasm unrelated to chemical treatment. Therefore, these tumours had no relationship to those occurring in the high-dose female group.

Details of the distribution provided by the reviewer with regard to chronic progressive nephropathy are presented in [Table 31](#). Chronic progressive nephropathy was much more prominent in males than in females and was considered not to be treatment related.

The mode of action involving key histopathology events leading to the development of renal tubule tumours exclusively in the high-dose female rats receiving 600 ppm of dithianon in the diet for 2 years appeared to involve nuclear enlargement with chronic cell loss and sustained cell proliferation localized to the P3 segment of proximal tubule in the outer stripe of outer medulla. Both the observed compound-related nephrosis and the increased incidence of renal tumours were restricted to the high-dose females, and there was concordance between the site of chronic injury and the site of tumour development. This mode of action is compatible with a non-genotoxic pathway of renal tumour development, implying a threshold. The histological re-evaluation indicated that 120 ppm was a no-observed-effect level (NOEL) for lesions associated with tumour development, but 20 ppm was a NOAEL for exacerbation of chronic progressive nephropathy, a rat-specific disease process not considered by the reviewer to be an expression of nephrotoxicity (Hard, 2009).

2.4 Genotoxicity

Dithianon was evaluated for its potential genotoxicity in a battery of 11 genotoxicity studies, 5 of which were in vitro studies and 6 were in vivo studies ([Table 32](#)). Most of the studies complied with GLP, and QA statements were attached.

On the basis of these studies, dithianon is concluded not to have mutagenic or genotoxicity properties either in vitro or in vivo.

Table 31. Group distribution of chronic progressive nephropathy severity

Dietary concentration (ppm)	Total number of rats in group	Total number of rats graded ^a	Number of rats with chronic progressive nephropathy grade of severity						
			0	1	2	3	4	5	6
Females									
0	70	65	6	36	14	9	0	0	0
20	70	68	11	28	14	9	6	0	0
120	70	68	4	21	21	17	5	0	0
600	70	68	0	15	19	27	5	1	1
Males									
0	70	66	4	20	29	10	2	1	0
20	70	na	na	na	na	na	na	na	na
120	70	45	3	16	14	10	1	1	0
600	70	66	0	6	14	18	10	2	16

From Hard (2009)

na, not assessed for chronic progressive nephropathy

^a Excludes rats that could not be graded for chronic progressive nephropathy because of other intercurrent disease process or postmortem autolysis.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation reproduction study, dithianon technical (purity 91.6%; batch No. 15C/86) was administered to Crl:CD(SD)BR strain Sprague-Dawley rats (28 of each sex per group and 25 of each sex per group in the P and F₁ generations, respectively) at a dietary concentration of 0, 35, 200 or 600 ppm during the premating (100 days), mating, gestation and lactation periods. At the start of the treatment, animals were 6 weeks old and weighed 163–207 g (males) and 121–166 g (females). The dietary concentrations were selected based on the results of a 90-day feeding study in rats, which showed decreased overall body weight gains, increased kidney, liver and adrenal gland weights and changes in haematological and clinical chemistry parameters for males and females and histopathological findings in the kidney in females at 1080 ppm. The dams (P generation) were allowed to rear their offspring (F_{1a} generation) until weaning. The F₁ parental animals were selected from the F_{1a} offspring and treated for a 100-day premating period and then mated. The F₁ parental animals were also allowed to rear their offspring (F_{2a} generation) until weaning. The litters for the F_{1a} and F_{2a} generations were culled on postnatal day 4 to a maximum of eight pups per litter. Feed consumption of the P and F₁ parents was determined weekly up to the mating period. After mating, female feed consumption was determined on days 0, 7, 14 and 20 of gestation and on days 1, 4, 7 and 14 postpartum.

For P and F₁ generation males treated with 35, 200 or 600 ppm dithianon in their diets, mean compound intake at the time of mating (week 14) was estimated to be equal to 1.7, 9.0 and 27.6 mg/kg bw per day, respectively. For females of both generations, mean compound intake at the time of mating was comparable to that during gestation and was estimated to be equal to 2.1, 11.4 and 34.9 mg/kg bw per day, respectively. During lactation, compound consumption increased for females of both generations. On average, dithianon consumption during lactation for P and F₁ females treated with 35, 200 and 600 ppm was 4.5, 22.3 and 72.6 mg/kg bw per day, respectively (Table 33).

In general, body weights of P and F₁ parents were determined once weekly during the premating and mating periods. P/F₁ females were weighed on days 0, 7, 14 and 20 of gestation and on days

Table 32. Results of genotoxicity studies on dithianon

Test system	Assay/strain/species	Batch No., purity (%)	Result	Reference
Gene mutation in bacterial cells ^a	Ames mutagenicity test, <i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	15C/86, 92	Negative	Mueller (1986, 1993)
Gene mutation in mammalian cells	HGPRT mammalian cell mutation, V79 Chinese hamster cells	30/84, 94.7	Negative	Miltener (1984a,b, 1993)
Gene mutation in mammalian cells	HGPRT mammalian cell mutation, V79 Chinese hamster cells	15C/86, 90	Negative without S9; equivocal with S9 (positive responses only at cytotoxic doses that exceeded target cytotoxicity)	van de Waart (1993a)
Cytogenetic tests	Chromosomal aberration assay, V79 Chinese hamster cells	15C/86, 92	Positive at cytotoxic concentration	Heidemann (1988)
DNA damage and repair	Unscheduled DNA synthesis, Wistar CF HB rat hepatocytes	15C/86, 92	Negative	Timm (1986)
Cytogenetic tests	Micronucleus, EMD: NMRI(SPF) mice	162/83, purity not stated	Negative	Schulze Schencking & Unkelbach (1984)
Chromosomal damage (clastogenicity)	Micronucleus, Wistar rat	DT2008-3473, 95.5	Negative	Schulz, & Landsiedel (2009a)
Chromosomal aberration	Bone marrow cells of Wistar rat	15C/86, 91.6	Negative	Voelkner (1990, 1993)
DNA damage and repair	Comet assay, Wistar rat ^b	DT2008-3473, 95.5	Equivocal	Schulz & Landsiedel (2009b); Schulz & Mellert (2009a)
Potential for DNA binding	Covalent binding index, Sprague-Dawley rat ^c	903-1101, > 95	Negative	Sagelsdorff (2009)
Potential to induce DNA repair synthesis	Unscheduled DNA synthesis, Wistar rat	DT2008-3473, 95.5	Negative	Schulz & Mellert (2009b)

DNA, deoxyribonucleic acid; S9, 9000 × g rat liver supernatant

^a Because of cytotoxicity observed with several of the bacterial strains (some cultures with no viability at the highest doses tested), the experiment was repeated with lower dose levels.

