ISOXAFLUTOLE

First draft prepared by
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Explanation

Isoxaflutole is the International Organization for Standardization–approved name for 5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service No. 141112-29-0. The company code for isoxaflutole is RPA 201772. Isoxaflutole is an isoxazole herbicide that is used as a pre-emergent or early post-emergence broadcast treatment for the control of broadleaf and grass weeds. Its primary target in plants is the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD); inhibition of the enzyme results in the bleaching of weeds due to the blockage of phenylquinone biosynthesis.

Isoxaflutole has not previously been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

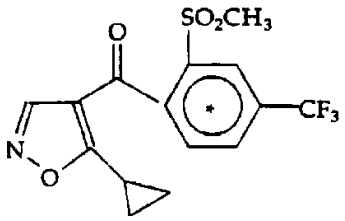
All critical studies contained statements of compliance with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

Absorption, distribution, metabolism and excretion of isoxaflutole following a single gavage low dose, high dose and repeated dosing (14 days) have been studied in rats. Fig. 1 shows the radiolabelling position of isoxaflutole used in the absorption, distribution, metabolism and excretion studies in rats.

Fig. 1. [^{14}C]Isoxaflutole (RPA 201772)



* denotes the position of the uniformly labelled phenyl ring

1.1 Absorption, distribution and excretion

In an absorption, distribution, excretion and metabolism study, [^{14}C]isoxaflutole (purity 98.7%) was administered to groups of male and female Sprague-Dawley (CD) rats (five of each sex per dose) by gavage at a single low oral dose (1 mg/kg body weight [bw]), repeated low oral dose (1 mg/kg bw in a 14-day repeated-dose series) and a single high dose (100 mg/kg bw). In addition, pharmacokinetics in blood was investigated using two groups of rats (five of each sex per dose) that received a single oral dose of [^{14}C]isoxaflutole at 1 or 100 mg/kg bw. Urine and faeces were collected at intervals of 0–24, 0–48, 0–96, 0–120, 0–144 and 0–168 hours, and tissues were collected at 168 hours post-dosing. Metabolite analysis was performed on the urine and faeces from animals of all dose groups and on the liver samples of male and female rats in the two low-dose groups (Filaquier, 1994). A separate time course tissue distribution study was conducted in Sprague-Dawley rats. In this study, [^{14}C]isoxaflutole (purity 98.7%) was administered to groups (16 of each sex per dose) of male and female Sprague-Dawley (CD) rats by gavage at a single low oral dose (1 mg/kg bw) and a single high oral dose (100 mg/kg bw). Four rats of each sex per dose were killed at 1, 24, 96 and 168 hours post-dosing, and isoxaflutole-derived radioactivity was measured in various tissues (Valles, 1999).

The total recovery of radioactivity in male and female rats ranged from 97.3% to 100.4% of the administered dose (Table 1). The mean total recovery of radioactivity from the males and females was 98.09%. As there were no data available for isoxaflutole from intravenous administration or bile duct–cannulated rats, the extent of absorption is interpolated from available urinary excretion data. Based on urinary elimination, it appears that about 60%, 67% and 36% of the administered dose were absorbed in the low-dose, repeated low-dose and high-dose groups, respectively (Table 1) in 168 hours. The mean maximum estimated proportion of the dose absorbed was calculated from the radioactivity detected in the urine, cage washes and tissues, which yielded about 73%, 75% and 39% for the low-dose, repeated low-dose and high-dose groups, respectively. The urine was the major route of elimination for the low-dose groups (about 69–74% of the dose), whereas faeces was the major route of elimination for the high-dose group (about 55–63% of the dose). These results suggest

that significant absorption of the test material occurred at the low dose; as the dose increased, there was saturation of absorption, resulting in a major portion of the parent compound being excreted unchanged.

The blood pharmacokinetic parameters for isoxaflutole are shown in Table 2. The maximal concentrations in blood (C_{max}) were achieved between 0.5 and 1 hour post-dosing. The results indicate a direct dose–response relationship with the maximal concentrations of [^{14}C]isoxaflutole found in the whole blood, with an indication of higher levels in the male rats. The time of maximal concentration (T_{max}) appeared shorter for the females, and the elimination phase half-lives were very similar between sexes and dose regimens. The results from blood/plasma pharmacokinetic groups indicated that [^{14}C]isoxaflutole and/or its metabolites have a mean β-phase elimination half-life of about 60 hours, irrespective of the dose level.

The tissue distribution results from the single and repeated low-dose groups indicated that only very low radioactive residue concentrations remained in the tissues 168 hours after dose administration (Table 3).

The liver and the kidney were the only two tissues containing mean residue concentrations above 0.22 µg equivalents (eq)/g, reaching maximum mean levels of approximately 4.6 µg eq/g in the liver and about 3.8 µg eq/g in the kidneys (Table 4). The high-dose group results indicated that the highest mean radioactive residue levels (both sexes) were found in the blood (7.7 µg eq/g), plasma (6.3 µg eq/g), liver (4.6 µg eq/g) and kidneys (3.4 µg eq/g).

Table 1. Recovery of radioactivity in rats in 168 hours after administration of [^{14}C]isoxaflutole

	% of radioactive dose recovered					
	Single low dose		Repeated low dose		Single high dose	
	Males	Females	Males	Females	Males	Females
Tissues	4.33	3.36	2.62	1.44	1.48	1.79
Cage wash	7.68	11.34	6.12	6.4	1.48	0.63
Urine	61.16	58.80	66.65	67.41	31.37	41.20
Faeces	26.06	26.94	24.04	24.72	63.00	55.23
Total recovery	99.23	100.44	99.43	99.97	97.33	98.85
Urinary excretion (urine + cage wash)	68.84	70.14	72.77	73.81	32.85	41.83
Estimated oral absorption (urine + cage wash + tissues)	73.17	73.50	75.39	75.25	34.33	43.62

Source: Filaquier (1994)

Table 2. Blood pharmacokinetic parameters for isoxaflutole in the rat based upon total radioactivity measurements.

Dose (mg/kg bw)	Sex	C_{max} (µg eq/g)		T_{max} (h)		Elimination half-life (h)	
		Mean	SD	Mean	SD	Mean	SD
100	Males	48.10	12.2	0.98	0.40	59.23	2.6
	Females	25.19	5.9	0.67	0.05	60.04	3.9
1	Males	0.50	0.10	1.03	0.35	61.05	6.1
	Females	0.27	0.05	0.52	0.04	59.49	8.4

C_{max}: maximum concentration in blood; eq: equivalents; SD: standard deviation; T_{max}: time to reach C_{max}
Source: Filaquier (1994)

Table 3. Mean percentage of administered radioactivity found in the tissues at 168 hours after administration of isoxaflutole

Group	Mean % of administered radioactivity	
	Males	Females
Single oral high dose	1.48	1.79
Single oral low dose	4.33	3.36
Repeated oral low dose	2.62	1.44

Source: Filaquier (1994)

Table 4. Distribution of radioactivity in rat tissues/organs at 168 hours after administration of [^{14}C]isoxaflutole

Tissue/organ	Isoxaflutole distribution (µg eq/g tissue, or ppm)					
	Single low dose		Multiple low dose		Single high dose	
	Males	Females	Males	Females	Males	Females
Liver	0.498	0.388	0.427	0.172	4.53	4.59
Kidneys	0.223	0.498	0.213	0.221	2.93	3.78
Heart	0.001	0.001	0.001	n.d.	1.85	3.19
Lungs	0.006	0.001	0.004	0.001	2.46	4.00
Brain	n.d.	n.d.	n.d.	n.d.	0.26	0.38
Spleen	n.d.	0.001	0.001	n.d.	1.52	1.91
Muscle	n.d.	n.d.	n.d.	n.d.	1.18	1.44
Fat	0.001	0.001	0.002	0.001	1.71	1.62
Gonads	n.d.	n.d.	n.d.	n.d.	0.80	2.36
GI tract + contents	0.004	0.005	0.007	0.004	2.03	1.60
Bone and marrow	n.d.	n.d.	n.d.	n.d.	0.84	1.09
Adrenal	0.002	0.002	n.d.	n.d.	2.32	2.69
Uterus	–	0.001	–	0.001	–	2.57
Eyes	n.d.	n.d.	n.d.	n.d.	0.65	0.74
Harderian gland	n.d.	n.d.	n.d.	n.d.	1.06	1.66
Residual carcass	n.d.	n.d.	n.d.	n.d.	0.72	0.93
Skin and fur	0.012	0.023	0.015	0.020	0.40	0.56
Blood	0.002	0.004	0.004	0.003	6.28	9.08
Plasma	0.001	0.002	0.005	0.004	5.22	7.28

eq: equivalents; GI: gastrointestinal; n.d.: not detected; ppm: parts per million
Source: Filaquier (1994)

The results from the tissue kinetic study (Valles, 1999) reflected those seen in the absorption, distribution, metabolism and excretion study (Filaquier, 1994); radioactivity was widely distributed in the tissues, with a predominance in the liver and kidney. The distribution of the absorbed radioactivity was found to be similar for both dose levels used (1 and 100 mg/kg bw), with the higher concentrations being observed in the gastrointestinal tract (at early sacrifice times), liver, kidney, plasma and cardiac blood. The levels observed in the liver and kidney remained among the highest at all sampling times in both dose groups and both sexes. The highest tissue concentrations were found at 1 hour post-administration at the low dose, whereas they were observed at 24 hours post-administration at the high dose, reflecting a rapid absorption in the former case. The distribution of radioactivity was comparable in males and females at both dose levels (Valles, 1999).

In the low-dose and repeated low-dose groups, [^{14}C]isoxaflutole was primarily excreted in the urine of rats (about 58.8–67.4% of the administered dose). In the two low-dose groups, approximately 24.0–26.8% of the administered dose was excreted in the faeces. In the high-dose group, the mean recoveries were mainly in the faeces, indicating saturation of absorption (Table 5). The majority of radioactivity was eliminated in the first 24 hours for the two low-dose groups and within the first 48 hours for the high-dose group. There was no observed sex-related difference in the elimination pattern in either the single or the repeated low-dose groups.

Table 5. Time course excretion of isoxaflutole-derived radioactivity in the urine and faeces following single low dose, repeated low dose and single high dose in rats

Route of excretion / time interval (h)	% of the administered dose					
	Single low dose		Repeated low dose		Single high dose	
	Males	Females	Males	Females	Males	Females
Urine						
0–24	55.40	48.71	60.27	61.98	23.95	29.98
0–48	57.86	52.79	63.67	64.30	29.29	37.83
0–72	59.20	54.91	64.64	65.41	30.24	39.50
0–96	59.93	56.34	65.33	66.29	30.67	40.16
0–120	60.46	57.43	65.85	66.78	30.97	40.61
0–144	60.83	58.22	66.30	67.13	31.19	40.92
0–168	61.16	58.80	66.65	67.41	31.37	41.20
Faeces						
0–24	20.03	18.10	18.50	22.61	46.26	30.83
0–48	24.46	24.74	22.75	23.78	59.06	50.01
0–72	24.92	25.68	23.50	24.17	60.84	53.14
0–96	25.15	26.16	23.74	24.44	61.66	53.93
0–120	25.37	26.53	23.87	24.58	62.23	54.45
0–144	25.51	26.76	23.97	24.66	62.66	54.91
0–168	26.05	26.94	24.04	24.72	63.00	55.23

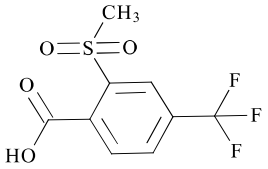
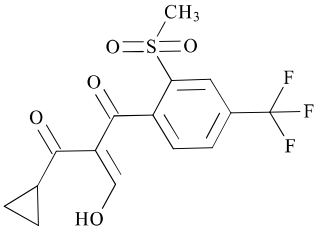
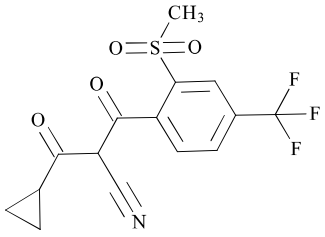
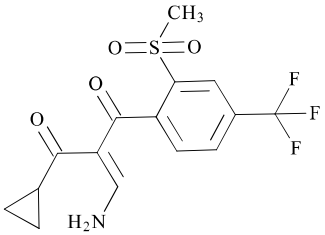
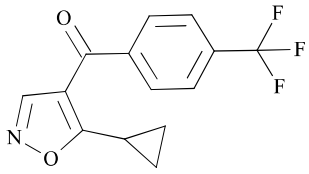
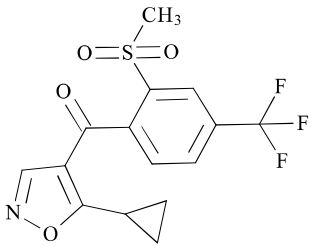
Source: Filaquier (1994)

1.2 Biotransformation

The metabolism of [^{14}C]isoxaflutole was qualitatively and quantitatively similar in both sexes of rats and was not influenced by repeated administration. The compound was rapidly and extensively metabolized, as seen by the lack of parent material in the urine of rats in the two low-dose groups; only traces were detected in the urine of rats in the high-dose group. Unchanged parent compound was detected in the faeces of rats from the high-dose group, indicating saturation of absorption of the compound. After oral administration, 9 metabolites were detected in the urine, and 11 metabolites were detected in the faecal extracts. Independent of the time period and the elimination route, the major metabolite detected in the urine (UMET/5) and faecal (FMET/7) extracts for the three dose groups was identified as RPA 202248 (3-cyclopropyl-2-[2-mesyl-4-trifluoromethylbenzoyl]-3-oxopropane nitrile). It is a diketonitrile derivative of the parent compound, which represented 70% of the radioactivity excreted in the urine and faeces. The metabolites UMET/1 and FMET/1 (more polar metabolite) were identified as RPA 203328 (2-mesyl-4-trifluoromethylbenzoic acid; minor metabolite); the metabolites UMET/2 and FMET/3 were not clearly identified, but suggested the presence of a strongly acidic proton or the presence of an unstable conjugate. The metabolites UMET/7 and FMET/9 were identified as an amine derivative from RPA 202248. The minor metabolites UMET/8 and FMET/10 were possibly the Des-SO_2Me derivatives from the parent compound. The unchanged isoxaflutole was detected as UMET/9 and FMET/11. There was no

indication of any metabolites resulting from Phase II (conjugation) reactions. The liver samples from the males and females from the two low-dose groups contained TMET/1 (RPA 202248) as a major metabolite, representing 33–77.91% of the initial radioactivity measured. TMET/2 (unidentified) was detected as a minor metabolite (0.28–2.15% of the initial radioactivity) (Table 6).

Table 6. Mean percentage of administered radioactivity associated with the radiolabelled components characterized/identified in the urine and faeces of rats following single oral low dose, repeated oral low dose and single oral high dose of isoxaflutole[a]

	Met. ID	**RPA 203328 (UMET/1 & FMET/1)**						**RPA 207048 (FMET/5)**					
	Structure												
	Group	SOHD		SOLD		ROLD		SOHD		SOLD		ROLD	
Sample	Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Urine	% dose	1.2	0.56	1.0	–	0.7	2.0	–	–	–	–	–	–
Faeces	% dose	2.4	2.0	0.99	0.58	0.58	0.57	1.9	1.3	–	–	–	–
Total	% dose	3.6	2.6	2.0	0.58	1.3	2.6	1.9	1.3	–	–	–	–
	Met. ID	**RPA 202248 (UMET/5, FMET/7 & TMET/1)**						**RPA 205834 (UMET/7 & FMET/9)**					
	Structure												
	Group	SOHD		SOLD		ROLD		SOHD		SOLD		ROLD	
Sample	Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Urine	% dose	28.2	36.2	60.1	58.8	63.8	63.9	0.79	2.3	–	–	–	–
Faeces	% dose	41.8	43.7	19.4	18.8	20.6	21.3	1.5	2.0	–	–	0.03	–
Total	% dose	70.0	79.9	79.5	77.6	84.4	852	2.29	4.3	–	–	0.03	–
	Met. ID	**RPA 205568 (UMET/8 & FMET/10)**						**Isoxaflutole (UMET/9 & FMET/11)**					
	Structure												
	Group	SOHD		SOLD		ROLD		SOHD		SOLD		ROLD	
Sample	Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Urine	% dose	0.11	0.42	–	–	–	–	0.22	0.05	–	–	–	–
Faeces	% dose	1.9	0.88	–	–	–	–	8.0	5.6	–	–	–	–
Total	% dose	2.0	1.3	–	–	–	–	8.2	5.7	–	–	–	–

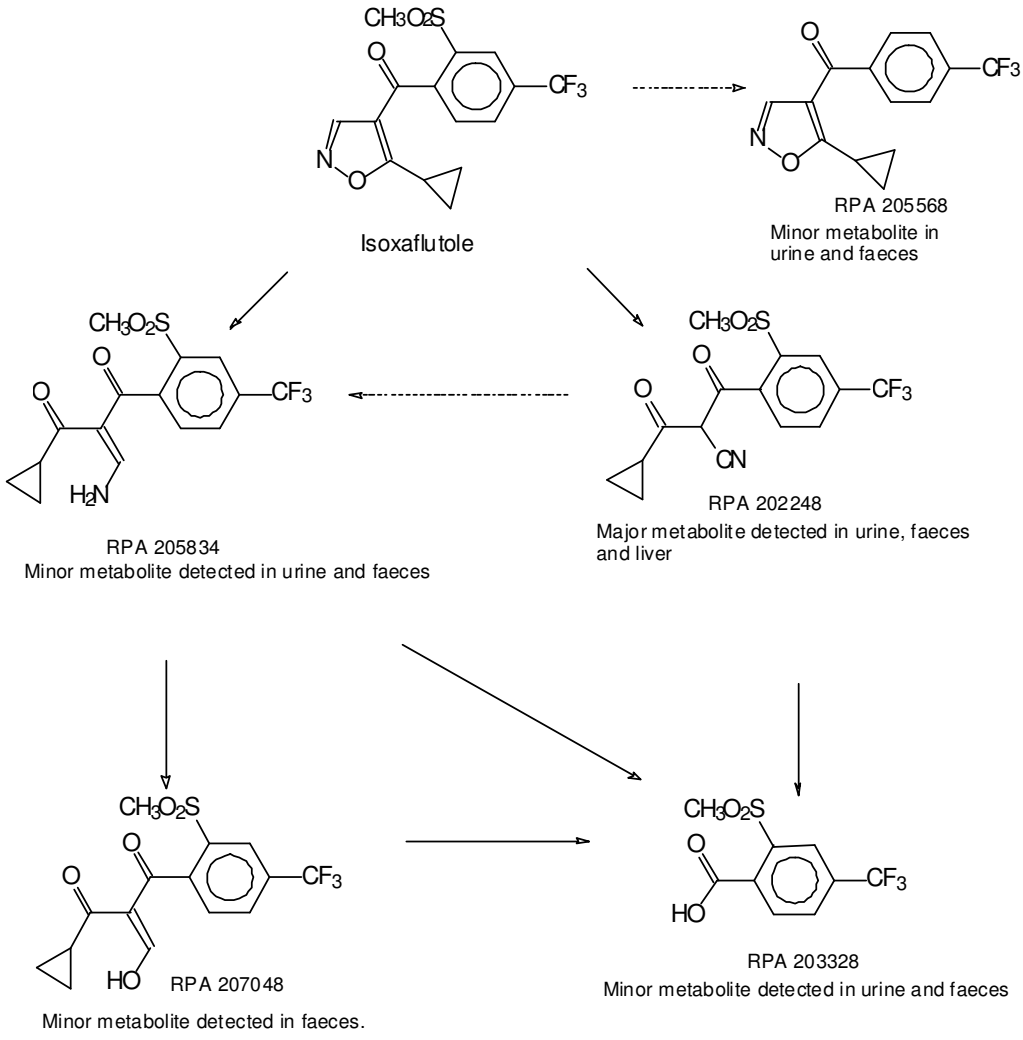
–: not detected; ID: identification; Met.: metabolite; SOHD: single oral high dose; SOLD: single oral low dose; ROLD: repeated oral low dose

[a] The data are presented in terms of percentage of administered dose and are means over the 0–168 h post-dosing period for both sexes.