^b In the present comet assay in kidney cells of male and female Wistar rats in vivo, single oral administration of dithianon did not lead to a clear and reproducible increase in the mean relative tail intensity at any dose level or exposure time. Increased levels of DNA damage observed only in single female rats are considered to be due to secondary effects induced by organ toxicity that is known to be expressed at more pronounced levels in the female than in the male rat with special patterns of cellular degeneration in the female rat kidney. Thus, under the experimental conditions of this study, dithianon is considered to be equivocal with respect to DNA damaging potential in the comet assay using kidney cells of male and female Wistar rats in vivo.

^c No DNA adduct formation could be detected in the liver or in the kidney of female rats after treatment with [ring-¹⁴C]- or [cyano-¹⁴C]-dithianon at a limit of detection of about 0.1 covalent binding index. This maximum possible DNA binding ability of dithianon is more than 5 orders of magnitude lower than the corresponding value for the strong genotoxic compound aflatoxin B₁ and more than 4000 times lower than the covalent binding index for the genotoxic compound 2-acetylaminofluorene. [³H]Acetylaminofluorene showed levels of DNA binding that were well within the expected range, thus confirming the sensitivity of the test system. Under the experimental conditions of this assay, dithianon was not considered to have DNA binding-mediated genotoxic potential.

1, 4, 7, 14 and 21 after birth. The F₁ and F₂ pups were weighed on the day of or the day after birth and on days 4, 7, 14 and 21 postpartum.

The state of health of parents and pups was checked at least once each day, and parental animals were examined for their mating and reproductive performances. Pups were sexed and evaluated as to health, and pup viability was recorded. All pups were examined macroscopically at necropsy. All P and F₁ parental animals were assessed by gross pathology (including testes and epididymides weight

Table 33. Mean dithianon intake for P and F₁ generation animals

Dietary concentration (ppm)	Mean dose (mg/kg bw per day)							
	Premating period (males)		Premating period (females)		Gestation period (females)		Lactation period (females)	
	Week 1	Week 14 ^a	Week 1	Week 14 ^a	Week 1	Week 3	Week 1	Week 3
P generation								
35	3.2	1.6	3.2	2.1	2.1	2.1	3.4	5.8
200	18.1	8.8	18.5	11.8	11.7	11.1	17.7	29.9
600	52.2	26.9	53.4	34.8	35.0	33.8	55.3	91.9
F₁ generation								
35	3.5	1.8	3.6	1.9	2.1	2.0	3.1	5.6
200	20.4	9.1	21.0	11.2	11.7	11.1	15.6	26.0
600	61.1	28.3	59.6	34.6	37.4	33.5	55.6	87.8

From Osterburg (1991)

^a Week 14 of the premating period was the last week prior to mating.

determination) and subjected to a histopathological examination of the organs of the reproductive system.

There were no treatment-related mortalities observed for either generation of parental animals. No treatment-related clinical signs were observed at any stage of the study. During the premating period, there were decreases in overall body weight gain for parental animals of the P generation (8.3% for males, 4.3% for females) and F₁ generation (4.3% for males, 3.9% for females) at 600 ppm, compared with the controls. The decreases in overall body weight gain were statistically significant for the P generation males. Feed consumption was slightly decreased during the premating period for males and females in the P and F₁ parental animals at 600 ppm. In the 600 ppm group, feed consumption for the P females was also reduced during the gestation period, compared with the controls (Tables 34–36).

There were no effects of treatment on mating performance or fertility indices for the P and F₁ parental animals. There were no treatment-related effects on gestational length, pup sex ratio, pup viability at birth and at weaning, and pup body weights for either generation. There were also no effects on physical or functional development of the offspring. At necropsy, no treatment-related macroscopic effects were observed in the parental animals or in the pups. There were no effects on the weight of the testes or the epididymides in the P or F₁ parental males; these were the only organs weighed. No treatment-related histopathological findings were observed in the reproductive organs of P and F₁ parental animals. Furthermore, no treatment-related malformations were observed in the F_{1a} or F_{2a} pups.

Based on these results, the NOAEL for parental toxicity was 200 ppm (approximately 9 mg/kg bw per day), based on reductions in body weight gain and feed consumption for P and F₁ parental animals at 600 ppm. The NOAEL for fertility and reproductive function was 600 ppm (equal to 27.6 mg/kg bw per day), the highest concentration tested.

The study complied with GLP requirements, and a QA statement was attached (Osterburg, 1991).

(b) Developmental toxicity

Rats

In a preliminary oral (gavage) embryotoxicity study, dithianon technical (purity 91.6%; batch No. 15C/86) was administered by intragastric intubation to groups of eight sexually mature and

Table 34. Mean body weight and feed consumption data for P males during the pre mating and mating periods^a

	Dietary concentration (ppm)			
	0	35	200	600
Number of rats dosed	28	28	28	28
Mean body weight (g)				
- Day 1	182.8	183.7	181.9	182.6
- Day 43	410.6	407.9	408.0	389.2*
- Day 99	518.6	521.9	518.3	490.3*
- Day 120	537.3	540.0	539.5	508.5*
- Days 1–99	335.8	338.2	336.4	307.4*
Mean feed consumption (g)				
- Days 1–3	18.0	18.0	17.7	18.1
- Days 8–10	24.6	24.2	24.1	22.8**
- Days 43–45	28.6	28.1	28.0	26.0**
- Days 99–101	26.8	26.1	26.0	24.8**

From Osterburg (1991)

Significantly different from the vehicle control values: * $P < 0.05$; ** $P < 0.01$ ^a Premating period: days 1–99 from the beginning of the study; mating period: days 100–120 from the beginning of the study.**Table 35. Mean body weight and feed consumption data for P females during the pre mating, gestation and lactation periods**

	Dietary concentration (ppm)			
	0	35	200	600
Premating period (days 1–99 from the beginning of the study)				
Number of rats dosed	28	28	28	28
Mean body weight (g)				
- Day 1	139.3	140.6	140.2	138.3
- Day 43	223.8	229.2	239.0**	219.2
- Day 99	259.7	266.8	281.0**	252.5
- Days 1–99	119.5	126.2	140.8**	114.3
Mean feed consumption (g)				
- Days 1–3	14.6	14.7	14.4	13.9
- Days 8–10	16.9	16.9	17.7	16.2
- Days 43–45	20.9	18.6	20.0	17.3
- Days 99–101	18.8	18.2	17.4	16.0**
Gestation period (gestation days 0–20)				
Number of rats dosed	27	27	26	26
Mean body weight change (g)	105.91	105.36	103.32	106.42
Mean feed consumption (g)	21.57	21.21	20.97	19.52**
Lactation period (lactation days 1–21)				
Number of rats dosed	27	27	26	26
Mean body weight change (g)	39.73	40.90	33.91	33.56
Mean feed consumption (g)	38.30	45.96*	41.43	38.45

From Osterburg (1991)

Significantly different from the vehicle control values: * $P < 0.05$; ** $P < 0.01$

Table 36. Mean body weight and feed consumption data for F_1 parental animals

	Dietary concentration (ppm)							
	0	35	200	600	0	35	200	600
	Males				Females			
Premating period (days 1–99 post-weaning)								
Number of rats dosed	25	25	25	25	24	25	25	25
Mean body weight (g)								
- Day 1	113.9	117.9	114.1	111.0	101.4	107.1	100.5	95.1
- Day 99	504.5	526.9	518.1	484.6	266.4	271.5	274.0	253.7
- Days 1–99	390.6	409.0	404.0	373.6	165.1	164.5	173.4	158.6
Mean feed consumption (g)								
- Days 1–3	17.4	18.3	17.2	16.9	16.3	15.4	15.4	13.4**
- Days 43–45	28.0	28.1	27.0	26.1	18.8	18.2	19.1	17.3
- Days 99–101	26.3	27.9	26.6	26.4	17.4	17.7	16.8	15.4**
Gestation period (gestation days 0–20)								
Number of rats dosed	—	—	—	—	22	22	23	25
Mean body weight change (g)	—	—	—	—	99.99	100.25	99.26	103.78
Mean feed consumption (g)	—	—	—	—	20.44	20.29	20.48	19.86
Lactation period (lactation days 1–21)								
Number of rats dosed	—	—	—	—	22	22	23	25
Mean body weight change (g)	—	—	—	—	35.57	39.89	27.75	40.75
Mean feed consumption (g)	—	—	—	—	Spilled			

From Osterburg (1991)

** Significantly different from the vehicle control values ($P < 0.01$)

mated female Sprague-Dawley Crl:CD(SD)BR strain rats at a dose of 20, 40, 70 or 100 mg/kg bw per day for 10 consecutive days from days 6 to 15 of gestation. A control group of eight rats received vehicle (1% carboxymethylcellulose in water). The dose volume was 10 ml/kg bw, adjusted daily on the basis of the individual body weights. The male rats were 14 weeks old when mating started, and the weight range was approximately 400–600 g. Female rats were approximately 8–12 weeks old and weighed between 170 and 254 g. All animals were examined at least once daily for signs of ill-health and behavioural changes; mortality checks were performed twice daily. Body weights were recorded on days 0, 6, 9, 12, 16 and 20 of gestation. On day 20 of pregnancy, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were removed and examined, and the following data were recorded: number of corpora lutea, number and position of implantations (live fetuses, early resorptions, late resorptions and dead fetuses), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations.

The results from this pilot range-finding study indicated maternal toxicity (markedly decreased [by approximately 85% of control] body weight gain during days 6–15) and developmental toxicity (slightly decreased [by approximately 14% of control] mean fetal weight) in the 100 mg/kg bw per day group (the highest dose tested). Maternal toxicity (moderately decreased [by approximately 40% of control] body weight gain during days 6–15) in the absence of developmental toxicity was observed at the next lower dose of 70 mg/kg bw per day.

Based on these results, the dose levels selected for the definitive developmental toxicity study in rats were 0, 20, 50, 70 and 100 mg/kg bw per day. The study was conducted in compliance with GLP, and a QA statement was attached (Mueller, 1989a, 1993b).

In a teratogenicity study, pregnant Sprague-Dawley Crl:CD(SD)BR strain rats (25–32 animals per group) received daily gavage doses of dithianon technical (purity 91.6%; batch No. 15C/86) at 0, 20, 50, 70 or 100 mg/kg bw per day during days 6–15 of gestation. The 0 and 70 mg/kg bw per day groups contained 32 rats, at the request of the study sponsor, in order to ensure that 20 pregnant animals were obtained in these groups. The 20 and 100 mg/kg bw per day groups contained 25 rats. The 50 mg/kg bw per day group (25 animals) was added after the initiation of the study, at the request of the sponsor, to provide an additional intermediate-dose group, and a second control group consisting of an additional 25 animals was added at the same time. The test material was administered as a suspension in 1% carboxymethylcellulose, at a dose volume of 10 ml/kg bw. The male rats were 14 weeks old when mating started, and the weight range was approximately 400–600 g. Female rats were approximately 8–12 weeks old and weighed between 170 and 254 g. Doses were selected for the definitive study based on results from the preliminary study described above (Mueller, 1989a). For the definitive study, all surviving animals were necropsied on day 20 of gestation, and the fetuses were removed and examined. All animals were examined at least once daily for signs of ill-health, toxicity and behavioural changes; mortality checks were performed twice per day. Body weights were recorded on days 0, 6, 9, 12, 16 and 20 of gestation. On day 20 of pregnancy, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were removed and examined, and the following data were recorded: number of corpora lutea, number and position of implantations (live fetuses, early resorptions, late resorptions and dead fetuses), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations and for skeletal or visceral abnormalities.

Treatment-related mortalities occurred during the gestation period in 1 of 32 and 5 of 25 animals at 70 and 100 mg/kg bw per day, respectively. Prior to their deaths, these animals appeared thin and pale and exhibited diarrhoea, lethargy and vaginal discharge. There were no abortions in any of the groups. Decreases in body weight gain (44%, 43% and 94%) and feed consumption (31%, 27% and 48%) were observed in the 50, 70 and 100 mg/kg bw per day groups, respectively, compared with the controls, during the dosing period. There were actual mean body weight losses observed at 70 mg/kg bw per day from days 6 to 9 of gestation (3 g) and at 100 mg/kg bw per day from days 6 to 12 of gestation (33 g). Necropsy of the maternal animals revealed a discoloration of the gastric wall in several animals given 70 and 100 mg/kg bw per day (Table 37).