Source: Adapted from Shipp (2012).

The proposed metabolic pathway for isoxaflutole in rats is shown in Fig. 2.

Fig. 2. Proposed metabolic pathway for isoxaflutole in rats



Source: Shipp (2012)

1.3 Dermal absorption

In a dermal absorption study, [^{14}C]isoxaflutole was dermally applied to male Crl:CD®BR rats at 0.865, 7.32 or 79 µg/cm^2. Four animals per dose were exposed for 0.5, 1, 2, 4, 10 and 24 hours. Carboxymethyl cellulose in water (1%) was used as a vehicle for dermal application. Small amounts of radioactivity were detected in/on the skin of the application site, accounting for 0.92–12.0% of the applied radioactivity. The amounts of radioactivity found in the blood, eliminated in the excreta and retained in the carcass were considered to be the direct dermal absorption of isoxaflutole by rats. The highest direct absorption was at the longest exposure time (24 hours after dose). It accounted for

4.49% (0.494 µg eq), 0.92% (0.846 µg eq) and 0.2% (1.97 µg eq) at 0.865, 7.32 and 79 µg/cm^2, respectively. The amount of dermal absorption of isoxaflutole was not proportional to the dose (Cheng, 1996).

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies with isoxaflutole (including skin and eye irritation and dermal sensitization studies) are summarized in Table 7.

Table 7. Acute toxicity of isoxaflutole

Species	Strain	Sex	Route	Purity; vehicle	Result	Reference
Rat	Sprague-Dawley	M + F	Oral	98.7%; 0.5% CMC	LD_{50} > 5 000 mg/kg bw	Allen (1993b)
Rat	Wistar	F	Oral	98.6%; 2% aqueous Cremophor EL	LD_{50} > 2 000 mg/kg bw	Eiben (2005b)
Rat	Wistar	M + F	Dermal	98.6%; wet gauze	LD_{50} > 2 000 mg/kg bw	Eiben (2005a)
Rabbit	New Zealand White	M + F	Dermal	98.7%; 0.5% CMC	LD_{50} > 2 000 mg/kg bw	Allen (1993d)
Rat	Sprague-Dawley	M + F	Inhalation (whole body)	98.3%	LC_{50} (4 h) > 5.23 mg/L	Jackson (1994)
Rabbit	New Zealand White	M + F	Skin irritation	98.7%; 0.5% CMC	Non-irritating	Allen (1993c)
Rabbit	New Zealand White	F	Skin irritation	98.6%; moistened with water	Non-irritating	Schüngel (2005b)
Rabbit	New Zealand White	M + F	Eye irritation	98.7%	Minimally irritating	Allen (1993a)
Rabbit	New Zealand White	F	Eye irritation	98.6%	Minimally irritating	Schüngel (2005a)
Guinea-pig	Dunkin Hartley	M + F	Skin sensitization (Buehler method)	99.4%; propylene glycol	Non-sensitizing	Rees (1992)
Guinea-pig	Hartley	M + F	Skin sensitization (Magnusson-Kligman)	99.6%; propylene glycol	Non-sensitizing	Rees (1996)
Guinea-pig	Hartley	F	Skin sensitization (Buehler method)	98.6%; polyethylene glycol	Non-sensitizing	Vohr (2005)

CMC; carboxymethyl cellulose; F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male

(a) Oral administration

Rats

One male and one female fasted Sprague-Dawley rat were treated orally, by gavage, with isoxaflutole (purity 98.7%) in 0.5% carboxymethyl cellulose at a single dose of 5000 mg/kg bw and observed for 5 days. A second group of five fasted male and five fasted female Sprague-Dawley rats was similarly treated at a single gavage dose of 5000 mg/kg bw. Animals were observed for mortality and clinical signs several times for the 1st day and once daily thereafter for 14 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals.

No deaths or clinical signs of an adverse reaction to treatment occurred, and there were no effects on body weight or macroscopic findings at necropsy in any animal. Based on these results, the acute oral median lethal dose (LD_{50}) was estimated to be greater than 5000 mg/kg bw (Allen, 1993b).

In a separate study, three fasted female Wistar rats were treated orally, by gavage, with isoxaflutole (purity 98.6%) in 2% aqueous Cremophor EL at a single dose of 2000 mg/kg bw and observed for 14 days. Another group of three female rats was similarly treated.

No deaths, clinical signs, effects on body weight or gross pathological findings at necropsy were observed. Based on these results, the acute oral LD_{50} was estimated to be greater than 2000 mg/kg bw (Eiben, 2005b).

(b) Dermal application

Rats

In an acute dermal lethality study, a group of five male and five female Wistar rats was treated with isoxaflutole (purity 98.6%) topically at a dose of 2000 mg/kg bw. The compound was applied to a wet gauze patch, then to the shorn skin using stretch tape and a jacket. After a 24-hour exposure period, the application sites were cleaned and evaluated according to the Draize method.

No deaths or systemic clinical signs of an adverse reaction to treatment occurred. There were no local signs of an effect of treatment at the application site, and there were no macroscopic findings at necropsy in any animal. All animals gained weight during the study. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw (Eiben, 2005a).

Rabbits

A group of five male and five female New Zealand White rabbits was treated with isoxaflutole (purity 98.7%) dispersed in aqueous solution of 0.5% carboxymethyl cellulose at a dose of 2000 mg/kg bw once for 24 hours by topical administration. Each application site was covered with surgical gauze and secured with adhesive and an elasticated corset. After a 24-hour exposure period, the application sites were cleaned and evaluated according to the Draize method. Animals were observed for mortality and clinical signs several times for the 1st day and once daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. After 14 days of observation post-treatment, the animals were subjected to necropsy and postmortem examination.

No deaths or clinical signs of an adverse reaction to treatment occurred. A very slight erythema at the treatment site was noted in two male rabbits and one female rabbit at 1 day after treatment and persisted in one of the male rabbits through the 2nd day after dosing. There were no macroscopic findings at necropsy in any animal. All animals gained weight during the study. Based on these results, the acute dermal LD_{50} was estimated to be greater than 2000 mg/kg bw (Allen, 1993d).

(c) Exposure by inhalation

Rats

A group of five male and five female Sprague-Dawley rats was exposed once for 4 hours, by whole-body exposure, to a dust atmosphere of isoxaflutole (purity 98.3%) at the maximum technically achievable concentration of 5.23 mg/L air. The animals were observed for 14 days post-treatment. Body weights were recorded on day 0 and weekly thereafter. All animals were subjected to necropsy and postmortem examination.

The mass median aerodynamic diameter of isoxaflutole in the chamber air was 3.1 µm, the standard geometric deviation was 1.95 and over 88% of the particles were 7 µm or less. No deaths occurred during the exposure or observation period. Clinical signs observed during exposure were limited to partial closing of the eyes and accumulation of the test compound on the fur. During the post-exposure observation period, there were no treatment-related clinical signs except for the presence of residual test compound on the fur immediately following exposure. Body weight gain was comparable to the control values at termination. There were no macroscopic findings at necropsy, except for the lungs of one male rat, which were slightly congested. Based on these results, the acute (4-hour) median lethal concentration (LC_{50}) was estimated to be greater than 5.23 mg/L air (Jackson, 1994).

(d) Dermal irritation

Rabbits

In a study of primary dermal irritation, one female and five male New Zealand White rabbits were dermally exposed to 0.5 g of isoxaflutole (purity 98.7%) moistened with 0.5 mL of 0.5% carboxymethyl cellulose and placed onto the shorn skin on the back of each rabbit under a 2.5 cm^2 gauze patch secured in position with surgical adhesive tape. The test material was in contact with the skin for 4 hours. After removal of the patch, the treated application site was washed off with water. Dermal irritation was scored at 1, 24, 48 and 72 hours after the removal of the patch using the Draize method. The animals were observed for 14 days post-treatment.

A very slight erythema was noted on one animal at 1 hour after removal of the patches. No other animals were noted with erythema, eschar or oedema at any time point. Based on the results of this study, isoxaflutole was not irritating to the skin of rabbits (Allen, 1993c).

In a separate primary dermal irritation study, three gauze patches, each containing 0.5 g isoxaflutole (purity 98.6%) moistened with water, were applied to the shorn skin of a female New Zealand White rabbit and secured with non-irritating tape. The first patch was removed after 3 minutes, and, in the absence of serious skin reactions, the second patch was removed after 1 hour. As observations of the animal indicated no hazard to animal welfare, the third patch was removed after 4 hours. Additionally, 0.5 g of isoxaflutole was applied to the shorn skin of two other rabbits under gauze patches secured with non-irritating tape for 4 hours.

No erythema, eschar or oedema was observed at any observation time point. Under the conditions of the study, isoxaflutole is not an irritant to the skin of the rabbit (Schüngel, 2005b).

(e) Eye irritation

Rabbits

In a primary eye irritation study, a volume of 0.1 mL (approximately 99 mg) of isoxaflutole (purity 98.7%) was instilled into the right conjunctival sac of three male and three female New Zealand White rabbits. The eyes were not washed. The ocular irritation was assessed approximately 1, 24, 48 and 72 hours after treatment.

Iridial inflammation was noted in the treated eye of one animal at 1 hour after treatment. Minimal to moderate conjunctival irritation was noted in all treated eyes at 1 hour after treatment. All

treated eyes appeared normal at the 24-hour observation. Based on the results of this study, isoxaflutole is minimally irritating to rabbit eyes (Allen, 1993a).

In a second primary eye irritation study, a volume of 0.1 mL (approximately 99 mg) of isoxaflutole (purity 98.6%) was instilled into the right conjunctival sac of three female New Zealand White rabbits. The eyes were not washed for 24 hours. The ocular irritation was assessed approximately 1, 24, 48 and 72 hours after treatment.

All three animals showed slight redness of the conjunctivae in the treated eye at 1 hour after compound application. Two animals had conjunctival redness that persisted through 24 hours after treatment. Based on the results of this study, isoxaflutole is minimally irritating to rabbit eyes (Schüngel, 2005a).

(f) Dermal sensitization

Guinea-pigs

The skin sensitization potential of isoxaflutole (purity 99.4%) was investigated in 10 male and 10 female Dunkin Hartley guinea-pigs using the modified Buehler method. The control group included five male and five female guinea-pigs. The shaven left flanks of 10 male and 10 female guinea-pigs were subjected to a 6-hour occluded topical application of 50% weight per volume (w/v) isoxaflutole in propylene glycol on days 1, 3, 5, 8, 10, 12, 15, 17 and 19 of the test period. On day 29, all test and control animals were challenged by 6-hour occluded topical applications of 50% and 10% w/v isoxaflutole in propylene glycol and propylene glycol alone to their shaven right flanks. Dermal responses to the challenge procedure were assessed approximately 24 and 48 hours after application of the occlusive dressings.

Applications of 50% w/v isoxaflutole in propylene glycol caused intermittent, very faint erythema throughout the induction period. There were no reactions in either the test or control animals after the challenge application of isoxaflutole at either 10% or 50% in propylene glycol. Based on the lack of response in the challenge phase, isoxaflutole is not considered to be a sensitizer using the modified Buehler method (Rees, 1992).

In a second study, the skin sensitization potential of isoxaflutole (purity 99.6%) was investigated in 10 male and 10 female Dunkin Hartley guinea-pigs using the maximization test. A concurrent positive control group was not included. Concentrations of 10% and 50% isoxaflutole in propylene glycol were used for intradermal induction, topical induction and challenge phases. Skin reactions to the challenge applications were evaluated 24 and 48 hours after patch removal.

Intradermal injection of 10% isoxaflutole in propylene glycol caused some slight erythema and skin discoloration, whereas inclusion of Freund's complete adjuvant caused slight to moderate erythema, skin discoloration and pallor. Topical application of isoxaflutole at 50% in propylene glycol caused isolated cases of exfoliation and eschar formation. Challenge application of isoxaflutole at either 50% or 10% in propylene glycol did not cause any dermal reaction in any animal at either 24 or 48 hours after removal of the material. Under the conditions of the guinea-pig maximization study, there was no indication of delayed contact hypersensitivity induced by isoxaflutole (Rees, 1996).

In a third study, the skin sensitization potential of isoxaflutole (purity 98.6%) was investigated in 20 female Crl:HA guinea-pigs using the maximization test. Ten females were used as controls. Isoxaflutole was applied to female guinea-pigs in polyethylene glycol 400 at concentrations of 5% for intradermal injection, 50% for topical induction and 50% for challenge application. Isoxaflutole in polyethylene glycol was injected into the skin either with or without Freund's complete adjuvant. Topical application of isoxaflutole was then conducted for a 48-hour period starting on study day 8. Finally, 3 weeks after the initial intradermal injection, new areas were shorn of hair, and isoxaflutole at 50% in polyethylene glycol 400 was applied for a 24-hour period. The

application sites were assessed at 24 and 48 hours after removal of the test item and scored for severity of reaction.

After the intradermal induction, animals in the control group and the test group showed strong effects up to encrustation at the injection sites of the first induction. No skin reactions were observed in the treated and control groups after the challenge phase. Under the conditions of the guinea-pig maximization study, there was no indication of delayed contact hypersensitivity induced by isoxaflutole (Vohr, 2005).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 28-day preliminary dietary study, groups of 10 male and 10 female CD-1 mice received isoxaflutole (purity 99.9%) via dietary administration at a concentration of 0, 175, 700, 2800 or 7000 parts per million (ppm) daily for 28 days. The dose levels were equal to 0, 29.4, 120.7, 474.6 and 1140.1 mg/kg bw per day for males and 0, 34.7, 142.9, 534.4 and 1347.4 mg/kg bw per day for females, respectively.

There were no treatment-related mortalities, clinical or ophthalmoscopic observations or treatment-related effects on body weight, body weight gain or feed consumption. The liver appeared to be the target organ. Significant treatment-related clinical chemistry findings included increased activities of alanine aminotransferase (ALAT) at 700 ppm and above and aspartate aminotransferase (ASAT) at 2800 ppm and above; alkaline phosphatase (AP) activity was increased at 7000 ppm in males, but not significantly (Table 8).

Table 8. Treatment-related clinical chemistry findings in the mouse 28-day study with isoxaflutole

Observation	Males					Females				
	0 ppm	175 ppm	700 ppm	2 800 ppm	7 000 ppm	0 ppm	175 ppm	700 ppm	2 800 ppm	7 000 ppm
Total bilirubin (µmol/L)	3.91	3.26**	2.22**	1.40**	1.14**	2.49	2.19	1.87	1.21**	1.00**
Total protein (g/L)	54.80	55.80	57.10	56.44	60.90**	54.50	54.10	55.00	56.80	61.40**
ASAT (IU/L)	41.40	40.30	44.80	59.89**	79.60**	58.80	58.60	58.50	67.70	65.30
ALAT (IU/L)	21.3	22.0	25.5	76.5**	133.5**	21.70	24.00	28.40*	36.40*	48.40**
AP (IU/L)	61.0	51.3	52.5	55.9	124.7	72.9	80.20	71.10	77.0	72.40
Creatinine (µmol/L)	39.10	32.30*	28.30**	31.22**	26.60**	35.30	26.30*	23.90**	22.70**	17.89**
Liver weight (g)	1.30	1.45*	1.57**	2.13**	2.82**	0.86	0.96	1.00**	1.30**	1.91**
Liver weight (% of body weight)	3.99	4.35*	4.72**	6.52**	8.79**	3.72	4.08*	4.23**	5.47**	7.86**

ALAT: alanine aminotransferase; AP: alkaline phosphatase; ASAT: aspartate aminotransferase; IU: International Units; *: $P < 0.05$; **: $P < 0.01$
Source: Esdaile & Dange (1994)

Increased liver weights were observed at all dose levels in both sexes. Significant treatment-related gross pathological observations were noted in the liver and included enlarged liver (males at 700, 2800 and 7000 ppm and females at 2800 and 7000 ppm) and white striations (both sexes at 7000 ppm). Treatment-related histopathological findings in the liver included centrilobular hepatocellular hypertrophy (both sexes at 700 ppm and above) and hepatocellular necrosis (males at 2800 and 7000 ppm and females at 7000 ppm). Other histopathological findings included increased extramedullary haematopoiesis in spleen (both sexes at 7000 ppm) and X-zone cell vacuolation in the adrenal glands (females at 7000 ppm).

In the absence of any other significant findings at 175 ppm, the increased liver weights were considered a minor adaptive change; therefore, the no-observed-adverse-effect level (NOAEL) was 175 ppm (equal to 29.4 mg/kg bw per day), based on increases in liver enzymes (ALAT and ASAT), clinical chemistry changes (decreased bilirubin and creatinine levels) and increased liver weight at 700 ppm (equal to 120.7 mg/kg bw per day) (Esdaile & Dange, 1994).

In a 90-day preliminary dietary study, groups of 12 CD-1 mice of each sex per dose level received 0, 50, 1000 or 2000 ppm isoxaflutole (purity 983 g/kg) via dietary administration daily for 13 weeks and 5 days. The dose levels were equal to 0, 7.6, 170.0 and 324.1 mg/kg bw per day for males and 0, 8.7, 181.2 and 376.2 mg/kg bw per day for females, respectively.