Treatment with dithianon did not affect pregnancy status, as the pregnancy rates for initial control, 20, 70 and 100 mg/kg bw per day groups were 72%, 100%, 81% and 79%, respectively. Similarly, the pregnancy rates for the second control and 50 mg/kg bw per day groups were both 80%. The mean numbers of corpora lutea and total implantation sites for the treated groups were similar to those of their respective control groups, and there was no treatment-related increase in preimplantation loss. An increased number of resorptions (early plus late resorptions) was observed in the 70 and 100 mg/kg bw per day groups compared with the control group. A total of 12 resorptions (from 11 of 23 litters) was observed in the initial control group. The total number of resorptions for the 70 and 100 mg/kg bw per day groups was 67 (from 17 of 25 litters) and 97 (from 11 of 15 litters), respectively. The total number of resorptions was also increased, relative to the controls, at 50 mg/kg bw per day. A total of 72 resorptions (from 13 of 20 litters) was observed at 50 mg/kg bw per day, compared with a total of 19 resorptions (from 10 of 20 litters) in the second control group. For the 50, 70 and 100 mg/kg bw per day groups, the increases in total resorptions reflect an increase in the number of females with 100% resorptions. No litter in either control group had a 100% resorption rate. However, for the 50, 70 and 100 mg/kg bw per day groups, the incidence of litters with 100% resorptions was 6 of 20 litters (30%), 5 of 25 litters (20%) and 8 of 15 litters (53%), respectively, and the number of resorptions attributed to these litters was 60, 53 and 78 early resorptions, respectively. Because of the increase in total resorptions, the mean number of resorptions (defined as the total number of resorptions divided by the total number of litters per dose group) was increased in the 50, 70 and 100 mg/kg

Table 37. Summary toxicity data of dams treated with dithianon

Maternal findings	Dose (mg/kg bw per day)					
	0	20	70	100	0	50
No. of rats in the study	32	25	32	25	25	25
No. of non-pregnant rats	9	0	6	5	5	5
No. of rats died or killed moribund	0	0	1*	5 ^a	0	0
No. of dams with total litter loss	0	0	5	8	0	6
No. of rats with live fetuses at necropsy (day 20)	23	25	20	7	20	14
No. of thin animals	0	0	3	13	0	2
Mean feed consumption (g)						
- Days 6–16	23.29	22.56	16.93**	12.10**	24.09	16.60**
- Days 0–20	23.05	22.72	20.16**	16.35**	23.73	19.97**
Mean body weight gain (g)						
- Days 0–6	24.58	25.58	25.83	31.61	29.54	30.01
- Days 12–16	26.85	24.91	23.74	28.23	25.96	21.90
- Days 16–20	49.87	47.03	50.09	58.09	44.92	48.14
- Days 6–16	51.09	50.01	29.26**	3.14**	49.25	27.53**
- Days 0–20	125.54	122.62	105.18**	93.50*	123.71	105.68*
Necropsy data						
Stomach – lumen, material	0	0	0	3	0	0
Gastric wall – greyish layer	0	0	4	5	0	0
100% intrauterine deaths	0	0	5	8	0	6

From Mueller (1991)

* $P < 0.05$; ** $P < 0.01$ ^a Pregnancy status not determined for the rat of the 70 mg/kg bw per day group and for one of the dead rats of the 100 mg/kg bw per day group.

bw per day groups compared with controls. The mean numbers of resorptions for the initial control, 70 and 100 mg/kg bw per day groups were 0.52 (12 resorptions divided by 23 litters), 2.68 (67 resorptions divided by 25 litters) and 6.47 (97 resorptions divided by 15 litters), respectively. Similarly, the mean numbers of resorptions for the second control and 50 mg/kg bw per day groups were 0.95 (19 resorptions divided by 20 litters) and 3.60 (72 resorptions divided by 20 litters), respectively. Therefore, developmental toxicity, with a subsequent reduction in the mean number of live fetuses, was indicated at dose levels of 50 mg/kg bw per day and above. The mean number of live fetuses at 70 and 100 mg/kg bw per day was 9.4 and 5.6, respectively, versus 12.1 in the initial control group. Similarly, the mean number of live fetuses at 50 mg/kg bw per day was 7.5 versus 10.7 in the second control group. The total number of resorptions (10 resorptions from 6 of 25 litters), the mean number of resorptions (0.40, or 10 resorptions divided by 25 litters) and the mean number of live fetuses (11.3) for the 20 mg/kg bw per day group were comparable to controls, and no developmental toxicity was indicated at the lowest dose tested. Mean fetal weight was statistically significantly lower at 100 mg/kg bw per day (2.9 g), compared with the initial controls (3.3 g). Mean fetal weights for the 20 and 70 mg/kg bw per day groups were 3.3 g and 3.2 g, respectively, and were not different from those of the initial controls. Similarly, mean fetal weights for the 50 mg/kg bw per day group and the second control group were both 3.5 g. There were no differences in the type or incidences of fetal external, visceral or skeletal malformations or variations in any of the treatment groups (Table 38).

In summary, the NOAEL for both maternal and developmental toxicity for dithianon (technical) in rats was 20 mg/kg bw per day, based on decreases in body weight gain and feed consumption

Table 38. Summary of caesarean section and litter data

	Dose (mg/kg bw per day)					
	0	20	70	100	0	50
No. of surviving pregnant rats intubated	23	25	25	15	20	20
No. of dams with total litter loss	0	0	5	8	0	6
No. of dams with live fetuses at necropsy (day 20)	23	25	20	7	20	14
Mean no. of corpora lutea per litter	15.4	14.7	14.4	17.1	13.9	14.4
Mean no. of implantations per litter	12.6	11.7	12.4	14.7	11.7	11.5
% preimplantation loss per litter	18.7	19.0	14.1	13.0	15.8	19.9
No. of early resorptions per litter – fetal incidence						
- Animals with live fetuses at necropsy	0.5	0.4	0.7	2.7	1.0	0.9
- All animals	0.5	0.4	2.6	6.5	1.0	3.6
No. of late resorptions per litter – fetal incidence						
- Animals with live fetuses at necropsy	0	0	0.1	0	0.1	0
- All animals	0	0	0	0	0.1	0
No. of dead fetuses	0	0	0	0	0	0
No. of live fetuses per litter (litter size)						
- Animals with live fetuses at necropsy	12.1	11.3	11.7	12.0	10.7	10.6
- All animals	12.1	11.3	9.4	5.6	10.7	7.5
% postimplantation loss per litter						
- Animals with live fetuses at necropsy	3.7	2.9	5.8	16.5	8.2	6.6
- All animals	3.7	2.9	24.6*	61.0*	8.2	34.6
Mean proportion of live male fetuses	50.3	47.1	49.6	47.0	51.4	52.9
Live fetal body weight (g)	3.3	3.4	3.2	2.9*	3.5	3.5