There were no treatment-related mortalities, clinical, ophthalmoscopic or gross pathological observations, or treatment-related effects on body weight, body weight gain, feed consumption or feed efficiency. The liver appeared to be the target organ. Significant treatment-related clinical chemical observations were increased ASAT and ALAT activities at 2000 ppm in both sexes (Table 9).

Table 9. Potentially treatment-related clinical chemistry parameters and liver weights in the mouse 90-day study with isoxaflutole

Observation	Males				Females			
	0 ppm	50 ppm	1 000 ppm	2 000 ppm	0 ppm	50 ppm	1 000 ppm	2 000 ppm
ALAT (IU/L)	42	36	53	126***	37	34	38	47*
ASAT (IU/L)	71	66	92	112*	75	73	78	93*
CPK (IU/L)	122	137	189	114	69	70	76	154***
Terminal body weight (g)	37.3	38.3	34.4	35.5	27.2	28.7	28.1	27.7
Liver weight (g)	1.64	1.80*	1.90*	2.28**	1.33	1.32	1.37	1.54**
Liver weight (% of body weight)	4.409	4.176	5.543**	6.402**	4.890	4.539	4.890	5.569**

ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; CPK: creatine phosphokinase; IU: International Units; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
Source: Chase (1994c)

Increased absolute liver weights were observed in males at 50 ppm and above (Table 9). Increased relative liver weights were observed in males at 1000 ppm and above. Macroscopic examination revealed that one male at 2000 ppm had a large liver, and two further males at 2000 ppm had pale livers. Periacinar hepatocytic hypertrophy was observed at 1000 ppm and above. At 50 ppm, the lowest dose tested, the only significant observation was an increase in absolute liver weight in males.

In the absence of a similar increase in females and in the absence of any biochemical, gross pathological or histopathological observations at 50 ppm in either sex, this was considered to be a minor adaptive change; therefore, the NOAEL was 50 ppm (equal to 7.6 mg/kg bw per day), based on increased ALAT and ASAT activities, increased absolute and relative liver weights and increased incidence of periacinar hepatocytic hypertrophy at 1000 ppm (equal to 170.0 mg/kg bw per day) (Chase, 1994c).

Rats

In a short-term dietary study, groups of 10 male and 10 female CD rats received isoxaflutole (purity 99.4%) via dietary administration at a constant dose of 0, 25, 100, 400 or 1000 mg/kg bw per day for 6 weeks followed by a 7-week reversibility period (without test article). All control animals were killed after 17 weeks.

No mortality was observed during the study. An increased incidence of unilateral or bilateral opaque eyes was noted in all male groups beginning in week 3, without a dose–response relationship. Opacities persisted through the 1st week of reversibility; however, most of them had resolved by the 2nd week of the reversibility phase of the study. At ophthalmology, focal corneal opacities were seen in all treated groups (apart from females receiving 25 mg/kg bw per day) from week 3 of treatment, but their incidence was not dose related. The males were more affected than the females. The severity of each lesion tended to increase throughout the treatment period; this sometimes included an increase in size and opaqueness, with an associated keratitis and/or vascularization. After 6 days of reversibility, the majority of lesions had nearly completely recovered, leaving only a slight corneal haze and/or ghost vessels. Within 20 days of the reversibility phase, all of the lesions had regressed.

During the treatment period, the body weight gain for males and females receiving 1000 mg/kg bw per day and females receiving 400 mg/kg bw per day was low compared with that of their controls (81% and 67% for males and females receiving 1000 mg/kg bw per day and 82% for females receiving 400 mg/kg bw per day). During the reversibility phase, treated animals gained more weight than did the controls. Body weight gain for animals receiving 25 or 100 mg/kg bw per day and males receiving 400 mg/kg bw per day was considered to be unaffected by treatment. Feed consumption was decreased in females at 1000 mg/kg bw per day during the treatment period, but similar to that of controls during the reversibility phase. For the treatment period, the overall feed conversion efficiency of males and females receiving 400 or 1000 mg/kg bw per day was low compared with that of the controls. For the reversibility period, the feed conversion efficiency was greater for these animals than for their controls. Urine analysis after 5 weeks of treatment showed decreased pH in males at the top three doses and the presence of total reducing substances in six males and six females receiving 1000 mg/kg bw per day and one male and one female at each of 400 and 100 mg/kg bw per day (Table 10). After 4 weeks of treatment, there were slight decreases in some red cell parameters and total white cell counts in males or females and an increase in prothrombin times in males (Table 10). In clinical chemistry, there were decreases in a number of parameters, primarily related to the effects of isoxaflutole on the liver, in both males and females.

No treatment-related effects were observed at necropsy of treated animals. No treatment-related effects were observed on organ weights. Histopathology of the eyes from 10 treated animals revealed epithelial thickening and vacuolation, subepithelial fibroblastic reaction and active stromal vascularization in some animals.

In conclusion, the dietary administration of isoxaflutole at 25, 100, 400 and 1000 mg/kg bw per day produced liver toxicity and corneal lesions in the eye visible by both gross inspection and ophthalmological examination. The corneal lesions were resolved by 3 weeks after the end of the treatment, and liver weights were comparable to control values in 7 weeks after the end of the treatment. Therefore, the lowest-observed-adverse-effect level (LOAEL) was 25 mg/kg bw per day, based on corneal opacities and effects on the liver observed at all doses (Chase, 1994a).

Table 10. Treatment-related haematology, clinical chemistry and urine analysis parameters in the 6-week dietary/7-week reversibility study with isoxaflutole in the rat

Observation	Males					Females				
	0 mg/kg bw per day	25 mg/kg bw per day	100 mg/kg bw per day	400 mg/kg bw per day	1 000 mg/kg bw per day	0 mg/kg bw per day	25 mg/kg bw per day	100 mg/kg bw per day	400 mg/kg bw per day	1 000 mg/kg bw per day
PCV (%)	45	44	46	43	43	43	44	44	44	41**
Hb (g%)	15.1	15.8	16.1	15.5*	15.3**	15.6	15.8	15.7	15.9	15.0
RBC (10^6/cm^2)	8.05	7.86	7.98	7.80	7.89	7.83	8.02	7.89	8.10	7.83
MCHC (%)	36	36	35*	36	36	36	36	36	36	37
MCV (µm^3)	55	56	57	55	54	55	55	55	55	52**
MCH (pg/cell)	20	20	20	20	19	20	20	20	20	19*
Lymphocytes (1000/cm^2)	18.0	18.5	14.7*	14.7	14.4*	13.9	13.1	12.9	12.5	11.2*
Prothrombin time (s)	12.1	13.2*	12.4	13.4*	15.4***	12.5	13.1*	12.8	12.6	12.3
AP (IU/L)	159	133*	113***	110***	91***	96	88	75***	68***	70***
ALAT (IU/L)	34	34	30	35	30	24	25	22	31	18
ASAT (IU/L)	92	80**	71***	72***	63***	70	69	70	77	59
Urine pH	7.4	7.2	6.9***	7.0**	6.6***	6.5	6.3	6.1**	6.5	6.4
Total reducing substances	0/10	0/10	1/10	1/10	6/10	0/10	0/10	1/10	1/10	6/10
Terminal body weight (g)	593.9	525.7	528.5	524.1	505.3	329.7	308.7	315.0	319.3	298.8
Liver weight (g)	24.0	20.6	22.1	21.8	21.5	12.5	11.7	12.6	12.3	11.8
Liver weight (% of body weight)	4.04	3.91	4.18	4.16	4.27	3.78	3.77	3.99	3.85	3.93

ALAT: alanine aminotransferase; AP: alkaline phosphatase; ASAT: aspartate aminotransferase; Hb: haemoglobin; IU: International Units; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean cell volume; PCV: packed cell volume; RBC: red blood cells; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
Source: Chase (1994a)

In a 90-day dietary study, groups of 10 CD rats of each sex per dose received isoxaflutole (purity 99.4%) at 0, 1.0, 3.0, 10 or 100 mg/kg bw per day via dietary administration daily for 13 weeks and 3 days.

There were no treatment-related mortalities and no treatment-related effects on body weight, body weight gain, feed consumption or feed efficiency. There were no clinical signs of toxicity except opaque eyes observed during the treatment period for four males and four females receiving 100 mg/kg bw per day and two males at 10 mg/kg bw per day. The eyes of some males receiving the highest dosage were also noted to be dull. There were no significant treatment-related effects on haematological, clinical chemistry or urine analysis parameters examined. Treatment-related findings in males at 100 mg/kg bw per day included increased absolute and relative liver weights, with an associated increased incidence of periacinar hepatocytic hypertrophy; these were considered to be an adaptive response and not adverse. Absolute and relative kidney weights were increased in males at 100 mg/kg bw per day. Significant treatment-related ophthalmoscopic, gross pathological and histopathological findings were observed in the eye in males at 10 mg/kg bw per day and in both sexes at 100 mg/kg bw per day. Clinical and ophthalmoscopic observations included increased incidences of bilateral and unilateral opaque eyes and focal corneal opacity, respectively. Focal

corneal opacity was first apparent during week 3 and persisted throughout the study. Significant gross pathological observations included an increased incidence of corneal opacity (unilateral and bilateral) in both sexes at 100 mg/kg bw per day. The overall incidence of corneal lesions was similar in both sexes, although the severity of the lesions was more significant in the males. The most notable histopathological findings included vacuolation (males and females) and superficial exfoliation of the epithelial cells (males and females), epithelial thickening (males), necrosis and inflammation (males and females), subepithelial fibroblastic reaction (males and females) and vascularization of the stroma (males and females). The changes were considered by the study author to be reversible after a short recovery period following cessation of treatment in the previous study (Chase, 1994a), with the exception of residual evidence of tissue repair detectable only by histopathological examination. There were no treatment-related effects at 1.0 or 3.0 mg/kg bw per day; therefore, the NOAEL for isoxaflutole was 3.0 mg/kg bw per day, based on haematological changes, corneal opacity and liver toxicity observed at 10 mg/kg bw per day (Chase, 1994b).

The tyrosine concentrations in the plasma samples taken at 13 weeks from the 13-week toxicity study by dietary administration to CD rats described above (Chase, 1994b) were determined in a separate study (Table 11). The mode of action of isoxaflutole is the inhibition of the HPPD enzyme, which results in an increase in tyrosine levels in the blood and other tissues.

Table 11. Plasma tyrosine concentrations in the rat 90-day study with isoxaflutole

	Males					Females				
	0 mg/kg bw per day	1.0 mg/kg bw per day	3.0 mg/kg bw per day	10 mg/kg bw per day	100 mg/kg bw per day	0 mg/kg bw per day	1.0 mg/kg bw per day	3.0 mg/kg bw per day	10 mg/kg bw per day	100 mg/kg bw per day
Tyrosine concentration (nmol/mL)	516.8	725.7	922.6	1 061	1 330	511.3	423.3	890.6	1 023	1 308
Fold increase	–	1.4	1.8	2.1	2.6	–	0.8	1.7	2.0	2.6

Source: Little (1993a)

Plasma tyrosine levels were increased for males in all dose groups and for females from 3.0 mg/kg bw per day and above (Little, 1993a).

Dogs

In a pilot study, isoxaflutole (purity 99.9%) was administered orally in gelatine capsules to one male and one female Beagle dog in order to determine the maximum tolerated dosage. Doses increased from 100 mg/kg bw per day for the first 3 days to 300, 550 and then 1000 mg/kg bw per day for 3 succeeding days at each dose.

There were no clinical findings, effects on body weight or feed consumption, or observations at gross necropsy that could be related to treatment. The maximum tolerated dose was therefore set at greater than 1000 mg/kg bw per day in the dog when administered over a 3-day period (Mondot, 1992a).

In a second pilot study, isoxaflutole (purity 99.9%) was administered orally in gelatine capsules to one male and one female Beagle dog to evaluate potential toxicity. One dog from each sex received a dose of 1000 mg/kg bw per day for 14 days.

There were no deaths, clinical signs, effects on body weight or feed consumption or ophthalmological findings. At necropsy, no detectable changes or variation from normal was noted in the treated dogs. In conclusion, isoxaflutole was well tolerated when administered orally in capsules to Beagle dogs at a dose of 1000 mg/kg bw per day (Mondot, 1992b).

In a third pilot study, one male and one female Beagle dog were administered isoxaflutole (purity 97%) orally in capsules at a dose of 1000 mg/kg bw per day for 6 weeks. After a washout period of 8 days with no dosing, both dogs were then administered isoxaflutole in the diet at 25 000 ppm for 14 days.

There was no effect of treatment on mortality, clinical signs, body weight, feed consumption, ophthalmological findings, urine analysis, haematology, clinical chemistry or macroscopic findings. AP increased in both sexes during the dosing periods and decreased during the washout period. Relative liver weight was slightly increased in both animals; in the female, histopathological findings included occasional medullary foci of mineralization in the kidney and congestion and minimal centrilobular rarefaction of hepatocytes in the liver.

In conclusion, the study results indicate that isoxaflutole is not markedly toxic to dogs after short-term administration of 1000 mg/kg bw per day (Brooker, 1994b).

In a 1-year toxicity study, isoxaflutole (purity 98.7%) was administered to five Beagle dogs of each sex per dose in the diet at a dose level of 0, 240, 1200, 12 000 or 30 000 ppm (equal to 0, 8.56, 44.81, 453 and 1265 mg/kg bw per day for males and 0, 8.41, 45.33, 498 and 1254 mg/kg bw per day, for females, respectively) for 52 weeks. The treated males in the 30 000 ppm treatment group were sacrificed after 26 weeks due to severe chronic reaction to the test substance.

No animals died during the study. No treatment-related ophthalmological abnormalities were noted. All males in the 30 000 ppm treatment group were killed, for humane reasons, after 26 weeks of treatment due to apparent anaemia, suspected from pallor of the gums and confirmed by haematology. Pale gums were recorded for one male and one female in the 12 000 ppm treatment group during weeks 28–51 and 23–27, respectively. There was a statistically significant decrease in mean body weight gain of females in the 12 000 and 30 000 ppm treatment groups over 52 weeks of treatment (Table 12). Mean body weight gain of males in the 12 000 ppm treatment group was lower than that of the controls, although the difference did not attain statistical significance. Weight gains at 26 weeks were 65% and 56% of control gains in males and females in the 12 000 ppm group, respectively. Feed consumption was comparable between the control and treatment groups.

Table 12. Body weight and body weight gain in the dog 52-week dietary study with isoxaflutole

Week(s)	Males					Females				
	0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm	0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm
0	9.3	9.5	9.4	9.6	9.3	9.0	9.2	9.0	8.7	9.0
13	10.6	11.1	10.7	10.6	9.6	10.6	11.1	10.3	9.4	9.5
26	11.0	11.5	10.8	10.7	9.5	10.8	11.7	10.8	9.7	9.6
52	11.4	11.8	11.3	10.8	–	11.2	12.2	11.2	9.9	9.8
0–26	1.7	2.0	1.4	1.1	0.2*	1.8	2.6	1.7	1.0*	0.6**
0–52	2.0	2.3	1.8	1.2	–	2.2	3.0	2.2	1.2*	0.8**

*: $P < 0.05$; **: $P < 0.01$
Source: Brooker (1994a)

Females in the 12 000 and 30 000 ppm treatment groups generally showed a significant decrease in mean red cell indices (haematocrit, red blood cells and haemoglobin) compared with controls from week 13 through week 52 (Table 13). Males in the 12 000 ppm treatment group did not generally exhibit these decreases on the basis of mean value, although individual animals did show decreased red cell indices from week 22. The 30 000 ppm group males exhibited a marked reduction in red cell indices through the 26-week treatment period. Occasional reduction in red cell parameters in females in the 1200 ppm treatment group was only slight and sporadic and was not statistically significant. An increase in platelet count was recorded for males and females receiving 12 000 ppm and females receiving 30 000 ppm isoxaflutole, with the differences compared with controls considered statistically significant at both doses on all occasions, with the exception of week 39. No treatment-related changes were found in bone marrow smears.

Male and female Beagles in the 12 000 ppm treatment group, females in the 30 000 ppm treatment group and males in the 30 000 ppm treatment group at week 26 had lower serum albumin levels from week 13 onwards compared with controls (Table 14). This also resulted in corresponding reductions in total protein and a lower albumin to globulin ratio. Both sexes in the 12 000 ppm group and females in the 30 000 ppm group had significantly higher group mean plasma ALAT and AP levels than controls. Females in the 30 000 ppm treatment group exhibited a consistently low plasma urea level. From week 13 on, a treatment-related lowering of serum calcium was observed in both sexes in the 12 000 and 30 000 ppm treatment groups.

There were no meaningful differences in urinary pH; any statistically significant differences were considered to be of no toxicological importance. In males at 30 000 ppm, at week 26, urine specific gravity and protein were increased. Ketones and total reducing substances were increased in weeks 13 and 26. In males in all dose groups, urine specific gravity was increased relative to controls during week 36. Urine specific gravity and urine protein were increased in females at 30 000 ppm, although these findings were not always increased in a statistically significant manner. Urine specific gravity was occasionally increased in males and females at 12 000 ppm from week 39 of the study. Increased urine ketones were noted in males and females at 12 000 ppm and in females at 30 000 ppm from week 13 and in males and females at 1200 ppm only in week 26. The incidence of total reducing substances was increased in both males and females at 12 000 ppm and in females at 30 000 ppm from week 39. The increases in urinary ketones and total reducing substances are considered to be related to the excretion of the active ingredient and/or tyrosine metabolites in the urine and are thus markers of exposure rather than of toxicity.

Absolute and relative liver weights were elevated for two males in the 240 ppm treatment group, three males and one female in the 1200 ppm treatment group and all animals in the 12 000 and 30 000 ppm treatment groups. The increases in liver weights at 240 and 1200 ppm were small in magnitude and were considered as adaptive responses by the study author. Increases in kidney weights (relative and absolute) compared with controls were seen in the 1200 and 12 000 ppm treatment groups and in females receiving 30 000 ppm. Increased adjusted thyroid weights were noted for both sexes in the 12 000 ppm treatment group and in the 30 000 treatment group females. Treatment-related gross pathological changes were limited to friable surfaces of livers from male and female dogs in the 12 000 and 30 000 ppm treatment groups. An increased incidence and degree of hypertrophy of thyroid follicular epithelium were seen in males receiving 12 000 ppm (trace to minimum) and males and females receiving 30 000 ppm (trace to moderate). Liver changes were characterized by hepatocellular swelling and/or clumping and margination of cytoplasm. Centrilobular necrosis and fibrosis were also seen in males receiving 12 000 and 30 000 ppm. Dilated centrilobular sinusoids were seen in one male receiving 30 000 ppm. Occasional vacuolated hepatocytes were seen in females receiving 12 000 and 30 000 ppm. This change was also seen in one female receiving 240 ppm, but this is considered unlikely to be of toxicological significance.

In conclusion, the NOAEL was 1200 ppm (equal to 44.81 mg/kg bw per day), based on reduced weight gains, increase liver weight, histopathological findings in the liver and changes in haematological and clinical chemistry parameters at 12 000 ppm (equal to 453 mg/kg bw per day) (Brooker, 1994a).

Table 13. Treatment-related haematology parameters in the dog 52-week dietary study with isoxaflutole

Observation	Week	Males					Females				
		0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm	0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm
PCV (%)	−2	40	41	41	42	40	44	41	40*	40	42
	−1	41	41	41	40	38	43	43	41	39	41
	13	46	49	50	50	40	56	51	48*	49*	50*
	22	40	–	–	40	27	49	–	–	41	43
	24	43	–	–	46	31	51	–	–	41	44
	26	47	49	49	47	28	55	52	51	46**	46**
	39	47	48	47	47	–	51	51	48	44*	46*
	52	49	52	52	53	–	58	53	53	49*	54*
Hb (g/dL)	−2	12.7	12.9	12.9	13.0	12.7	13.8	13.5	12.6	12.7	13.4
	−1	13.0	13.3	13.0	12.4	12.3	13.6	13.8	13.5	12.7	12.9
	13	12.9	14.1	13.8	14.2	11.1	15.6	14.6	13.8	14.3	14.5
	22	13.5	–	–	13.5	8.8	16.3	–	–	13.9	14.3
	24	14.2	–	–	14.8	9.5	16.4	–	–	13.5	13.9
	26	14.2	15.1	14.9	14.1	8.0	16.8	15.9	15.7	14.2*	14.0**
	39	14.9	15.0	14.6	14.4	–	15.9	16.1	15.1	13.7*	14.0*
	52	15.4	15.7	15.3	15.4	–	17.5	15.7	15.8	14.3*	15.5*
RBC (× 10^6/mm^3)	−2	5.1	5.1	5.3	5.2	5.1	5.6	5.3	5.1*	5.0*	5.3
	−1	5.2	5.2	5.2	4.9	5.0	5.6	5.5	5.3	4.9*	5.2
	13	5.4	5.7	5.9	6.0	4.9	6.9	5.9*	5.8*	6.1*	6.4*
	22	5.5	–	–	5.5	3.6	6.7	–	–	5.6	6.1
	24	5.7	–	–	6.0	3.9	6.7	–	–	5.4	5.9
	26	5.8	6.0	6.2	5.8	3.3	6.9	6.3	6.4	5.8*	6.0**
	39	6.2	6.1	6.2	6.0	–	6.7	6.5	6.1	5.7	6.0
	52	6.2	6.3	6.6	6.6	–	7.2	6.4*	6.5*	6.0*	6.9*
MCHC (%)	−2	31.5	31.6	31.4	31.2	31.8	31.6	32.5**	31.9	32.2	32.2
	−1	31.5	32.0	31.5	31.4	32.1	31.8	32.4	32.7*	32.7*	31.8
	13	27.7	28.7	27.9	28.6	27.6	27.9	28.5	28.8*	29.0*	28.8*
	26	30.5	30.9	30.4	29.6	28.0	30.5	30.5	31.1	30.8	30.7
	39	31.6	31.3	31.0	30.7	–	31.2	31.3	31.6	30.8	30.5
	52	29.7	30.4	29.4	29.1	–	29.9	29.9	30.1	29.2	28.9*
Platelets (×10^3/mm^3)	−2	421	389	366	400	437	288	389	374	391	408*
	−1	371	363	317	365	383	345	374	356	367	350
	13	361	337	352	407	423	297	320	320	419*	426*
	26	357	353	380	476	334	308	324	334	459*	421*
	39	359	358	365	401	–	328	319	306	439	425
	52	345	396	357	411	–	305	356	320	471*	414*

Hb: haemoglobin; MCHC: mean corpuscular cell haemoglobin; PCV: packed cell volume; RBC: red blood cells; *: $P < 0.05$; **: $P < 0.01$

Source: Brooker (1994a)

Table 14. Treatment-related clinical chemistry parameters in the dog 52-week dietary study with isoxaflutole

End-point	Week	Males					Females				
		0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm	0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm
Albumin (g/dL)	–2	2.6	2.7	2.7	2.7	2.7	2.7	2.6	2.6	2.7	2.6
	–1	2.6	2.6	2.6	2.5	2.6	2.6	2.7	2.6	2.6	2.6
	13	2.7	2.7	2.6	2.3**	2.1**	2.9	2.8	2.7	2.3**	2.2**
	26	2.7	2.8	2.6	2.3*	2.0**	2.9	3.0	2.9	2.4**	2.3**
	39	2.8	2.9	2.6	2.3*	–	2.9	3.0	2.8	2.3**	2.3**
	52	2.8	2.8	2.6	2.3**	–	3.0	2.8	2.8	2.2**	2.3**
Globulin (g/dL)	–2	2.5	2.5	2.4	2.6	2.5	2.6	2.4	2.3*	2.4	2.5
	–1	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.3	2.2	2.4
	13	2.7	2.6	2.6	2.7	3.1	2.7	2.4	2.4	2.6	2.7
	26	3.0	2.6	2.9	2.8	3.3	2.8	2.6	2.7	2.9	2.7
	39	2.8	2.8	3.1	3.0	–	2.8	2.8	2.7	3.0	2.9
	52	2.8	2.6	2.8	2.9	–	2.8	2.9	2.8	3.4	3.0
Albumin/ globulin ratio	–2	1.05	1.06	1.10	1.04	1.06	1.03	1.11	1.13	1.10	1.08
	–1	1.09	1.11	1.07	1.05	1.06	1.06	1.13	1.16	1.16	1.09
	13	1.03	1.05	0.97	0.83	0.71**	1.08	1.20	1.11	0.88**	0.81**
	26	0.90	1.09	0.92	0.82	0.62**	1.05	1.15	1.11	0.87	0.85
	39	0.99	1.03	0.89	0.78	–	1.04	1.07	1.06	0.82*	0.79**
	52	0.98	1.08	0.95	0.81	–	1.08	0.98	1.04	0.69**	0.77**
Total protein (g/dL)	–2	5.2	5.2	5.1	5.2	5.2	5.3	5.0	5.0*	5.1	5.1
	–1	5.0	5.0	5.0	4.9	5.0	5.0	5.0	4.9	4.8	5.1
	13	5.4	5.3	5.2	5.0	5.2	5.6	5.2	5.1*	4.9**	5.0**
	26	5.7	5.4	5.5	5.1*	5.3*	5.7	5.6	5.6	5.3	5.0*
	39	5.6	5.6	5.7	5.4	–	5.7	5.7	5.5	5.3	5.2
	52	5.6	5.4	5.4	5.2	–	5.7	5.6	5.6	5.6	5.2
AP (mU/mL)	–2	239	240	249	249	297	306	224	208	244	275
	–1	235	245	235	241	266	286	218	205	240	268
	13	175	183	188	468**	636**	192	142	169	354**	490**
	26	217	160	167	610**	1 100**	169	141	158	540**	744**
	39	124	156	165	646**	–	146	141	150	479**	813**
	52	105	129	145	502**	–	136	117	155	498**	1 029**
ALAT (mU/mL)	–2	20	21	22	20	25	23	23	24	25	21
	–1	19	20	21	20	22	22	22	23	22	22
	13	21	28	23	31	72**	26	32	26	40*	50*
	26	21	27	24	28	38*	25	27	27	43*	40*
	39	22	29	24	31	–	24	27	24	39*	43*
	52	18	27	23	33**	–	25	25	22	27	29

Table 14 (continued)

End-point	Week	Males					Females				
		0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm	0 ppm	240 ppm	1 200 ppm	12 000 ppm	30 000 ppm
GGT (mU/mL)	26	3	3	3	4	5	3	3	2	3	4**
	39	2	< 3	< 2	3	–	< 2	< 2	< 2	3	4*
	52	3	< 3	< 3	3	–	3	< 1	< 2	2	3
5′-Nucleotidase (mU/mL)	26	< 0.4	< 0.4	0.2	< 1.6	2.6**	< 0.5	< 0.5	< 0.3	1.5*	3.3**
	39	0.1	0.2	0.5	2.6**	–	0.5	0.2	0.7	1.7*	3.5**
	52	< 0.2	< 0.2	< 0.2	1.3**	–	< 0.4	< 0.1	< 0.3	1.0*	3.1**
Calcium (meq/L)	–2	5.8	5.7	5.6	5.8	5.7	5.8	5.7	5.6	5.7	5.9
	–1	5.7	5.6	5.6	5.6	5.6	5.7	5.7	5.5	5.6	5.6
	13	5.3	5.4	5.3	5.1*	5.0**	5.5	5.3*	5.3*	5.1**	5.0**
	26	5.4	5.3	5.3	5.1*	5.0**	5.5	5.5	5.4	5.1**	5.1**
	39	5.2	5.1	5.1	5.0*	–	5.3	5.2	5.1	5.0	5.0
	52	5.1	5.1	5.1	4.9*	–	5.4	5.1*	5.2*	4.9**	4.9**
Urea (mg/dL)	–2	23	22	21	23	20	20	20	21	27	20
	–1	27	27	26	21*	25	23	26	24	26	23
	13	33	33	30	26	28	35	34	33	30	26
	26	29	26	24	20	30	27	28	27	25	21
	39	29	25	23	22	–	30	26	26	27	20**
	52	33	27*	27*	21**	–	30	24	27	24*	21**

ALAT: alanine aminotransferase; AP: alkaline phosphatase; eq: equivalents; GGT: gamma-glutamyltransferase; U: units; *: $P < 0.05$; **: $P < 0.01$
Source: Brooker (1994a)

(b) Dermal application

Rats

In a 21-day dermal toxicity study, eight CD rats of each sex per group were treated topically with isoxaflutole (purity not reported) at a dose of 0, 10, 100 or 1000 mg/kg bw per day, 8 hours/day, for 21 days. The test material was applied in 0.5% w/v methyl cellulose in purified water daily at a dosing volume of 2 mL/kg bw.

There were no deaths or signs of systemic toxicity observed during the study. Dermal irritation, including slight erythema and slight exfoliation on days 3 and 4, was observed in one female at 1000 mg/kg bw per day. Because of the isolated nature of the incidence, this finding was attributed to incidental causes. Mean body weights, body weight gains (days 0–20), mean feed consumption and mean feed efficiency of the treated animals did not significantly differ from those of the controls. There were no treatment-related changes in the haematology or clinical chemistry parameters. The lymphocyte counts of treated animals were slightly lower than those of the control animals, the values were within the normal ranges and there was no dose–response relationship. No treatment-related effects were noted. There were no treatment-related changes on gross necropsy examination of the animals. At 1000 mg/kg bw per day, the absolute liver weights of female rats and relative liver weights of male and female rats were higher (> 10%) than those of the control animals. This finding was considered to be treatment related but an adaptive response. Although increases (7%) in the liver weights were also noted at 100 and 10 mg/kg bw per day, the differences were not statistically significant. The only findings on histopathology involved the skin. These were confined to encrustations seen in one female rat from the 10 mg/kg bw per day group, two females from the

100 mg/kg bw per day group and one male from the 1000 mg/kg bw per day group at necropsy. These changes were of slight and minimal degree and showed no relationship to treatment.

In conclusion, based on lack of systemic toxicity, the NOAEL was 1000 mg/kg bw per day, the highest dose tested (Cummins, 1994).

(c) Exposure by inhalation

No studies are available.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 78-week carcinogenicity study, isoxaflutole (purity 98.7%) was given as a dietary admix to 64 or 76 mice of each sex per dose at 0, 25, 500 or 7000 ppm daily (equal to 0, 3.2, 64.4 and 977.3 mg/kg bw per day for males and 0, 4.0, 77.9 and 1161.1 mg/kg bw per day for females, respectively). Interim sacrifices were made at 26 weeks (12 mice of each sex at the 0 and 7000 ppm doses) and at 52 weeks (12 mice of each sex at all dose levels).

There was no treatment-related effect on mortality. The survival rates of the treated groups were not significantly different from those of the controls. There were no clinical signs, including palpable swellings, that differed between control and treated animals. Body weights and body weight gains were significantly lower than those of controls for male mice in the 500 and 7000 ppm treatment groups and for female mice in the 7000 ppm treatment groups. Feed consumption was unaffected by treatment; however, feed efficiency was lower in both sexes compared with controls at 7000 ppm during the first 14 weeks of the study (not determined after week 14). No treatment-related ocular changes were observed during the study. Blood from the control and 7000 ppm treatment groups was examined only for differential leukocyte counts by Romanowsky stain and direct visual count. No treatment-related differences in this parameter were observed. Blood from the 25 and 500 ppm treatment groups was not examined. Absolute and relative liver weights of male and female mice in the 7000 ppm treatment group were significantly higher (24–207%, $P < 0.01$) than those of the controls after 26, 52 and 78 weeks of treatment. There were significant differences in the absolute and relative adrenal weights of females in the 7000 ppm treatment group at 26 weeks and of males in the 7000 ppm treatment group at 52 weeks. However, in the absence of any histopathological findings, effects on adrenal weights were not considered as adverse. Macroscopic examination revealed a higher incidence of enlarged or swollen livers and/or liver masses and/or "areas of change" on the livers of male and female mice in the 7000 ppm treatment group. No other treatment-related gross postmortem differences were observed between mice in the 7000 ppm and the control groups.

Significant increases in microscopic liver abnormalities were observed in both sexes as early as the 26-week interim kill and were detected with increasing frequency at longer sacrifice intervals. At the 26-week kill, all of the high-dose males (12/12, $P < 0.001$) and females (12/12, $P < 0.001$) had developed periacinar hepatocytic hypertrophy. Other significant liver lesions at the 26-week kill of high-dose animals were hepatocyte necrosis (males 10/12, $P < 0.001$; females 7/12, $P < 0.01$) and pigmented Kupffer cells (males 10/12, $P < 0.001$). There were also non-significant incidences in high-dose males of periacinar hepatocytic fatty vacuolation (2/12) and pigment-laden hepatocytes (3/12), compared with none in controls. At 52 weeks, there were significant increases in periacinar hepatocytic hypertrophy in males and females, and periacinar hepatocytic fatty vacuolation decreased in males and increased in females ($P < 0.001$ and $P < 0.01$, respectively) in the 7000 ppm group. In addition, the high-dose males showed significant increases in other hepatocyte abnormalities. A significant increase in spleen extramedullary haematopoiesis (7/12, $P < 0.01$) was observed in high-dose males killed at 52 weeks. Male mice in the 500 ppm group exhibited a significant increase in periacinar hepatocytic hypertrophy (7/12, $P < 0.01$, versus none in controls).

Notable non-neoplastic lesions observed in mice in the 78-week terminal group are summarized in Table 15. Findings from animals sacrificed at termination and those having unscheduled deaths are presented separately, as each category was subjected to separate statistical

Table 15. Incidences of treatment-related non-neoplastic lesions in mice fed isoxaflutole for 78 weeks

Site and lesion	No. observed/no. examined			
	0 ppm	25 ppm	500 ppm	7 000 ppm
Males				
Liver				
Periacinar hepatocytic hypertrophy	0/37[a]	0/31	2/37	14***/36
	0/15[b]	*0/21*	*0/15*	*4/16*
Individual hepatocyte necrosis	4/37	5/31	12*/37	25***/36
	1/15	*0/21*	*1/15*	*8*/16*
Pigment-laden hepatocytes	0/37	0/31	0/37	6*/36
	0/15	*0/21*	*0/15*	*5*/16*
Erythrocyte-containing hepatocytes	0/37	1/31	4/37	11***/36
	NR	*NR*	*NR*	*NR*
Pigment-laden Kupffer cells	1/37	2/31	4/37	31***/36
	1/15	*0/21*	*1/15*	*12***/16*
Periacinar hepatocytic fatty vacuolation	17/37	17/31	14/37	9/36
	2/15	*0/21*	*1/15*	*2/16*
Basophilic foci	1/37	2/31	0/37	9**/36
	0/15	*0/21*	*0/15*	*1/16*
Clear cell foci	5/37	0/31	3/37	11/36
	NR	*NR*	*NR*	*NR*
Increased ploidy	0/37	0/31	2/37	10***/36
	0/15	*0/21*	*0/15*	*2/16*
Spleen				
Extramedullary haematopoiesis	3/37	4/6	4/8	11*/36
	4/15	*8/21*	*7/15*	*7/15*
Thyroid				
Amyloidosis	2/37	–/0	–/0	12**/36
	3/15	*2/21*	*0/15*	*6/16*
Females				
Liver				
Periacinar hepatocytic hypertrophy	0/43	0/39	1/39	17***/46
	0/9	*0/13*	*2/13*	*1/6*
Individual hepatocyte necrosis	0/43	1/39	0/39	8**/46
	1/9	*0/13*	*0/13*	*0/6*
Pigment-laden hepatocytes	0/43	0/39	0/39	0/46
	0/9	*0/13*	*1/13*	*0/6*
Erythrocyte-containing hepatocytes	0/43	1/39	1/39	9**/46
	NR	*NR*	*NR*	*NR*
Pigment-laden Kupffer cells	7/43	4/39	2/39	10/46
	1/9	*1/13*	*2/13*	*1/6*
Periacinar hepatocytic fatty vacuolation	13/43	9/39	7/39	26*/46
	0/9	*1/13*	*1/13*	*1/6*
Basophilic foci	0/43	0/39	0/39	0/46
	0/9	*0/13*	*0/13*	*0/6*

Table 15 (continued)

Site and lesion	No. observed/no. examined			
	0 ppm	25 ppm	500 ppm	7 000 ppm
Clear cell foci	0/43	0/39	0/39	0/46
	NR	*NR*	*NR*	*NR*
Increased ploidy	0/43	0/39	0/39	0/46
	0/9	*0/13*	*0/13*	*0/6*
Spleen				
Extramedullary haematopoiesis	3/43	3/8	4/10	12*/46
	6/9	*8/13*	*5/13*	*5/6*
Thyroid				
Amyloidosis	1/43	–/0	–/0	14***/46
	0/9	*0/13*	*0/13*	*0/6*

NR: not reported; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

[a] Data are from the mice killed on schedule at 78 weeks.

[b] Data in italics are from unscheduled deaths occurring throughout the 78-week study period.