From Mueller (1991)

* $P < 0.05$

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. Because developmental toxicity was observed only at dose levels that were maternally toxic, dithianon is not selectively toxic to the fetus. Dithianon is not teratogenic in Sprague-Dawley rats.

The study was conducted in compliance with GLP, and a QA statement was attached (Mueller, 1991).

Rabbits

In a preliminary oral (gavage) embryotoxicity study, dithianon technical (purity 91.6%; batch No. 15C/86) was administered to groups of eight sexually mature and mated female New Zealand White rabbits at a dose level of 10, 20 or 40 mg/kg bw per day for 13 consecutive days from day 6 to day 18 of gestation. A control group of eight rabbits received vehicle (1% carboxymethylcellulose in water). The dose volume was 10 ml/kg bw, adjusted daily on the basis of the individual body weights. The male rabbits were at least 30 weeks old when mating started, and the weight range was approximately 5–5.5 kg. The sexually mature female rabbits were approximately 14–17 weeks old and weighed between 3.1 and 4 kg. All animals were examined at least once daily for signs of ill-health and behavioural changes; daily mortality checks were performed twice per day. Body weights were recorded on days 0, 6, 9, 12, 15, 19, 24 and 28 of gestation. On day 28 of pregnancy, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were

removed and examined, and the following data were recorded: number of corpora lutea, number and position of implantations (live fetuses, early resorptions, late resorptions and dead fetuses), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations.

The results of this range-finding study indicated moderate maternal toxicity (i.e. decreased body weight gain with a body weight loss between days 6 and 9 and abortions in two of nine rabbits) and developmental toxicity (i.e. increased post-implantation loss resulting from an increased number of early resorptions) at a dose of 40 mg/kg bw per day. The range-finding study also showed that an intermediate dose of 20 mg/kg bw per day did not produce any adverse maternal or developmental effects.

Based on the results indicated above, the doses selected for the definitive developmental toxicity study in the rabbit were 0, 10, 25 and 40 mg/kg bw per day. The study was conducted in compliance with GLP, and a QA statement was attached (Mueller, 1989b, 1993a).

On the basis of the doses selected from the range-finding study, four groups of pregnant New Zealand White rabbits (20 per group) were used in a definitive developmental toxicity study. Dithianon technical (purity 91.6%; batch No. 15C/86) was administered by oral gavage at doses of 0, 10, 25 or 40 mg/kg bw per day during days 6–18 of gestation. The test material was administered once per day as a suspension in aqueous carboxymethylcellulose (1%) at a dose volume of 10 ml/kg bw. The male rabbits were healthy and sexually mature. The sexually mature female rabbits were approximately 14–17 weeks old and weighed between 2.8 and 4.4 kg. All animals were examined at least once daily for signs of ill-health and behavioural changes; daily mortality checks were performed twice per day. Body weights were recorded on days 0, 6, 9, 12, 15, 19, 24 and 28 of gestation. On day 28 of pregnancy, the animals were sacrificed and examined macroscopically for pathological changes. The ovaries and uteri were removed and examined, and the following data were recorded: number of corpora lutea, number and position of implantations (live fetuses, early resorptions, late resorptions and dead fetuses), individual fetal weights and sex of the fetuses. All fetuses were examined for external malformations and for skeletal or visceral abnormalities.

No treatment-related mortalities were reported. Three abortions were noted at 40 mg/kg bw per day, compared with no abortions at 25 mg/kg bw per day, one abortion in the 10 mg/kg bw per day group and one abortion in the control group. The abortions in the 40 mg/kg bw per day group were considered to be treatment related, whereas the abortion in the 10 mg/kg bw per day group was considered incidental because the incidences were comparable in the 10 mg/kg bw per day and control groups.

Maternal toxicity, expressed as dose-dependent decreases in body weight gain and feed consumption compared with controls, was observed for the 25 and 40 mg/kg bw per day groups. In the 40 mg/kg bw per day group, between gestation days 6 and 19, body weight gain was decreased by 58% and feed consumption was decreased by 38% compared with controls, with an actual 108 g loss in body weight from days 6 to 19. In the 25 mg/kg bw per day group, a 48% decrease in body weight gain and a 29% decrease in feed consumption were noted, compared with controls, during gestation days 6–19. In the 10 mg/kg bw per day group, there was a slight decrease in feed consumption (4% compared with controls) during the treatment period, but this was not considered adverse because it did not result in a decrease in body weight gain in this group (Table 39). No treatment-related macroscopic effects were observed at necropsy in the maternal animals.

Developmental toxicity was observed in the 40 mg/kg bw per day group and consisted of a statistically significant increase in the incidence of postimplantation loss (41.5% versus 7.7% in the controls) due to an increase in the number of early resorptions (31 from 10 litters versus 8 from 4 litters in the controls). This resulted in a statistically significant reduction in the mean number of fetuses per doe in the 40 mg/kg bw per day group (3.7 versus 6.9 in the controls). There were no treatment-related effects on preimplantation or postimplantation losses in the other groups. There

Table 39. Maternal parameters measured in a rabbit developmental study with dithianon

Maternal findings	Dose (mg/kg bw per day)			
	0	10	25	40
No. of females in the study	20	20	20	20
No. of non-pregnant females	4	1 ^a	0	1
No. of pregnant females that died	0	4	3	4
No. of females that aborted and were therefore killed	1	1	0	3
No. of females with 100% intrauterine deaths	1	0	0	2
No. of females with live fetuses at necropsy	14	15	17	10
Mean feed consumption (g/day per animal)				
- Days 6–19	211.45	190.58	150.79	131.88
- Days 0–28	206.56	186.21	163.50	167.51
Mean body weight gain (g)				
- Days 0–6	303.39	265.34	234.14	282.46
- Days 6–19	366.85	350.92	191.31	153.87
- Days 19–24	142.32	64.77	154.11	135.98
- Days 24–28	138.59	53.30	102.56	95.88
- Days 0–28	968.14	787.40	675.65	675.71

From Mueller (1990)

^a This animal was also found dead during the study.

were no effects of treatment on fetal weight or on the incidence of malformations or variations at any of the doses tested.