Source: Chase (1995b)

analysis. Male and female mice in the 7000 ppm treatment group exhibited an increased incidence of periacinar hepatocytic hypertrophy, necrosis of individual hepatocytes and erythrocytes in hepatocytes, pigment-laden hepatocytes (only in males) and Kupffer cells, compared with the controls; male mice also exhibited basophilic foci of hepatocellular alteration and increased ploidy. Male mice in the 500 ppm group exhibited necrosis of individual hepatocytes (12/37, $P < 0.05$). In addition, mice in the 7000 ppm treatment group had a higher incidence of systemic amyloidosis, involving the kidneys, small intestine, stomach, thyroid, mesenteric lymph nodes and heart. Both sexes were significantly affected at the high dose. Amyloidosis of the thyroid occurred in 12/36 ($P < 0.01$) terminal kill males, 6/16 unscheduled male deaths and 14/46 ($P < 0.001$) terminal kill females at the high dose.

No neoplastic lesions that were considered to have been test article related were observed among males or females sacrificed at 26 weeks or among females in the 52-week interim group. At the 52-week sacrifice, 7/12 ($P < 0.05$) males at 7000 ppm showed an increased incidence of adenomas; only 1/12 males in the 500 ppm group developed carcinoma. For the 78-week terminal groups, Table 16 summarizes the data for unscheduled deaths and scheduled sacrifices, respectively, and the overall incidence. The tumour incidence among unscheduled deaths was similar to that observed in the scheduled sacrifices. There was a significant increase in the occurrence of adenomas (7/16, $P < 0.05$) in males at 7000 ppm. In animals sacrificed on schedule, significant ($P < 0.01$) increases in the incidence of adenomas and carcinomas were observed in the 7000 ppm males, and adenomas were increased significantly ($P < 0.001$) in the 7000 ppm females. The overall incidences of adenomas and carcinomas in both sexes at 7000 ppm were each considerably higher than the maxima of the historical control ranges. In males at 500 ppm, adenomas and carcinomas occurred with frequencies of 17% and 15%, higher than the corresponding mean historical control values of 15% and 6%. Statistical analysis of neoplastic tissue data indicated significant positive treatment-related trends in the incidence of benign, malignant and combined benign/malignant liver tumours. Pair-wise comparisons between the 7000 ppm treatment group and the controls for benign and combined tumour incidences were statistically significant.

No liver tumours were observed in mice sacrificed at 26 weeks. Adenomas appeared at approximately 1 year in controls and all dose groups of males. Carcinoma appearance, however, showed a clear trend from 78 days in controls to 47 days in the 7000 ppm dose group (the data were not analysed statistically). In females, no adenomas were observed before 77 weeks in any group. Carcinomas were not seen in any control or treated female through 500 ppm; in the 7000 ppm group,

Table 16. Overall incidence of liver tumours in mice fed isoxaflutole (78-week terminal phase; scheduled + unscheduled sacrifices)

	0 ppm	25 ppm	500 ppm	7 000 ppm	Historical controls[a]	
					Mean	Range
Unscheduled deaths						
Males						
No. examined	15	21	15	16	–	–
Hepatocellular adenoma	1 (7%)	1 (7%)	1 (7%)	7* (44%)	–	–
Hepatocellular carcinoma	1 (7%)	3 (14%)	3 (20%)	4 (25%)	–	–
Adenomas and/or carcinomas combined	2 (13%)	4 (19%)	4 (27%)	10[b] (63%)	–	–
Females						
No. examined	9	13	13	6	–	–
Hepatocellular adenoma	0 (0%)	0 (0%)	0 (0%)	2 (33%)	–	–
Hepatocellular carcinoma	0 (0%)	0 (0%)	0 (0%)	1 (17%)	–	–
Adenomas and/or carcinomas combined	0 (0%)	0 (0%)	0 (0%)	3 (50%)	–	–
Scheduled sacrifice						
Males						
No. examined	37	31	37	36	–	–
Hepatocellular adenoma	8 (22%)	9 (29%)	8 (22%)	20** (56%)	–	–
Hepatocellular carcinoma	3 (8%)	2 (6%)	5 (14%)	13** (36%)	–	–
Adenomas and/or carcinomas combined	11 (30%)	11 (35%)	10[b] (27%)	28[b] (78%)	–	–
Females						
No. examined	43	39	39	46	–	–
Hepatocellular adenoma	0 (0%)	1 (3%)	1 (3%)	13*** (28%)	–	–
Hepatocellular carcinoma	0 (0%)	0 (0%)	0 (0%)	3 (7%)	–	–
Adenomas and/or carcinomas combined	0 (0%)	1 (3%)	1 (3%)	15[b] (33%)	–	–
Unscheduled deaths and scheduled sacrifice combined						
Males (n = 52/group)						
Hepatocellular adenoma	9 (17%)[†]	10 (19%)	9 (17%)	27* (52%)	15.0%	3.8–23.1%
Hepatocellular carcinoma	4 (8%)[†]	5 (10%)	8 (15%)	17* (33%)	6.28%	1.9–11.5%
Adenomas and/or carcinomas combined	13 (25%)[†]	15 (29%)	14 (27%)	38* (73%)	Data not provided	

Table 16 (continued)

	0 ppm	25 ppm	500 ppm	7 000 ppm	Historical controls[a]	
					Mean	Range
Females (n = 52/group)						
Hepatocellular adenoma	0 (0%)†	1 (2%)	1 (2%)	15* (29%)	0.27%	0–2.0%
Hepatocellular carcinoma	0 (0%)‡	0 (0%)	0 (0%)	4 (8%)	0.55%	0–2.0%
Adenomas and/or carcinomas combined	0 (0%)†	1 (2%)	1 (2%)	18* (35%)	Data not provided	

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$ for unscheduled deaths and scheduled sacrifice group; *: $P < 0.001$ (pair-wise comparison); †: $P < 0.001$; ‡: $P < 0.05$ (trend test) for combined group

[a] Historical control data obtained from Chase (1995b); incidence data from seven studies showed that of the 366 males and 366 females examined, 55 males (15.03%) and 1 female (0.27%) had adenomas and 23 males (6.28%) and 2 females (0.55%) had carcinomas.

[b] Some animals developed both adenoma and carcinoma.

Source: Chase (1995b)

the onset of carcinomas was 60 weeks. Only 1/12 males at 500 ppm among unscheduled deaths in the 52-week interim sacrifice developed hepatocellular carcinoma.

In conclusion, the NOAEL for this study was 25 ppm (equal to 3.2 mg/kg bw per day), based on increased liver weights and increased histopathological changes (increased incidence of periacinar hepatocytic hypertrophy and necrosis of the individual hepatocytes) at 500 ppm (equal to 64.4 mg/kg bw per day). The NOAEL for carcinogenicity was 500 ppm (equal to 64.4 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas and carcinomas in both sexes at 7000 ppm (equal to 977.3 mg/kg bw per day) (Chase, 1995b).

Rats

In a combined chronic toxicity and carcinogenicity study, isoxaflutole (purity 98.3–99.2%) was continuously administered to 75 Sprague-Dawley rats of each sex per dose at a dietary level of 0, 0.5, 2, 20 or 500 mg/kg bw per day for 104 weeks. An additional 20 rats of each sex per group were treated for 52 weeks, after which 10 rats of each sex per group were killed and the remainder were held for a maximum of 8 weeks without treatment in order to assess reversibility of treatment-related changes.

There was no treatment-related effect on mortality. Mortality was statistically significantly reduced at 500 mg/kg bw per day in both males and females in the oncogenicity phase of the study. Significant treatment-related clinical findings were observed in both sexes at 500 mg/kg bw per day and included opaque eyes, thin body build, abnormal gait and limited use of hindlimbs. From the 1st week of the study, mean body weight gains in the 500 mg/kg bw per day group rats were lower than those of controls; from weeks 0 through 104, body weight gains were decreased by 36% and 49% ($P < 0.01$) in males and females, respectively. These decreased body weight gains were considered to be treatment related. During the 6-week recovery phase (following 52 weeks of treatment), the 500 mg/kg bw per day group male and female rats had 104% and 59% weight gain increases, respectively, over the controls ($P < 0.01$ in males only), indicating partial recovery. Body weight gains of animals in the 0.5, 2 and 20 mg/kg bw per day groups were unaffected by treatment. Feed consumption in the 500 mg/kg bw per day group females (104-week terminal phase) was decreased (4–17%) at four consecutive 6-month intervals; overall feed consumption was 12% lower than in controls. No adverse effects on feed consumption were seen in males. Feed consumption for treated animals was similar to that of control rats during the 6-week recovery period. Feed conversion efficiency in the 104-week terminal phase rats, calculated through week 14, was lower in the 500 mg/kg bw per day group males

(12%) and females (19%) compared with controls. Ophthalmoscopic examinations (Table 17) revealed treatment-related corneal lesions, ranging from small focal superficial opacities to large corneal opacities with associated vascularization and iritis in males at 20 mg/kg bw per day and in both sexes at 500 mg/kg bw per day, with the rate of incidence greater in females and severity of the lesions greater in males.

Table 17. Number of rats with treatment-related corneal lesions[a]

Study week	Number observed/number examined				
	0 mg/kg bw per day	0.5 mg/kg bw per day	2 mg/kg bw per day	20 mg/kg bw per day	500 mg/kg bw per day
Males					
5	0/45	0/45	0/45	0/45	28/45 (62%)
11	0/45	0/45	0/45	0/45	27/45 (60%)
23	0/45	0/45	0/45	2/45 (4%)	29/45 (64%)
37	0/44	0/45	0/45	1/45 (2%)	32/44 (73%)
49	0/44	0/45	0/45	4/45 (9%)	33/44 (75%)
75	0/25	0/25	0/25	2/25 (8%)	18/25 (72%)
101	0/25	0/25	0/25	4/25 (16%)	20/25 (80%)
Females					
5	0/45	0/45	0/45	0/45	30/45 (67%)
11	0/45	0/45	0/45	0/45	38/45 (84%)
23	0/45	0/45	0/45	0/45	42/45 (93%)
37	0/44	0/44	0/45	1/45 (2%)	39/44 (89%)
49	0/43	0/44	0/45	1/45 (2%)	39/44 (89%)
75	0/25	0/25	0/25	0/25	23/25 (92%)
101	0/25	0/25	0/25	0/25	16/25 (64%)

[a] Expressed as the number of animals with the reported finding in one or both eyes.
Source: Chase (1995a)

During 102 weeks of treatment, a few changes in platelet count as well as erythrocyte count and mean haemoglobin values were seen in the 500 mg/kg bw per day group rats. These differences, although occasionally statistically significant, were generally minor and not dose related and therefore were judged not to be of toxicological concern. These changes were no longer apparent after a 7-week recovery period (following 52 weeks of treatment). There were treatment-related clinical chemistry and urine analysis findings in both sexes at 20 and 500 mg/kg bw per day; however, after a 7-week recovery period, there were no findings that were considered to be biologically or toxicologically significant in either sex. Significant ($P < 0.05$, 0.01 or 0.001) decreases in AP (decreased 3–49%), ALAT (decreased 14–53%) and ASAT (decreased 14–53%) activities and changes in levels of urea (increased 16–31%), glucose (decreased 0–18%), potassium (increased 6–17%), chloride (decreased 2–5%) and total plasma protein (increased 7–18%) were seen in one or both sexes primarily in the 500 mg/kg bw per day dose group in the 104-week study. Cholesterol levels were increased throughout the dosing period in the 500 mg/kg bw per day dose group animals (32–87% increases over controls; $P < 0.05$). In the 20 mg/kg bw per day males, cholesterol was elevated (29–54%; $P < 0.05$). Levels were also increased at the 6-, 50-, 78- and 102-week intervals in the 20 mg/kg bw per day dose group females (21–48%; $P < 0.05$ or 0.01). The overall evidence is inconclusive with respect to the biological importance of the elevated cholesterol levels. Urine analysis at 50 weeks indicated changes in pH (decreased 4–22%), urinary output (decreased 40% only in week 77 in females; male urinary output slightly increased [about 20%] in both week 50 and week 77), specific gravity (increased 1–3%), total reducing substances, ketones and colour. These findings were detected primarily in the 500

mg/kg bw per day group. In females at 20 mg/kg bw per day, specific gravity was increased at 77 weeks of treatment. After 101 weeks of treatment, significant differences were seen only in urinary pH (decreased 6%) in males and specific gravity (increased 2%) in females. Urine was positive for total reducing substances after 50 weeks for rats receiving 500 mg/kg bw per day, but not at 101 weeks. Furthermore, after the 6-week recovery period, the urinary parameters in the control and treated rats were similar.

Among animals sacrificed at 52 weeks, in males at 20 mg/kg bw per day and in both males and females at 500 mg/kg bw per day, absolute and relative liver weights were increased relative to controls. Absolute and relative thyroid weights were increased in males and relative thyroid weight was increased in females at 500 mg/kg bw per day (Table 18). After the 8-week recovery period (following 52 weeks of treatment), absolute and relative thyroid weights remained increased in the high-dose females. Relative kidney weight in males at 500 mg/kg bw per day was 19% higher than in the controls. In animals sacrificed at 104 weeks, in males at 20 mg/kg bw per day and in both males and females at 500 mg/kg bw per day, absolute and relative liver weights were increased relative to controls. In males at 500 mg/kg bw per day, absolute and relative kidney weights were increased compared with control animals. In females at 500 mg/kg bw per day, the absolute and relative weights of the uterus and cervix weighed together were increased relative to controls. Treatment-related gross pathological findings at 20 and 500 mg/kg bw per day included swollen livers (males at 20 and 500 mg/kg bw per day), "areas of change" on lungs (males at 500 mg/kg bw per day), masses (males and females at 500 mg/kg bw per day) in the liver, opaque eyes (males at 20 and 500 mg/kg bw per day) and dark enlarged thyroids with masses (males at 20 and 500 mg/kg bw per day). At 20 mg/kg bw per day, the incidence of enlargement of the thyroid was increased in males only.

Table 18. Terminal body weight and organ weights in the rat oncogenicity study with isoxaflutole

Observation	Males					Females				
	0 mg/kg bw per day	0.5 mg/kg bw per day	2 mg/kg bw per day	20 mg/kg bw per day	500 mg/kg bw per day	0 mg/kg bw per day	0.5 mg/kg bw per day	2 mg/kg bw per day	20 mg/kg bw per day	500 mg/kg bw per day
12 months										
N	10	10	10	10	10	7	9	10	10	10
Terminal body weight (g)	835.3	852.5	770.0	759.8	680.3*	414.7	428.5	412.8	449.6	303.1
Liver weight (g)	22.8	26.1	24.8	28.5*	40.8**	14.0	15.1	13.5	15.7	18.8**
Liver weight (% of body weight)	2.75	3.01	3.25	3.78**	6.11**	3.46	3.50	3.27	3.48	6.38**
Thyroid weight (g)	0.027	0.033	0.031	0.030	0.047*	0.021	0.021	0.020	0.027	0.023
Thyroid weight (% of body weight)	0.003 2	0.003 9	0.004 0	0.004 0	0.006 9**	0.005 3	0.004 8	0.004 8	0.006 0	0.007 9*
12 months + 8 weeks of reversibility										
N	9	9	10	10	9	10	10	10	10	8
Terminal body weight (g)	803.4	856.6	812.1	868.7	737.6	445.2	485.8	488.5	446.7	384.1
Liver weight (g)	25.1	28.1	23.8	31.0*	24.9	14.8	16.4	15.6	15.2	14.6

Observation	Males					Females				
	0 mg/kg bw per day	0.5 mg/kg bw per day	2 mg/kg bw per day	20 mg/kg bw per day	500 mg/kg bw per day	0 mg/kg bw per day	0.5 mg/kg bw per day	2 mg/kg bw per day	20 mg/kg bw per day	500 mg/kg bw per day
Liver weight (% of body weight)	3.11	3.28	2.93	3.58	3.40	3.34	3.37	3.21	3.40	3.83
Thyroid weight (g)	0.029	0.033	0.034	0.043	0.035	0.020	0.020	0.021	0.021	0.028*
Thyroid weight (% of body weight)	0.003 7	0.003 8	0.004 2	0.004 9	0.004 9	0.004 5	0.004 3	0.004 4	0.004 7	0.007 3**
24 months										
N	34	25	34	37	46	35	23	25	22	48
Terminal body weight (g)	802.0	850.8	870.9	798.1	568.7**	548.7	551.1	564.5	543.6	347.0**
Liver weight (g)	27.4	25.0	30.3	31.4*	42.8**	20.5	21.7	19.2	22.0	25.5**
Liver weight (% of body weight)	3.55	2.99*	3.58	4.08	7.62**	3.81	3.95	3.43*	4.11	7.51**
Thyroid weight (g)	0.051	0.055	0.046	0.063	0.055	0.046	0.031	0.042	0.034	0.029
Thyroid weight (% of body weight)	0.006 1	0.006 5	0.005 3	0.008 2	0.009 5**	0.009 5	0.005 8	0.006 9	0.006 4	0.008 5
Kidney weight (g)	5.47	5.06	5.84	5.77	6.37*	3.96	3.82	3.66	3.67	3.26**
Kidney weight (% of body weight)	0.723	0.616	0.695	0.744	1.137**	0.767	0.728	0.661	0.697	0.952**
Uterus weight (g)	–	–	–	–	–	0.85	1.00	0.78	0.84	1.10*
Uterus weight (% of body weight)	–	–	–	–	–	0.163	0.191	0.141	0.165	0.334**

*: $P < 0.05$; **: $P < 0.01$

Source: Chase (1995a)

At terminal sacrifice, treatment-related non-neoplastic findings in the liver included periacinar hepatocytic hypertrophy (both males and females at 20 and 500 mg/kg bw per day), focal cystic degeneration (males at 20 and 500 mg/kg bw per day), midzonal foamy hepatocytes (males at 20 mg/kg bw per day and both males and females at 500 mg/kg bw per day), portal tract senile changes in bile duct (both males and females at both 20 and 500 mg/kg bw per day), basophilic and clear cell foci (females at 500 mg/kg bw per day) and pigment-laden hepatocytes (females at 500 mg/kg bw per day). Treatment-related lesions of the eye were observed in males and included increased incidences of keratitis (2, 20 and 500 mg/kg bw per day), vascularization of the stroma (500 mg/kg bw per day), epithelial thickening (20 and 500 mg/kg bw per day) and superficial exfoliated epithelial cells (500 mg/kg bw per day). Other treatment-related non-neoplastic findings included increased incidences of thyroid cystic follicular hyperplasia, axonal and myelin sciatic nerve degeneration, and focal degeneration and inflammation of the thigh muscle in males at 20 and 500 mg/kg bw per day and in females at 500 mg/kg bw per day.