In summary, the NOAEL for maternal toxicity was 10 mg/kg bw per day, based on the reductions in body weight gain and feed 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 postimplantation 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. Thus, dithianon (technical) is not teratogenic in New Zealand White rabbits.

The study was conducted in compliance with GLP, and a QA statement was attached (Mueller, 1990).

2.6 Special studies

(a) Subacute neurotoxicity

Rats

Dithianon technical (purity 95.5%; batch No. AC12395-26) was administered to groups of 10 male and 10 female Wistar CrI Glx BrI Han:WI strain rats by gavage for 4 weeks at a dose of 0 (Group 0, vehicle control), 15 (Group 1), 30 (Group 2) or 60 (Group 3) mg/kg bw per day. Feed and water consumption were determined once a week. Body weight was determined once a week and on the days when functional observational batteries (FOBs) were performed. The animals were examined for clinical signs of toxicity or mortality at least once a day. The ages of the animals were 32–36 days (males) and 31–35 days (females). Only animals free from clinical signs of disease were used for the study. Detailed clinical examinations in an open field were conducted prior to the start of

the administration period and weekly thereafter, except for the weeks when FOBs were carried out. FOBs (including auditory startle response test and grip strength tests of forelimbs and hindlimbs) and motor activity measurements were performed on days –1 and 27. At the end of the study, the first five animals of each sex per dose were sacrificed by perfusion fixation and subsequently examined neuropathologically (brain, spinal cord, optic nerve, peripheral nervous system). The remaining animals were sacrificed without further examination.

During week 1, feed consumption was statistically significantly reduced only for animals at 60 mg/kg bw per day (highest dose tested), specifically by 16.9% for males and by 13.9% for females, compared with controls. This treatment-related effect resulted in a statistically significantly decreased body weight change at 60 mg/kg bw per day for the 4-week treatment period, specifically by 25.3% for males and by 17.5% for females. No treatment-related effect on water consumption was observed. No clinical signs of neurotoxicity were observed in treated animals, up to 60 mg/kg bw per day (the highest dose tested), for the following sets of detailed measurements: daily observations, weekly clinical examinations, FOB (including home cage observations, open-field observations and sensorimotor tests/reflexes) and motor activity assessment. Other non-neurotoxic, treatment-related clinical observations (including open-field observations) were noted, indicative of general systemic toxicity, for males and females at both 30 and 60 mg/kg bw per day. Specifically, slight to severe urine-smearing anogenital region was seen in over half of the males and females at 60 mg/kg bw per day during weeks 3 and 4, whereas this observation was noted in only a few rats at 30 mg/kg bw per day, and only with slight severity. In addition, piloerection was observed sporadically in half of the males and females at 60 mg/kg bw per day. Also, several male animals at 60 mg/kg bw per day showed slight to moderate salivation predominately before treatment for several days of the study. Because this finding was primarily observed before administration of the test compound, it was assessed as a conditioning reflex rather than an adverse or toxic effect. In addition, dark discoloured urine was observed in over half of the males and females at 60 mg/kg bw per day, whereas it was observed in only a few male and female rats at 30 mg/kg bw per day. Importantly, this observation is probably related to the physical property (solid brown colour) of the test article. Therefore, this finding was considered to be substance related but not adverse.

For the FOB parameters, only rearing (an open-field observation) was statistically significantly reduced in males at 60 mg/kg bw per day on day 27 (–79.6%), which was considered to be substance related. Importantly, this reduction in rearing was considered to be related to general systemic toxicity rather than neurotoxicity. Other open-field observations (including abnormal movements and impairment of gait), home cage observations (including abnormal movements and impairment of gait) and sensorimotor tests/reflexes (including coordination of movements such as righting response) in the FOB demonstrated no test substance-related findings up to 60 mg/kg bw per day (the highest dose tested). There were no neuropathological changes and no specific clinical signs of neurotoxicity observed up to 60 mg/kg bw per day (the highest dose tested). For motor activity measurements, a statistically significantly reduced value was obtained only in male animals at 60 mg/kg bw per day on day 27, including the earliest 5–6 intervals considered to assess exploration behaviour. Importantly, this reduction in motor activity was considered to be related to general systemic toxicity rather than neurotoxicity. As mentioned above, no treatment-related effects were found in the neuropathological examinations, including organ weights, gross lesions and detailed neuropathology of perfusion-fixed tissues (including brain, spinal cord, optic nerve and peripheral nervous system).

Based on these results (including clinical observations of smeared anogenital region with urine and dark discoloured urine at 30 mg/kg bw per day), the NOAEL for general systemic toxicity was 15 mg/kg bw per day. For neurotoxicity, the NOAEL was 60 mg/kg bw per day (the highest dose tested) for both sexes.

The study was conducted in compliance with GLP, and a QA statement was attached (Kaspers, Kaufmann & Ravenzwaay, 2003).

(b) *Short-term study (7 days) on nephrotoxicity*

Rats

In a short-term study, groups of 15 male (approximate weight 75 g) and 15 female (approximate weight 125 g) Crl:CD(SD)BR strain Sprague-Dawley rats received dithianon technical (purity 90%; batch No. 15C/86) in the diet at a concentration of 0, 120, 600 or 1080 ppm (equivalent to 0, 12, 60 and 108 mg/kg bw per day, respectively, in both sexes). After exposure for 2, 4 or 7 days, five animals of each sex per group were killed. The objectives of the study were to 1) define the nephrotoxic potential of dithianon in Sprague-Dawley rats; 2) establish a no-effect level for renal damage; and 3) investigate further the sex difference in the renal toxicity of dithianon.

The kidneys were examined macroscopically, microscopically and at the ultrastructural level. Exposure to dithianon at levels up to 1080 ppm did not result in any adverse clinical signs. Body weight gain was decreased for males and females at 1080 ppm at each time interval during the study. The decreases in body weight gain for males and females, respectively, were 61% and 31% at 2 days, 43% and 43% at 4 days and 25% and 45% at 7 days, compared with controls. A transient, slight reduction in feed consumption was noted in animals receiving 1080 ppm. There was also an increased incidence of pale kidneys at necropsy in the 600 and 1080 ppm treatment groups.

Histopathological evaluation of the kidneys revealed hydropic degeneration of the proximal tubular epithelial cells in both males and females receiving 600 or 1080 ppm at 4 days and 7 days. The incidence and severity of the lesions were increased in females compared with the corresponding males. At day 7, there was evidence of active, tubular epithelial cell degeneration. In females, the newly regenerated tubular epithelial cells showed signs of hydropic degeneration, demonstrating the continued susceptibility of these cells to damage. In contrast, males showed no evidence of further degeneration in any regenerative tubular epithelial cells, suggesting that adaptation to the toxic effects had occurred at day 7. Electron microscopy of sections from animals at 600 and 1080 ppm demonstrated that the mitochondria in the proximal tubular cells were the target for the toxic effects of dithianon. In females fed 1080 ppm, further change consisting of dilatation of the endoplasmic reticulum was observed. The ultrastructural changes were consistent with the findings observed histopathologically as hydropic degeneration. Although slight focal changes were seen ultrastructurally at 120 ppm in female rats, these changes were described as having only minimal severity, both quantitatively and qualitatively, and occurred only at 2 days. Therefore, these minimal focal changes at 120 ppm were considered to be unrelated to treatment (Table 40).

In conclusion, the NOAEL for light and electron microscopic changes in the kidney was 120 ppm (equivalent to 12 mg/kg bw per day) in females for short-term exposure of dithianon technical for up to 7 days.

The study was conducted in compliance with GLP, and a QA statement was attached (Price, 1991).

(c) *Short-term study (28 days) on renal cell turnover*

Rats

Dithianon technical (purity 90%; batch No. 15C/86) was administered to female Crl:CD(SD)BR strain Sprague-Dawley rats (approximate weight 125 g) (five per group per time point) at a dose of 20, 120 or 600 ppm (equivalent to 2, 12 and 60 mg/kg bw per day) for 7, 14 and 28 days in order to measure effects on renal cell turnover and to correlate any findings with light microscopic observations. Continuous labelling of deoxyribonucleic acid (DNA) was achieved with Alzet osmotic pumps containing 5-bromo-2'-deoxyuridine implanted subcutaneously in anaesthetized rats 7 days prior to necropsy.

At each of the time points (7, 14 and 28 days), all designated rats were sacrificed. Animals were examined for signs of macroscopic external and internal abnormalities; body and individual

Table 40. Incidence of hydropic degeneration and multiple foci of basophilic tubules in male and female rats fed dithianon for 2, 4 and 7 days

	Exposure time (days)											
	2				4				7			
	Dietary concentration (ppm)											
	0	120	600	1080	0	120	600	1080	0	120	600	1080
Males												
No. of animals examined	5	5	5	5	5	5	5	5	5	5	5	5
No abnormalities	4	2	4	4	5	5	3	0	4	4	1	2
Tubular dilatation	0	2	1	1	0	0	0	0	0	0	0	0
Hydropic degeneration	0	0	0	0	0	0	1	3	0	0	2	1
Interstitial cell infiltration	1	1	0	0	0	0	0	0	0	0	0	0
Basophilic tubules												
- Few	0	2	0	0	0	0	1	2	1	1	1	1
- Several	0	0	0	0	0	0	0	0	0	0	1	2
Females												
No. of animals examined	5	5	5	5	5	5	5	5	5	5	5	5
No abnormalities	3	3	3	3	3	4	2	1	4	5	1	0
Tubular dilatation	1	1	0	0	0	0	0	0	0	0	0	0
Hydropic degeneration	0	0	0	0	0	0	3	4	0	0	4	4
Interstitial cell infiltration	0	0	2	1	0	1	0	0	0	0	0	0
Basophilic tubules												
- Few	2	0	1	2	2	1	1	0	1	0	0	0
- Several	0	0	0	0	0	0	0	0	0	0	1	4

From Price (1991)

kidney weights were recorded. Sections of both kidneys were fixed in 10% neutral buffered formalin for histological examination after staining with haematoxylin and eosin. Sections of the cortex of the right kidney were fixed in 4% glutaraldehyde buffered with sodium cacodylate at 0.1 mol/l, pH 7.4, for ultrastructural examination. Sections of kidney (4 µm) were cut, dewaxed and incubated with antibodies and stained with diaminobenzidine. The number of dark-staining nuclei was counted and compared with control values.

Hydropic degeneration was observed in proximal tubular epithelial cells after dietary exposure to 600 ppm dithianon for 7, 14 and 28 days. This lesion was accompanied by islands of basophilic tubules, indicative of active regeneration. Continuous labelling techniques using 5-bromo-2'-deoxyuridine showed an increase in tubular cell turnover rate at 600 ppm, consistent with the histopathological results. No increase in tubular cell turnover rate was observed at 20 or 120 ppm.

It was concluded that persistent cellular damage to proximal tubular epithelial cells triggered a regenerative response of basophilic tubules, which is the basis of the indirect mechanism in the development of proliferative lesions (including tumours) following longer-term (2-year) dietary exposure of female rats to 600 ppm. In this study, the NOAEL for the repeated cellular degenerative/regenerative responses was 120 ppm (approximately 12 mg/kg bw per day), according to histopathological and DNA labelling criteria. Neither the degenerative/regenerative (cell turnover) responses nor renal tumours (in the carcinogenicity study) were observed at concentrations of 20 and 120 ppm.

Although atypical hyperplasia was observed at 120 ppm in 4 of 70 female rats following 104 weeks of treatment, progression to neoplasia did not occur at this dose.

The study was conducted in compliance with GLP, and a QA statement was attached (Price, 1993).