There were no treatment-related neoplastic lesions detected in the animals at the interim kill or on completion of the recovery period. The incidences of selected neoplastic lesions detected in animals that were killed or died during the study and at the 104-week terminal sacrifice are presented in Table 19. Treatment-related neoplastic lesions were detected in the livers and thyroid glands. In the 500 mg/kg bw per day group rats (both sexes), there were significant increases in the incidences of hepatocellular adenomas, hepatocellular carcinomas, and combined adenomas plus carcinomas (41% and 62% for males and females, respectively). In both sexes at 500 mg/kg bw per day, the incidence of carcinomas (23% and 32% for males and females, respectively) contributed to the overall increase in liver tumour incidence; animals with carcinomas accounted for over half of the total number of animals bearing adenomas and/or carcinomas. Thyroid follicular cell adenomas showed a significantly increased incidence ($P < 0.01$) in males at 500 mg/kg bw per day, but not females, although there were positive trends for both sexes ($P < 0.05$). There were no treatment-related neoplastic lesions detected in the 0.5, 2 or 20 mg/kg bw per day dose group rats.

Table 19. Overall incidence of liver and thyroid tumours in rats fed isoxaflutole (104-week terminal phase)[a]

Site/tumour	0 mg/kg bw per day	0.5 mg/kg bw per day	2 mg/kg bw per day	20 mg/kg bw per day	500 mg/kg bw per day	Historical controls[b]	
						Mean (%)	Range (%)
Males							
Liver							
Hepatocellular adenoma	2/75 (2.7)[‡c]	3/75 (4.0)	5/75 (6.7)	6/75 (8.0)	14**/75 (18.7)	2.51	0–10
Hepatocellular carcinoma	5/75 (6.7)[‡]	1/75 (1.4)	4/75 (5.3)	2/75 (2.7)	17**/75 (22.7)	2.28	0–6
Hepatocellular adenomas and/or carcinomas combined	7/75 (9.3)[†]	4/75 (5.3)	9/75 (12)	8/75 (10.7)	31***/75 (41.3)	Data not provided	
Thyroid							
Follicular cell adenoma	3/74 (4.1)[†]	1/72 (1.3)	5/74 (6.8)	7/75 (9.3)	15**/75 (20)	3.04	0–6.4
Follicular cell carcinoma	0/74	1/72	2/74	1/75	3/75	–	–
Females							
Liver							
Hepatocellular adenoma	4/75[†] (5.3)	2/75 (2.7)	1/75 (1.3)	0/75 (0)	29***/74 (39.2)	1.19	0–3.6
Hepatocellular carcinoma	0/75[†] (0)	0/75 (0)	1/75 (1.3)	0/75 (0)	24***/74 (32.4)	0.00	0–0
Hepatocellular adenomas and/or carcinomas combined	4/75[†] (5.3)	2/75 (2.7)	2/75 (2.7)	0/75 (0)	46***/74 (62.2)	Data not provided	
Thyroid							
Follicular cell adenoma	1/74[‡] (1.4)	0/73 (0)	1/73 (1.4)	4/74 (5.4)	3/73 (4.1)	0.72	0–2.0
Follicular cell carcinoma	0/74 (0.0)	1/73 (1.4)	1/73 (1.4)	0/74 (0.0)	2/73 (2.7)	–	–

**: $P < 0.01$; ***: $P < 0.001$ (pair-wise analysis); †: $P < 0.001$; ‡: $P < 0.05$ (trend analysis)

[a] Includes scheduled kills and unscheduled deaths.

[b] Historical control incidence data from eight studies, 440 males and 420 females.

[c] Percentage of animals with specific lesions.

Source: Chase (1995a)

In males, the earliest adenoma was observed at the 52-week interim sacrifice (approximately 365 days). Otherwise, there was no indication of a treatment-related decrease in the latency period in males. In females at 500 mg/kg bw per day, the first liver adenoma and carcinoma appeared considerably earlier (427 and 426 days, respectively) than did these tumours in controls (728 days at the terminal kill). The first thyroid tumour appeared in the 20 and 500 mg/kg bw per day females somewhat sooner than in controls, 576 and 623 days, respectively, versus 714 days for controls.

In conclusion, the NOAEL was 2 mg/kg bw per day, based on liver, thyroid, ocular and nervous system toxicity in males and liver toxicity in females seen at 20 mg/kg bw per day. An increased incidence of adenomas and carcinomas of the liver was found in male and female rats at 500 mg/kg bw per day. In male rats, an increase of thyroid follicular cell adenomas was also observed at 500 mg/kg bw per day (Chase, 1995a).

2.4 Genotoxicity

The results of studies of genotoxicity with isoxaflutole are summarized in Table 20. All the studies, either in vitro or in vivo, were negative. Isoxaflutole is not considered to possess any mutagenic or genotoxic potential.

Table 20. Results of studies of genotoxicity with isoxaflutole

Type of study	Organism/cell line	Dose range tested	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	50–1 000 µg/plate (in acetone)	98.7	Negative ±S9 mix	Percy (1993)
Chromosomal aberration	Human lymphocytes	0–500 µg/mL (in acetone)	99.4	Negative ±S9 mix	Dance (1993)
Chromosomal aberration	Human lymphocytes	0–300 µg/mL (–S9 mix; in acetone) 0–600 µg/mL (+S9 mix; in acetone)	98.7	Negative ±S9 mix	Dance (1992)
Gene mutation in mammalian cells	Chinese hamster lung cells (V79)	50–100 µg/mL (in acetone)	99.4	Negative ±S9 mix	Lloyd (1992)
Gene mutation in mammalian cells	Mouse lymphoma L5178Y cells	0–600 µg/mL (in acetone)	98.7	Negative ±S9 mix	Strang (1993)
In vivo					
Mouse micronucleus	Male and female CD-1 mice	0, 200, 1 000 and 5 000 mg/kg bw (gavage in corn oil)	98.7	Negative	Edwards (1993)
DNA repair UDS assay (mouse spot test)	Male Sprague-Dawley rats	0, 600 and 2 000 mg/kg bw (gavage in 1% aqueous methyl cellulose)	99.8	Negative	Proudlock, Gant & Dawe (1997)

DNA: deoxyribonucleic acid; S9: 9000 × *g* supernatant fraction from rat liver homogenate; UDS: unscheduled DNA synthesis

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In a multigeneration reproductive toxicity study, male and female Crl:CD®BR VAF/Plus® rats (30 of each sex per group) were fed diets containing varying concentrations of isoxaflutole (purity 98.7%) to provide constant doses at all phases of the study of 0, 0.5, 2, 20 and 500 mg/kg bw per day. Adults of the F_0 generation were maintained on control or treatment diets for a 10-week premating period, then throughout mating, gestation and lactation of the F_1 litters. At weaning, the F_1 pups were then fed the same dose level of isoxaflutole as their parents for at least 10 weeks between weaning and mating and throughout mating, gestation and lactation of the F_2 litters. Ophthalmological examinations were conducted prior to the study start, on all adults during the final week of the premating periods, on at least two F_2 pups per litter between lactation days (LDs) 16 and 28 and on all F_2 weanlings receiving 20 or 500 mg/kg bw per day prior to the end of the study. Pairings were on a one-to-one basis within treatment groups. Litters were culled to contain no more than eight pups, wherever possible four males and four females, on LD 4. At necropsy of adults, the reproductive tract, liver and eyes were weighed and/or preserved for histopathological examination, as appropriate. The reproductive tract, liver and eyes were examined by histopathology in pups, but organ weights were not determined.

There were no treatment-related mortalities or clinical signs observed in any dose group or generation. In the F_0 group, at 500 mg/kg bw per day, body weights were significantly decreased relative to controls at all phases of the study. Body weight gain was statistically significantly reduced in 500 mg/kg bw per day males from week 1. In males, the body weight gain was reduced by 14.6% compared with controls during premating (0–10 weeks). Body weight gain was reduced in a statistically significant manner in females at 500 mg/kg bw per day during premating (21.6% compared with controls), gestation days (GDs) 7–14 (27.17% compared with controls) and LDs 0–4 (1.2 g loss of body weight). Females at 500 mg/kg bw per day lost significantly less weight than controls on LDs 14–21 and gained significantly more weight on LDs 4–7. There was no effect on either body weight or body weight gain at 0.5, 2 or 20 mg/kg bw per day in the F_0 group.

F_1 males and females at 500 mg/kg bw per day began the second generation premating period at significantly reduced mean body weights. These differences from control were observed in both sexes throughout the premating period. During the 10-week premating interval, mean body weight values for F_1 males were 18–26% less than those of controls for the 500 mg/kg bw per day dose group; for F_1 females, mean body weight values were 11–20% less than those of controls. For F_1 males, the body weight gain over the 10-week premating interval was significantly lower (15% during weeks 0–10) at 500 mg/kg bw per day than in controls. For F_1 females, the body weight gain was lower (11% during weeks 0–10) at 500 mg/kg bw per day than in controls. Premating body weight and body weight gain values for F_0 and F_1 rats at lower dose levels were not adversely affected by treatment with isoxaflutole. There were no treatment-related ophthalmological findings in the F_0 adults. At 500 mg/kg bw per day, a compound-related increased occurrence of chronic keratitis was noted in F_1 males (both eyes: 13/30; right eye: 5/30; left eye: 5/30) and females (both eyes: 14/30; right eye: 2/30; left eye: 3/30). This finding was not noted in controls or at any lower dose. At 500 mg/kg bw per day, terminal body weights of animals in both generations were lower (9–20%) than those of the controls. There was a compound-related significant increase in mean absolute and relative liver weights in F_0 adults at 20 and 500 mg/kg bw per day and in F_1 adults at 500 mg/kg bw per day when compared with controls. The relative liver weight in F_1 males was increased (12%) at 20 mg/kg bw per day; the absolute liver weight was unaffected. Significant decreases in absolute and/or relative weights of the epididymis, seminal vesicle, testis and ovary in F_0 and F_1 males/females at 20 and/or 500 mg/kg bw per day were attributed to lower body weights. Necropsy observations reported at 500 mg/kg bw per day for F_0 and F_1 adult rats revealed a treatment-related liver effect consisting of mottled liver (F_0: 6/30 and 4/30; F_1: 6/30 and 2/30, in males and females, respectively, versus none in controls). At 20 and 500 mg/kg bw per day, compound-related changes observed in the liver consisted of centrilobular hypertrophy in males and females as well as vacuolation in males from both

generations. These changes were severe and frequent in males at 500 mg/kg bw per day. At 500 mg/kg bw per day, subacute bilateral or unilateral inflammation of cornea was noted in F_1 generation males and females.

Treatment-related clinical signs in the F_1 offspring were limited to some observations of enlarged eyes, eyes opaque and eyes darker than normal. There were no treatment-related clinical signs for F_2 pups. There were no biologically significant differences in mean litter size, mean number of live and dead offspring and sex ratio between treated and control groups in either generation. At 20 and 500 mg/kg bw per day, there were significant decreases in the viability indices (parental and F_1 animals) and mean body weights of male and female offspring of both generations compared with controls. At 500 mg/kg bw per day, there were significant decreases in the viability indices of F_1 and F_2 animals. Significant decreases in live birth indices observed at 0.5, 20 and 500 mg/kg bw per day in the F_0 generation were not considered to be treatment related because this effect was not seen in the F_1 generation. There were no significant differences in gestation index, weaning index or mean number of pups delivered. There were no differences in sex ratio. The viability index, however, was reduced at 20 and 100 mg/kg bw per day in F_1 pups and at 500 mg/kg bw per day in F_2 pups; the decreased viability index was associated with a dose-related increase in pup mortality during LDs 0–4. A compound-related decrease in mean pup body weight of both the F_1 (11–35%) and F_2 (8–29%) pups was noted at 500 mg/kg bw per day from postnatal day (PND) 0 to PND 21. Ophthalmic examination revealed chronic keratitis in F_2 male and female pups (LDs 16–28) and weanlings (days 20–37 of age) at 500 mg/kg bw per day. Additionally, F_2 pups at 500 mg/kg bw per day had a low incidence of inflammation of the iris, as well as retinal and vitreous bleeding. At 500 mg/kg bw per day, there was an increase in the number of F_1 pups and litters, culled on postpartum 4, with no milk in the stomach. At 500 mg/kg bw per day, there was a slight increase in the incidence of underdeveloped renal papilla in pups from both generations. Organ weights were not determined in weanlings of either the F_1 or the F_2 generation. There were no treatment-related findings at any dose in the F_1 or F_2 weanlings.

In conclusion, the NOAEL for parental systemic toxicity and offspring toxicity was 2 mg/kg bw per day. The parental systemic toxicity LOAEL of 20 mg/kg bw per day was based upon increased liver weights, liver hypertrophy and vacuolation. The offspring toxicity LOAEL of 20 mg/kg bw per day was based on decreased pup weights and reduced pup viability. The NOAEL for reproductive toxicity was 500 mg/kg bw per day, the highest dose tested (Henwood, 1995).

(b) Developmental toxicity

Rats

In a developmental toxicity study, isoxaflutole (purity not specified; lot/batch 40 ADM 93) was administered to 25 female Sprague-Dawley rats by gavage in a volume of 10 mL/kg bw at a dose level of 0, 10, 100 or 500 mg/kg bw per day from GDs 6 to 15, inclusive. The test material was suspended in 0.5% aqueous carboxymethyl cellulose. Females were weighed on days 0, 3, 6–16 inclusive, 18 and 20 of gestation. Feed consumption and water consumption were recorded periodically throughout the study. At necropsy on GD 20, the reproductive tracts were dissected and examined. Fetuses were weighed, examined externally and then either dissected immediately for visceral examination followed by fixation and skeletal staining or fixed for serial sectioning.

No mortality was noted. Compound-related increased salivation was observed in 10 dams at 500 mg/kg bw per day, within 1.5 hours of dosing, beginning from GD 7 through GD 15. Similar findings were also noted in one dam at 100 mg/kg bw per day on GD 7. Compound-related decreases in body weight and body weight gain were observed at 500 mg/kg bw per day. At this dose level, significant decreases in body weight gain were observed for GDs 8–12 (19%), GDs 6–15 (19%) and GDs 0–20 (6%). During GDs 16–20, a slight compensatory increase was noted for this dose group. Maternal body weight was slightly decreased on GDs 8 through 15 (1–3%) during the treatment period and remained lower (10%) from GD 16 through GD 20, compared with controls, during the post-treatment period. A compound-related significant decrease (10%, $P < 0.01$) in feed consumption was observed at 500 mg/kg bw per day only during GDs 6–8. A significant increase (9–11%, $P < 0.01$) in feed consumption was noted at 100 and 500 mg/kg bw per day during post-treatment, on GDs

16–17. No compound-related effect on water consumption was observed. No compound-related gross pathological findings were noted. There was no effect on the number of corpora lutea or on the incidence of preimplantation or postimplantation losses or resorptions.

There was no effect of treatment on the number of viable fetuses or sex ratio. At 100 and 500 mg/kg bw per day, fetal weights were decreased significantly (8% and 14%, respectively, $P \leq 0.001$) in a dose-related manner when compared with controls. External examination revealed that there was a dose-related increase in the incidence of small fetus and a decrease in the incidence of large fetus at 100 and 500 mg/kg bw per day. Visceral anomalies were observed at 500 mg/kg bw per day and included subcutaneous oedema and a slight space between body wall and organs. The skeletal anomalies seen at 100 mg/kg bw per day included decreases in sternebral (nos 3, 4 and 5), metacarpal and metatarsal ossification. Additional anomalies noted at 500 mg/kg bw per day consisted of a lack of ossification of the 1st thoracic vertebral centrum and one or more pubic bones, incomplete ossification of caudal vertebrae, an increase in the number of 14th ribs or enlarged 14th ribs and presacral vertebrae, and asymmetrical pelvis.

In conclusion, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on the decreased body weight gain observed at 500 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on decreased fetal weight and delayed ossification observed at 100 mg/kg bw per day (Reader, 1995b).

Rabbits

In a developmental toxicity study, isoxaflutole (purity not specified) was administered to 25 female New Zealand White rabbits by gavage in a dosing volume of 5 mL/kg bw at a dose level of 0, 5, 20 or 100 mg/kg bw per day from GDs 6 to 19, inclusive. The test material was suspended in 1% aqueous carboxymethyl cellulose. Body weight and feed consumption were measured throughout the study. At necropsy on GD 29, the reproductive tracts were dissected and examined. The fetuses were weighed and examined externally and viscerally. The heads of one third of the fetuses were then fixed for serial sectioning, with the torsos and all remaining fetuses fixed for skeletal examination.

No compound-related mortalities were noted. The incidental findings included death of one control and one mid-dose (20 mg/kg bw per day) female on GD 10 and GD 19, respectively. Necropsy of these animals revealed that these deaths were incidental. Compound-related clinical signs observed at 100 mg/kg bw per day included increased number of does with little diet consumed and few faeces in the cages beginning from GD 6 through GD 19. Body weights in the groups receiving 5 or 20 mg/kg bw per day were unaffected by treatment. From GD 10 (after 4 days of treatment), significant body weight losses were recorded in the 100 mg/kg bw per day group. Body weight gain was lower than that of the controls from the start of treatment. A large number of animals in this group continued to lose weight until GD 16, but after cessation of treatment, the rate of body weight gain was higher than that of the controls. The initial deficit was, however, not fully regained by GD 29. A compound-related significant decrease (15–24%, $P < 0.05$) in feed consumption was observed at 100 mg/kg bw per day, during GDs 6–19. A compensatory increase (17–32%, $P < 0.01$) in feed consumption was noted during post-treatment, on GDs 20–28. No adverse effects on feed consumption were noted at lower dose levels. No compound-related gross pathological findings were noted.

The numbers of corpora lutea and implantations were unaffected by treatment (Table 21). At 100 mg/kg bw per day, the mean number of late resorptions was slightly higher than that of concurrent and historical controls (1.5 versus 0.7 resorptions/litter in concurrent control; historical control range: 0.4–1.3). This resulted in an increased postimplantation loss (21% versus 11% in control) and reduced the mean number of viable pups (8.3/litter versus 9.8/litter). In addition, mean fetal weight was slightly but non-significantly lower (3%) than in controls.