(d) S-phase response study (7 days and 28 days)

Rats

The aim of these studies was to determine whether dithianon induced a cell proliferation response in the kidneys of rats after oral administration over periods of 7 and 28 days. Dithianon (purity 95.5%; batch No. DT2008-3473) was administered to groups of eight male and eight female CrI:CD (SD) strain Sprague-Dawley rats (61–63 days old) at a dietary concentration of 0, 120 or 600 ppm (equal to 0, 6.5 and 32.6 mg/kg bw per day for males and 0, 8 and 40.6 mg/kg bw per day for females) over a period of 7 days. In another study, similar dietary concentrations (equal to 0, 6.8 and 33.5 mg/kg bw per day for males and 0, 8.2 and 43.5 mg/kg bw per day for females) were administered over a period of 28 days. Feed consumption and body weights were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. One week prior to necropsy, osmotic minipumps containing 5-bromo-2'-deoxyuridine were implanted subcutaneously. Cell proliferation (S-phase response) and apoptosis (TUNEL)¹ were evaluated in the kidneys.

Irrespective of the duration of administration of dithianon (7 days or 28 days), dietary concentrations of 120 and 600 ppm in male Sprague-Dawley rats caused a marginal exacerbation of basophilic tubules, cell proliferation and secondary apoptosis without overt cytotoxicity of the kidney parenchyma, representing a first-grade chronic progressive nephropathy. Chronic progressive nephropathy is a rat-specific, spontaneously occurring kidney alteration.

In female Sprague-Dawley rats given similar dietary concentrations, the 7-day study showed specific degenerative lesions in the kidney parenchyma (outer stripe of outer medulla and cortex) as well as a significant increase of cell proliferation in high-dose animals. Administration of the high dose to female rats for 28 days affected body weight. Moreover, specific degenerative lesions in the kidney parenchyma (outer stripe of outer medulla and cortex) as well as a significant increase of cell proliferation were observed. Additionally, the high dose significantly increased apoptosis in the kidney cortex.

The studies were conducted in compliance with GLP, and QA statements were attached (Kaspers et al., 2009a,b,c,d).

2.7 Studies on metabolites

No known toxic metabolites were found in either animal or plant studies.

3. Observations in humans

Personnel handling dithianon in manufacturing, research and product formulation underwent regular medical examinations. This surveillance programme was not aimed to specifically identify

¹ TUNEL, or terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP) nick end labelling, is a common method for detecting DNA fragmentation that results from apoptotic signalling cascades. The assay relies on the presence of nicks in the DNA that can be identified by terminal deoxynucleotidyl transferase, an enzyme that will catalyse the addition of dUTPs that are secondarily labelled with a marker. It may also label cells that have suffered severe DNA damage (Gavrieli, Sherman & Ben Sasson, 1992).

dithianon-related symptoms or diseases. The only effects reported have been skin-related effects, such as erythema, swelling and itching. The information available does not allow a firm conclusion as to whether all these skin reactions should be interpreted as primary irritation or whether some may be true allergic reactions.

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. Epicutaneous patch testing has demonstrated that sensitization to products containing dithianon may occur. However, it is unclear whether pure dithianon is a potential sensitizer or whether the allergic reactions are due to excipients, impurities or decomposition products.

No observations regarding health effects after exposure of the general public are known (Holmsen, 2009).

4. Literature review

A literature review was performed to find an explanation for the observed sex- and species-specific induction of kidney tumours (seen only in female rats after long-term treatment at high doses, not in male rats and not in male or female mice). No straightforward explanation for the sex- and organ-specific occurrence of dithianon-induced tumours in the female rat kidney was found. Moreover, no other chemical was found that exclusively induces kidney tumours in female rats. An expert reviewer has discussed possible reasons for the sex-specific occurrence of dithianon-induced tumours in the female rat kidney. The most promising hypothesis explaining the sex-specific induction of kidney tumours was associated with the involvement of cyclooxygenase-2 (COX-2). Prostaglandin H-synthase can oxidize lipophilic xenobiotics if they have a sufficiently low redox potential, which is the case for many phenolic compounds and aromatic amines. This is assumed to be possible for dithianon or dithianon metabolites, which undergo oxidative metabolism. COX-2 is present in the proximal straight tubules of female but not male F344/NSlc rats (immunohistochemically and morphometrically determined, but no activities were measured; the rats were 3 months old) (Ichii et al., 2008). As the proximal straight tubules appear to be a site of origin of the sex-specific dithianon-induced kidney tumours of the female rats, this difference in the occurrence of COX-2 may be significant. The metabolic formation of the many terminal sulfonic acid derivatives observed in the metabolism studies (M216F005, M216F006, M216F007, M216F009, M216F011, M216F013, M216F014, M216F016, M216F018, M216F025) may occur via reactive intermediates (sulfenic and sulfinic acid derivatives). If COX-2 is a significant contributor to this metabolic route of dithianon, the occurrence of COX-2 in the proximal straight tubules exclusively in female compared with male rat kidney may be related to the sex-specific occurrence of kidney tumours in the female rat (Oesch, 2009). However, this hypothesis for the involvement of COX-2 in kidney tumour formation in female rats cannot be correct, as the kidney tumour has been reported in Sprague-Dawley rats and not in F344/NSlc rats. Further evidence from experimental results for the involvement of COX-2 in kidney tumour formation is not available at the present time.

Comments

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 hours. There were no substantial dose- or sex-related differences in the absorption, elimination or distribution of radioactivity following oral administration of [¹⁴C]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 hours. 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 (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 an 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 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 males and 0, 0.67, 3.0 and 12.6 mg/kg bw per day in females). 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 males and 0, 1.6, 7.9 and 37.5 mg/kg bw per day in females, respectively. 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 feed 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 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 males and females.

These findings were further substantiated by a 28-day dietary renal turnover study in female rats. Continuous labelling techniques using 5-bromo-2'-deoxyuridine 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 a concentration 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 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 9.0 mg/kg bw per day), based on reductions in feed 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 toxicity 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 feed 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 feed 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 postimplantation 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.

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 ^a	Toxicity	180 ppm, equal to 14.6 mg/kg bw per day	1080 ppm, equal to 86.7 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 toxicity	600 ppm, equal to 27.6 mg/kg bw per day ^b	—
		Parental toxicity	200 ppm, equal to 9.0 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, eye irritation	Severe irritant
Guinea-pig, dermal sensitization	Sensitizer (Magnusson & 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	Not relevant
Lowest relevant inhalation NOAEC	Not relevant

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 postimplantation loss in the presence of maternal toxicity; not teratogenic

Lowest relevant developmental NOAEL	25 mg/kg bw per day in rabbits
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Neurotoxicity/delayed neurotoxicity

Not neurotoxic

Other toxicological studies

Mechanistic studies on kidney toxicity
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Medical data

Local skin and eye irritation effects in exposed plant workers and operators
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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

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