Table 21. Caesarean section observations in a rabbit developmental toxicity study with isoxaflutole

Parameter	0 mg/kg bw per day	5 mg/kg bw per day	20 mg/kg bw per day	100 mg/kg bw per day
No. of animals assigned	25	25	25	25
No. of animals mated	25	25	25	25
No. of animals pregnant	20	21	23	20
Pregnancy rate (%)	100	100	100	100
No. died/no. non-pregnant	0	0	0	0
No. died/no. pregnant	1	0	1	0
No. non-pregnant	5	4	1	5
No. aborted	0	0	0	1
No. of premature deliveries	0	0	0	0
Total no. of corpora lutea	247 (19)	280 (21)	291 (23)	236 (19)[a]
No. of corpora lutea/dam	13.0 ± 3.1[b]	13.3 ± 2.2	12.7 ± 2.3	12.4 ± 2.3
Total no. of implantations	210	247	248	201
No. of implantations/dam	11.1 ± 3.2	11.8 ± 2.8	10.8 ± 3.8	10.6 ± 3.1
Total no. of live fetuses	186	210	209	159
No. of live fetuses/dam	9.8 ± 2.7	10.0 ± 2.4	9.0 ± 3.3	8.3 ± 2.7
Total no. of resorptions	24	37	39	42
- No. of early resorptions	10	13	21	13
- No. of late resorptions	14	24	18	29
No. of resorptions/dam	1.3 ± 1.1	1.8 ± 1.3	1.7 ± 1.3	2.2 ± 1.5
Total no. of dead fetuses	0	0	0	0
No. of dead fetuses/dam	0	0	0	0
Fetal weight/litter (g)	38.9 ± 1.6	40.0 ± 1.8	40.8 ± 1.6	37.9 ± 1.5
Preimplantation loss (%)	16.3	12.7	16.2	15.9
Postimplantation loss (%)	11.4	15.0	15.7	20.9
Sex ratio (% male)	54	51	46	48

[a] One dam aborted on GD 26; data excluded from analysis.
[b] Mean ± standard deviation.
Source: Reader (1995a)

The incidental finding noted was occurrence of small fetuses (Table 22) in all dose groups. Single fetuses were noted at necropsy to have anomalies in three litters at 20 mg/kg bw per day and one litter at 100 mg/kg bw per day. As there was no relationship to treatment and only one fetus per litter was affected, these observations were considered to be not related to treatment. There were no visceral findings observed during fetal examination that were considered to be related to treatment. Serial sections of fetal heads revealed an increased incidence of fetuses (litters) with incisors not erupted at 100 mg/kg bw per day, suggesting a delay in development. At examination of the skeletons, there was a dose-related increase in the incidence of 27 presacral vertebrae from 5 mg/kg bw per day. At the low dose, the incidence of this observation was only slightly outside the historical control data, and there was no increase in the number of ribs observed. At 20 mg/kg bw per day, there was an increased incidence of 13 pairs of ribs as well as an increased incidence of 27 presacral vertebrae. Additionally, the ossification of the heads of long limb bones, metacarpals, phalanges and centrales was reduced at 20 mg/kg bw per day. At 100 mg/kg bw per day, the incidence of 13 pairs of ribs, rudimentary first ribs and 27 presacral vertebrae was increased, whereas the incidence of the ossification of the heads of long limb bones, metacarpals, phalanges and pubic bones was decreased.

Table 22. Fetal weight and skeletal findings in the rabbit developmental toxicity study with isoxaflutole

Observation	Fetuses or litters	0 mg/kg bw per day	5 mg/kg bw per day	20 mg/kg bw per day	100 mg/kg bw per day	Control data	
						721 fetuses	11 studies
No. of fetuses	–	186	210	209	159	–	–
No. of litters	–	19	21	23	19	–	–
Small fetus (< 2.0 g)	Fetuses	20.4	13.3	18.2	19.5	11.15	0.0–21.4
	Litters	10	13	11	8	–	–
Ribs 12/12	Fetuses	61.8	49.5	23.4	15.7	53.63	35.3–65.1
	Litters	17	19	15	8	–	–
Ribs 12/13	Fetuses	9.7	12.9	14.8	9.4	12.33	6.8–16.9
	Litters	14	15	16	11	–	–
Ribs 13/13	Fetuses	28.5	37.6	61.2	74.8	33.98	22.7–57.3
	Litters	14	17	22	19	–	–
Rudimentary 1st rib or ribs	Fetuses	0.0	0.0	0.0	1.9	0.10	0.0–1.0
	Litters	0	0	0	2	–	–
26 presacral vertebrae	Fetuses	83.3	65.2	51.7	13.8	76.68	65.8–88.4
	Litters	19	20	22	11	–	–
27 presacral vertebrae	Fetuses	16.7	34.8	47.8	86.2	23.05	11.6–34.2
	Litters	12	19	20	18	–	–
Incomplete ossification, heads, long limb bones	Fetuses	46.2	40.0	45.0	44.0	42.52	31.6–50.0
	Litters	18	20	21	17	–	–
Heads of long limb bones unossified	Fetuses	7.5	11.4	22.0	32.7	8.94	3.9–19.3
	Litters	7	9	12	13	–	–
Incomplete ossification, one or both centrales	Fetuses	0.5	1.4	5.7	3.8	2.10	0.0–4.4
	Litters	1	2	5	5	–	–
Incomplete ossification, metacarpals or phalanges	Fetuses	6.5	6.7	15.3	13.2	5.01	0.8–11.5
	Litters	8	7	11	11	–	–
Metacarpals and/or phalanges unossified	Fetuses	4.3	7.6	16.7	10.7	6.41	2.4–12.0
	Litters	7	6	9	10	–	–
Incomplete ossification of pubic bones	Fetuses	1.6	1.4	2.9	7.5	2.32	0.0–4.7
	Litters	2	3	3	7	–	–
Incisors not erupted	Fetuses	13.1	22.1	18.1	37.0	16.78	0.0–30.0
	Litters	6	9	9	12	–	–

Source: Reader (1995a)

In conclusion, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased maternal body weight, decreased feed consumption and increased numbers of resorptions seen at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 20 mg/kg bw per day,

based on slightly delayed development of the fetuses, decreased fetal weights and delayed ossification at 100 mg/kg bw per day (Reader, 1995a).

2.6 Special studies

(a) Acute neurotoxicity

Rats

In an acute neurotoxicity study, CD rats (10 of each sex per group) received a single oral gavage administration of isoxaflutole (purity 99.2%) in 0.5% aqueous methyl cellulose at a dose of 0 (vehicle only), 125, 500 or 2000 mg/kg bw. Animals were fasted for about 18 hours prior to administration of the test article. Neurobehavioural tests were conducted on 10 animals of each sex per group and consisted of functional observational battery and evaluation of motor activity. Based on pharmacokinetic studies, these tests were performed on day 1 (within 1–2 and 2–4 hours, respectively, after dosing) and on days 8 and 15 post-dosing. The animals were observed for mortality and clinical signs of toxicity for 14 days post-dosing.

No treatment-related clinical signs were observed in animals receiving doses up to 2000 mg/kg bw. No compound-related effects on body weight, body weight gain or feed consumption were noted. There were no significant differences in group motor activity values among the control and treated animals on days 1, 8 and 15. The decrease in group mean motor activity values for high-dose males on day 8 compared with controls during the 1-hour measurement period was not statistically significant and was considered to be incidental. A significant decrease in mean forelimb grip strength in mid-dose males on day 8 was considered incidental. Additional incidental findings noted during the functional observational battery tests consisted of absence of response to a tail pinch in a few males and females from various dose groups on days 1, 8 and 15, absence of pupillary response in two mid-dose males on day 15 and piloerection in one or two males in the controls and each dose group on day 15. Landing foot splay was statistically significantly decreased relative to controls in males at 2000 mg/kg bw on study days 1 and 15 and in males from 500 mg/kg bw on study day 15 (Table 23). However, this is not considered to be related to treatment, as control values increased on study days 1 and 15, the measurements in treated animals were similar to those in controls during the pretest period and there were no other findings related to hindlimb function in this study.

Table 23. Effects of isoxaflutole on functional observational battery in male rats

Dose level (mg/kg bw)	Trial	Mean forelimb grip strength (g)				Mean hindlimb grip strength (g)				Mean landing foot splay (cm)			
		Pre-test	1 day	8 days	15 days	Pre-test	1 day	8 days	15 days	Pre-test	1 day	8 days	15 days
Control	1	319	409	886	564	191	173	499	534	6.0	7.4	6.2	7.8
	2	702	409	858	501	211	170	588	460	6.5	6.9	6.6	7.8
125	1	375	415	701	516	167	157	495	518	5.8	6.3	5.8	6.4
	2	411	362	769	508	176	154	538	446	5.9	6.5	5.9	6.0
500	1	386	451	725	557	213	123	542	530	4.8	6.2	4.9	5.5**
	2	385	390	566**	469	229	142	502	459	5.5	6.3	5.2	6.0*
2 000	1	407	481	778	542	247	121	538	557	5.5	5.1*	5.2	4.7**
	2	418	422	718	442	201	150	542	433	5.8	5.3	5.5	5.3**

*: $P < 0.05$; **: $P < 0.01$
Source: Mandella (1995a)

There were no compound-related pathological findings in the central or peripheral nervous system in treated animals examined histopathologically. The brain and pituitary weights between control and treated animals were comparable.

The NOAEL for systemic toxicity and neurotoxicity was 2000 mg/kg bw. A LOAEL was not observed in this study (Mandella, 1995a).

(b) Subchronic neurotoxicity

Rats

In a subchronic dietary neurotoxicity study, isoxaflutole (purity 99.2%) was administered to CD rats (10 of each sex per group) at a dietary level of 0, 25, 250 or 750 mg/kg bw per day for 90 days. Stability, homogeneity and concentrations in the diet were confirmed analytically. All animals were euthanized following 13 weeks of treatment. All animals were observed twice daily for mortality and moribundity. Detailed physical examinations were performed weekly. Individual body weights and feed consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals prior to the initiation of treatment and during study weeks 5, 9 and 13. Ophthalmic examinations were not performed. At terminal sacrifice, all animals were subjected to complete gross pathological examination; abnormal tissues were preserved in 10% neutral buffered formalin. Five animals of each sex from the high-dose group were selected for neuropathological evaluation and were anaesthetized and killed by perfusion fixation. Animals from the control and high-dose groups were subjected to neuropathological examinations of the central and peripheral nervous systems. All abnormal tissues were preserved in 4% paraformaldehyde and glutaraldehyde in phosphate-buffered saline. Brain and pituitary weights from each animal were determined.

The test article was homogeneously distributed in the diets, and diets were stable for 14 days at the ambient temperature. The concentration analyses of all test samples indicated values within ±5% of the target concentrations.

No treatment-related deaths occurred. At 250 mg/kg bw per day, one male was sacrificed moribund on day 46 of the study. This isolated finding was considered to be incidental. There were no treatment-related clinical signs of toxicity observed in treated animals. The mean body weights for high-dose males were consistently lower (6–13%) than those of controls throughout the study period, resulting in an overall decrease in body weight gain of 19% over 13 weeks (Table 24). For high-dose females, the mean body weight and body weight gains were comparable over 13 weeks, with the exception of a slight decrease in week 2 and weeks 11–13, resulting in an overall decrease in body weight gain of 9% over 13 weeks. Increases in body weight and body weight gain in low- and mid-dose females were not considered to be toxicologically significant. No effect on feed consumption was noted.

Table 24. Body weight in male and female rats in the 90-day neurotoxicity study with isoxaflutole

Week	Males				Females			
	0 mg/kg bw per day	25 mg/kg bw per day	250 mg/kg bw per day	750 mg/kg bw per day	0 mg/kg bw per day	25 mg/kg bw per day	250 mg/kg bw per day	750 mg/kg bw per day
0	194.3	195.5	191.0	191.7	146.4	152.7	154.7	153.5
5	412.2	407.4	389.7	367.0**	219.2	241.7*	237.4	222.2
9	504.1	498.2	477.2	445.2**	246.4	274.5*	265.8	245.9
13	558.7	541.6	523.2	486.8**	260.7	299.7**	280.7	258.0
Body weight gain (g), weeks 0–13	364.4	346.1	332.2	295.1	114.3	147.0	126.0	104.5
% change from controls	–	−5	−9	−19	–	+29	+11	−9

*: $P < 0.05$; **: $P < 0.01$

Source: Mandella (1995b)

Motor activity was unaffected by the treatment. Functional observational battery findings observed during the 13-week treatment period are summarized in Table 25. At 25 mg/kg bw per day and above, decreases in both forelimb and hindlimb grip strengths were noted in treated males during week 13. A non-significant decrease in forelimb grip strength was also noted during week 13. The decrease in landing foot splay in females at 25 mg/kg bw per day in week 9 was considered incidental. Additional findings consisted of moderate difficulty in handling, piloerection and absence of a tail pinch response seen prior to treatment and/or with similar frequency in the control group. There were no macroscopic or microscopic findings that were considered to be related to treatment in either males or females at any dietary concentration of isoxaflutole. No treatment-related lesions of the nervous system were noted in high-dose animals.

Table 25. Effect of isoxaflutole on functional observational battery in male rats

Dose level (mg/kg bw)	Trial no.	Mean forelimb grip strength (g)				Mean hindlimb grip strength (g)				Mean landing foot splay (cm)			
		Pre-test	Week 5	Week 9	Week 13	Pre-test	Week 5	Week 9	Week 13	Pre-test	Week 5	Week 9	Week 13
Control	1	396	619	880	993	255	315	727	639	4.6	7.5	6.7	6.3
	2	369	619	787	901	230	270	753	734	4.7	7.2	6.4	6.4
25	1	359	712	821	913	196	325	766	498	5.2	6.9	5.9	5.9
	2	449	625	581	731	232	254	674	504**	5.5	7.1	6.4	5.7
250	1	420	689	704	779	269	283	776	586	5.3	7.4	6.3	5.4
	2	426	643	719	740	244	272	701	562*	5.5	7.2	6.7	5.7
750	1	329	639	818	670**	267	280	797	476*	5.1	6.4	6.3	6.4
	2	414	614	669	765	235	258	695	583	5.1	6.8	6.6	6.4

*: $P < 0.05$; **: $P < 0.01$
Source: Mandella (1995b)

A NOAEL for systemic toxicity was not identified, as only limited parameters were evaluated in this study. The NOAEL for neurotoxicity was 750 mg/kg bw per day, the highest dose tested (Mandella, 1995b).

(c) Developmental neurotoxicity

Rats

In a developmental neurotoxicity study, isoxaflutole (purity 99.15%) in 1% methyl cellulose was administered by gavage in a dosing volume of 5 mL/kg bw to pregnant Crl:CD®(SD)IGS BR (25 per dose) from GD 6 to LD 10 at a dose of 0, 5, 25 or 250 mg/kg bw per day. Parental dams (P) were allowed to deliver naturally. All P females were killed on LD 21. On PND 4, eight pups (four of each sex) per litter were randomly selected in order to reduce variability among the litters; the remaining offspring were weighed and euthanized. The acquisition of balanopreputial separation and vaginal patency was assessed for each pup, and body weight was measured on the day of acquisition. Pups were assessed for acoustic startle response on PND 20 or 60, for locomotor activity on PND 13, 17, 21 or 61 and for swimming ability and learning and memory beginning on PND 22 or 62. Subsequently, 10 pups of each sex per group were selected for neurobehavioural testing and neuropathological examination. Pups not selected for behavioural and neuropathological evaluations were terminated on PND 28 or 29. Morphometric analyses, as required by Organisation for Economic Co-operation and Development and United States Environmental Protection Agency guidelines, were not performed on offspring (PND 11 or 72), as the evaluation of brains by light microscopy did not reveal any structural abnormalities, nor were there any clear functional differences between the control and treated groups.

No unscheduled parental deaths occurred during the study. Clinical or functional observational battery signs, gross pathology, pregnancy rate, number of implantations per dam,

gestation length and sex ratio were unaffected by treatment. No treatment-related differences in live litter size, postnatal survival, sex ratios, clinical signs or sexual maturation were observed in any treated group. Pup swimming ability, learning, memory, motor activity, auditory startle response, brain weights and dimensions, and neuropathology were unaffected by the test substance.

Body weights and body weight gains for the P females are presented in Table 26. At 5 and 25 mg/kg bw per day, there were no effects on maternal body weight, body weight gain or feed consumption. Body weights were slightly decreased ($P \leq 0.05$) in the 250 mg/kg bw per day P females during GDs 9–15 (decrease 4–6%) and LDs 1–10 (decrease 7–10%). Body weight gains were decreased ($P \leq 0.0$l) in the 250 mg/kg bw per day P females during GDs 6–9 (decrease 91%) and 9–12 (decrease 39%). Body weight gains were also decreased for the entire gestation treatment interval (GDs 6–20; decrease 14%, $P \leq 0.01$) and overall gestation (GDs 0–20; decrease 11%, $P \leq 0.05$). During the lactation treatment interval (LDs 1–10), body weight gains were comparable with those of controls in all treated groups. Feed consumption was unaffected by the treatment at 5 and 25 mg/kg bw per day. At 250 mg/kg bw per day, feed consumption was statistically significantly reduced compared with that of controls throughout gestation and the first 4 days of lactation.

Table 26. Selected mean body weights for P females (n = 23–25) administered isoxaflutole from GD 6 to LD 10

Treatment interval (days)	Mean body weights ± SD (g)			
	0 mg/kg bw per day	5 mg/kg bw per day	25 mg/kg bw per day	250 mg/kg bw per day
Gestation				
0	261 ± 14.7	258 ± 11.9	256 ± 131	258 ± 14.1
6	294 ± 19.8	292 ± 11.9	286 ± 15.4	291 ± 15.1
9	305 ± 18.7	304 ± 13.3	297 ± 17.9	292* ± 12.2 (↓4)
15	341 ± 28.4	334 ± 21.7	332 ± 21.3	320** ± 15.4 (↓6)
20	411 ± 35.3	408 ± 20.1	403 ± 26.6	392 ± 19.3
Treatment (6–20)	117 ± 25.0	116 ± 13.9	116 ± 17.4	101** ± 16.7 (↓14)
Overall (0–20)	150 ± 27.6	149 ± 18.8	146 ± 19.4	133* ± 19.1 (↓11)
Lactation				
1	309 ± 30.6	305 ± 22.7	297 ± 19.4	279** ± 18.5 (↓0)
4	326 ± 32.3	321 ± 20.3	315 ± 19.5	300** ± 17.1 (↓8)
10	351 ± 26.7	344 ± 20.0	337 ± 20.4	325** ± 23.6 (↓7)
21	345 ± 23.0	341 ± 19.3	336 ± 21.2	333 ± 17.6
Treatment (1–10)	43 ± 12.0	40 ± 17.6	40 ± 11.9	46 ± 18.2
Post-treatment (10–21)	−6 ± 15.2	−3 ± 14.7	−1 ± 14.0	8** ± 16.9 (↑133)
Overall (1–21)	37 ± 21.2	36 ± 17.6	39 ± 15.6	54** ± 20.8 (↑46)

*: $P \leq 0.05$; **: $P \leq 0.01$
Source: Nemec (1999)

Decreased ($P \leq 0.05$) survival was observed in the 250 mg/kg bw per day pups during PNDs 0–1 only (93.2% treated versus 98.3% controls). No treatment-related clinical signs were observed. Body weights of offspring in the 5 and 25 mg/kg bw per day groups were comparable with those of the controls. In the 250 mg/kg bw per day pups, body weights were decreased ($P \leq 0.05$) in the males from PND 1 to PND 28 (decreased 7–12%) and from PND 49 to PND 63 (decreased 4–5%). In the females, body weights were decreased ($P \leq 0.05$) sporadically between PND 1 and PND 35 (decreased 5–12%). Decreased ($P \leq 0.05$) body weight gains were observed sporadically in the males between PND 1 and PND 28 (decreased 6–21%) and only during PNDs 4–7 in the females (decreased

15%). Absolute brain weights were also reduced (11–12%) in males and females on PND 11 at 250 mg/kg bw per day, an effect likely related to the decreased body weights and body weight gains observed in these animals. No morphometric measurements of the brain were performed in this study.

In conclusion, the maternal NOAEL was 25 mg/kg bw per day, based on decreased maternal body weight, body weight gain and feed consumption at 250 mg/kg bw per day. The offspring toxicity NOAEL was 25 mg/kg bw per day, based on decreased pup survival, body weight and body weight gain at 250 mg/kg bw per day. In the absence of any neurotoxic findings, the NOAEL for neurotoxicity in the rat was 250 mg/kg bw per day, the highest dose tested (Nemec, 1999).

(d) Microsomal enzymes

A special study was conducted to establish the dose–response relationship and to investigate the role of the mixed-function oxidase system with respect to liver enlargement in isoxaflutole-treated mice. Groups of 25 male CD-1 mice received isoxaflutole (purity 99.6%) in their diet at a dose level of 0, 175, 700, 2800 or 7000 ppm (equivalent to 0, 26.3, 105, 420 and 1050 mg/kg bw per day) for 14 days.

There were no mortalities, no treatment-related clinical observations and no effects on body weight or feed consumption. Isoxaflutole administration caused an increase (≥ 11%) in absolute and relative liver weights in mice at and above 700 ppm. This increase was attributed to the induction of mixed-function oxidase enzymes in the liver. The total cytochrome P450 levels were increased in a dose-dependent manner; this was statistically significant at and above 700 ppm. Analysis of P450 isoenzymes indicated that the elevated P450 levels were mainly due to a significant increase in absolute and relative pentoxyresorufin *O*-dependentylase (PROD) activity (P450 2 family, B1 isoenzymes) at 175 ppm and above and at 700 ppm and above, respectively, and to a significant increase in absolute and relative benzoxyresorufin *O*-debenzylase (BROD) activity (P450 2B family, B1 and B2 isoforms) at 175 ppm and above. Absolute ethoxyresorufin *O*-deethylase (EROD) activity (P450 1 family, A1 isoenzymes) was significantly increased at 2800 and 7000 ppm. Absolute methoxyresorufin *O*-demethylase (MROD) activity (P450 1 family, A2 isoenzymes) was significantly increased at 700 ppm and above, but there was no dose–response relationship. Absolute lauric acid 11- and 12-hydroxylase activities (peroxisome proliferation) were significantly increased at 7000 ppm. The results suggest that isoxaflutole caused a dose-related increase in liver weight in male mice, owing to marked elevation in cytochrome P450 enzymes of the P450 2B family, similar to phenobarbital. It does not appear to induce other P450 isoenzymes significantly or cause peroxisome proliferation. There was no NOAEL. The LOAEL was 175 ppm (equivalent to 26.3 mg/kg bw per day) on the basis of elevated absolute and relative BROD activity and absolute PROD activity (Price, 1994a).

In another study conducted to establish the dose–response relationship and to investigate the role of the mixed-function oxidase system with respect to liver enlargement in isoxaflutole-treated rats, groups of five male Sprague-Dawley CD1 rats received isoxaflutole (purity 99.6%) in the diet at a dose level of 0, 10, 100 or 400 mg/kg bw per day for 14 days.

There were no mortalities, no treatment-related clinical observations and no effects on body weight or feed consumption. Isoxaflutole administration caused an increase (≥ 33%) in absolute and relative liver weights in rats at 100 and 400 mg/kg bw per day. This increase was attributed to induction of mixed-function oxidase enzymes in the microsomal fraction of the homogenized liver. The total cytochrome P450 levels were increased in a dose-dependent manner; this was statistically significant at all dose levels. The specific forms of isoenzymes responsible for this increase were PROD and BROD enzymes, the induction of which may be attributed to the P450 2B family (i.e. phenobarbital type). Therefore, isoxaflutole appears to function as a phenobarbital-type inducer of the P450 2B family. There was no increase in other P450 isoenzyme levels, including MROD and EROD, nor did the test compound induce lauric acid hydroxylases, which are associated with peroxisome proliferation. Analysis of P450 isoenzymes revealed a statistically significant increase in absolute and relative (to total liver P450) PROD (P450 2 family, B1 isoenzymes) and BROD (P450 2B family, B1

and B2 isoforms) activities at all dose levels. Absolute EROD activity (P450 1 family, A1 isoenzymes) was significantly, but not dose-relatedly, increased in all dose groups and was decreased in relation to total liver P450 at 100 and 400 mg/kg bw per day (statistically significant at 400 mg/kg bw per day). MROD activity (P450 1 family, A2 isoenzymes) was not markedly altered in comparison with other P450 isoenzymes. Absolute lauric acid 11- and 12-hydroxylase activities (peroxisome proliferation) were significantly increased at 400 mg/kg bw per day.

The results suggest that isoxaflutole caused a dose-related increase in liver weight in male rats that may be due to marked elevation in cytochrome P450 enzymes of the P450 2B family (PROD and BROD), similar to phenobarbital. It does not appear to induce other P450 isoenzymes significantly or cause peroxisome proliferation. There was no NOAEL. The LOAEL was 10 mg/kg bw per day on the basis of elevated absolute and relative PROD and BROD activities (Price, 1994b).

(e) Tyrosine levels

The plasma samples taken from the 2-week dietary study in mice (Price, 1994a) were analysed for tyrosine concentrations. Plasma tyrosine levels increased at all doses (175–7000 ppm) studied relative to the untreated control mouse plasma. No dose–response relationship was observed. The results of this study suggest that the supplementation of the mouse diet with isoxaflutole for 14 days resulted in an elevation of tyrosine concentrations in the plasma. A no-effect level was not observed and is therefore assumed to be less than 175 ppm (Little, 1993b).

The plasma samples taken from the 2-week dietary study in Sprague-Dawley CD1 rats (Price, 1994b) were analysed for tyrosine concentrations. Plasma tyrosine levels increased at all doses (10–400 mg/kg bw per day) studied compared with that of the untreated control rat plasma. Plasma tyrosine levels increased 3-fold compared with control levels at the lowest dose applied (10 mg/kg bw per day), increasing to 3.6- and 3.5-fold at 100 and 400 mg/kg bw per day, respectively. No dose–response relationship was observed. The results of this study suggest that the supplementation of the rat diet with isoxaflutole for 14 days resulted in an elevation of tyrosine concentrations in the plasma. A no-effect level was not observed and is therefore assumed to be less than 10 mg/kg bw per day (Little, 1993c).

(f) Thyroid mechanism

The mechanism of action of isoxaflutole on the thyroid was investigated in male Sprague-Dawley rats. In this study, isoxaflutole (purity 99.6%) was administered in the diet to male Sprague-Dawley rats (12 per dose) at a dose level of 0 or 500 mg/kg bw per day for 14 days. A third group (positive control) of rats received sodium phenobarbital by gavage at 80 mg/kg bw per day and an untreated diet. Following the treatment period, the liver enzyme activities, including cytochrome P450 and *p*-nitrophenol uridine diphosphate-glucuronosyltransferase (UDPGT), as well as thyroxine (T_4) and triiodothyronine (T_3) levels were monitored, and thyroid weights were determined. The rate of T_4 disappearance from blood was measured in rats (six animals from each group) after intravenous administration of sodium [^{125}I]thyroxine. The effects on blood concentration half-life, thyroid gland iodine uptake and thyroid weights were measured.

There were no mortalities, no treatment-related clinical observations and no treatment-related effects on body weight, body weight gain, feed consumption or feed efficiency. Isoxaflutole administration caused more than a 2-fold increase in Phase I (cytochrome P450–dependent mixed-function oxidase system, as indicated by increased PROD activity) and Phase II (as indicated by increased UDPGT activity) enzymes, which resulted in increased clearance of ^{125}I-labelled T_4 from the blood, as indicated by a shorter half-life and decreases in plasma T_4 levels. In addition, there were increases in liver and thyroid weights. The plasma T_3 level was unaffected. The significant reduction in the level of circulating T_4 was possibly the result of enhanced glucuronidation by hepatic UDPGT and a rapid systemic clearance of total ^{125}I-labelled T_4 in the isoxaflutole-treated group. Following intravenous administration of ^{125}I-labelled T_4, the thyroid iodine uptake was slightly higher and thyroid weights were significantly higher in isoxaflutole-treated rats compared with controls. The results of the study (Table 27) appear to support the hypothesis that the increased incidence of thyroid

tumours in male rats at 500 mg/kg bw per day in the carcinogenicity study may be due to an imbalance of thyroid hormones created by an induction of UDPGT followed by decreased plasma T_4 levels, increased clearance of T_4 and a decreased half-life for T_4.

Table 27. Comparison of isoxaflutole and phenobarbital in rats

Parameters measured	Dose groups[a]		
	Control	Isoxaflutole	Phenobarbital
Absolute liver weight (g) (% of control)	13.9	21.1** (152%)	19.8** (143%)
Relative liver weight (g) (% of control)	5.07	7.5 ** (149%)	7.3** (145%)
Microsomal protein (mg/g liver) (% of control)	18.1	31.6** (175%)	24.6** (136%)
P450 (nmol/g liver)	16.1	57.9**	41.3**
Absolute thyroid weight (mg) (% of control)	18.7	20.1 (107%)	23.6* (126%)
UDPGT (mmol/g liver per hour)	57	216*	169**
PROD (nmol/g liver per hour)	5.1	137**	104**
T_4 (ng/dL)	5.7	3.2**	4.9**
T_3 (µg/dL)	74	68	69
[^{125}I]T_4 K_{el} (/h)	0.040 7	0.052 0***	0.042 8
[^{125}I]T_4 $t_{½}$ (h)	17.0	13.3	16.2
[^{125}I]T_4 clearance (mL/min)	0.038	0.065***	0.049*

K_{el}: mean terminal rate constant; PROD: pentoxyresorufin *O*-dependylase; $t_{½}$, half-life; T_3: triiodothyronine; T_4: thyroxine; UDPGT: uridine diphosphate-glucuronosyltransferase; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

[a] Doses: isoxaflutole = 500 mg/kg bw per day; phenobarbital = 80 mg/kg bw per day.

Source: Chambers (1995)

Isoxaflutole appears to act like a phenobarbital-type inducer of hepatic Phase I and Phase II drug-metabolizing enzymes. The development of thyroid tumours in male rats treated with isoxaflutole at 500 mg/kg bw per day may be secondary to the treatment-related effects on the liver, which, in turn, produced alterations in thyroid–pituitary hormonal feedback mechanisms and a concomitant hormonal imbalance (Chambers, 1995).

To examine the potential for isoxaflutole to cause hepatic cell proliferation in the rat and to determine the reversibility of any effects observed, groups of 10 female rats were administered isoxaflutole (purity 99.43%) at varying concentrations to provide constant doses of 0, 2, 20, 50, 200 and 500 mg/kg bw per day for either 2 or 13 weeks. Reversibility groups of 10 animals were fed diets that provided 0 or 500 mg/kg bw per day for either 2 or 13 weeks, then transferred to control diets for a further 2 weeks. For the week prior to termination, animals were administered bromodeoxyuridine in the drinking-water to assess cell proliferation.

There were no deaths or clinical signs of toxicity during the study. Body weights and/or body weight gains at the terminal sacrifice were statistically significantly reduced for animals in the 500 mg/kg bw per day dose group for 2 weeks as well as for those given 200 or 500 mg/kg bw per day for 13 weeks. Corresponding decreases in feed consumption were seen for animals in these groups. Liver weights (absolute and relative to body weight) were increased in rats exposed to 200 and 500 mg/kg bw per day for 2 weeks as well as those exposed for 13 weeks. The hepatocyte labelling index (LI; indicator of hepatic cell proliferation) was increased at 2 and 13 weeks for animals given 200 and 500 mg/kg bw per day.

In the recovery group of rats treated with 500 mg/kg bw per day for 2 weeks and then placed on control (isoxaflutole-free) diet for 2 weeks, body weight gain, liver weights (absolute and relative) and LI were similar to those of controls. These results indicate that isoxaflutole-induced responses are

rapidly reversible. Reversibility of hepatic changes was also seen in rats treated with 500 mg/kg bw per day for 13 weeks followed by 2 weeks on control diet.

In conclusion, isoxaflutole demonstrated dose- and time-dependent effects on hepatocyte proliferation similar to those produced by other non-genotoxic hepatocarcinogens, such as phenobarbital. Hepatocyte proliferative effects were observed only at the highest doses of isoxaflutole, were correlated with changes in liver weight and were shown to be reversible upon cessation of isoxaflutole exposure. These data support the hypothesis that cell proliferation is the non-genotoxic mode of action for isoxaflutole tumorigenicity and that this response is dose dependent and reversible (Moser, 2001).

In a comparative tyrosine tolerance study, isoxaflutole (purity 98.7%) or 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione (NTBC; purity 99.8%), a therapeutic agent, was administered via gavage to male Sprague-Dawley rats (five per dose) at a dose level of 0 or 10 mg/kg bw per day for 1 week. The animals then received tyrosine at 500 mg/kg bw per day on the day of treatment and on days 2, 3 and 8 after the test substance administration. Urine collected at 4, 8 and 24 hours was analysed for tyrosine metabolites.

Administration of tyrosine to rats pretreated with isoxaflutole or NTBC increased the urinary excretion of tyrosine metabolites, *N*-acetyl tyrosine, 4-hydroxyphenyl acetate (HPAA) and 4-hydroxyphenyl lactate (HPLA). The effect of isoxaflutole was reversible within 48 hours after administration, whereas that of NTBC was not completely reversed by 1 week after administration. The results of this functional assay suggest that both isoxaflutole and NTBC affect the main catabolic pathway for tyrosine by inhibiting the liver enzyme HPPD (Esdaile, 1995).

In a comparative metabolism study, isoxaflutole (purity 98.3%) was administered to groups (five per species) of male Sprague-Dawley (CD) rats and CD-1 mice by gavage at a single dose (10 mg/kg bw) followed 1 hour later with a single oral dose of [^{14}C]tyrosine (500 mg/kg bw). The total radioactivity in the urine and expired carbon dioxide was estimated at intervals of 0–5, 5–12, 12–24 and 12–48 hours. Metabolite analysis was performed on the urine of the rat and the mouse to analyse the quantitative differences in their ability to utilize a bypass metabolic route for the blocked tyrosine pathway via HPLA and HPAA.

For both species, a major portion of the administered dose of [^{14}C]tyrosine was eliminated via urine and expired air. Urinary elimination (mice: 19.90%; rats: 8.42%) was predominant in the mouse, whereas a significant portion of radiolabel was predominantly excreted via the expired air as carbon dioxide in the rat (mice: 13.23%; rat: 20.41%) during the first 48 hours following administration of [^{14}C]tyrosine. High-performance liquid chromatographic analysis of [^{14}C]tyrosine metabolites found in the urine of both species revealed higher amounts of two major metabolies, HPLA and HPAA, in the mouse than in the rat. The enzymatic hydrolysis of conjugates indicated that some metabolites were excreted as glucuronides and/or sulfates in urine; these did not include HPLA and HPAA.

This study demonstrated species-related qualitative and quantitative differences in the excretion of tyrosine following single simultaneous administrations of isoxaflutole and [^{14}C]tyrosine to male mice and rats. The results suggest that the elimination of tyrosine as HPLA and HPAA is more efficient in the mouse than in the rat, with twice as much of the administered dose of [^{14}C]tyrosine observed in the mouse urine as in the rat urine (Filaquier, 1995).

It was hypothesized, following the development of several herbicides sharing an HPPD-inhibiting mode of action, that a number of effects (organ weight and histopathology in liver, kidney, pancreas, thyroid; histopathology in eye; fetal skeletal development) were not primary toxic effects of the compounds, but were secondary to the increased plasma tyrosine concentrations observed in the rat.

The objective of this study was to measure blood tyrosine levels in the rat following daily oral gavage administration of NTBC between days 1 and 18, together with administration of diet containing 2% weight per weight (w/w) L-tyrosine between days 15 and 19. NTBC (purity 99.7%), an HPPD inhibitor, was administered at a dose level of 5, 10, 20 or 40 µg/kg bw per day. Each group consisted of three female Sprague-Dawley rats. A control group received the vehicle alone (demineralized water). Body weights were recorded for all females on days 1, 5, 8, 12, 15 and 19. Feed consumption was measured for all females during the intervals days 1–5, 5–8, 8–12, 12–15 and 15–19. Clinical observations were recorded daily. Blood samples were collected on days 15 and 19 and subsequently analysed for tyrosine levels. At scheduled sacrifice, the pancreas and thyroid gland were preserved for possible histological examination.

There were no mortalities during the study. At NTBC doses from 10 µg/kg bw per day, one or more animals in each group were noted with white areas on the eye (bilateral) on study day 19. There were no clinical findings at an NTBC dose of 5 µg/kg bw per day. At an NTBC dose of 40 µg/kg bw per day, there was an overall mean body weight loss of 4 g between study days 1 and 19, compared with a mean body weight gain of 21 g in the controls over the corresponding period. At an NTBC dose of 40 µg/kg bw per day, mean feed consumption was reduced by 22% between study days 15 and 19, when compared with the controls. The effect was statistically significant ($P < 0.05$). On study day 15, prior to the addition of 2% tyrosine to the diet, plasma tyrosine concentrations at an NTBC dose of 5 µg/kg bw per day were similar to those measured in the control group, whereas plasma tyrosine was increased from an NTBC dose of 10 µg/kg bw per day (Table 28). On study day 19, after the animals were fed diets containing 2% tyrosine from study days 15 to 19 while also being treated daily with NTBC by oral gavage on study days 15 through 18, plasma tyrosine concentrations were increased in all groups including controls. The increase was dose related in the groups administered NTBC.

Table 28. Plasma tyrosine concentration after administration of NTBC and dietary tyrosine in rats

Study day	Plasma tyrosine concentration (mg/L)				
	Control	5 µg/kg bw per day NTBC	10 µg/kg bw per day NTBC	20 µg/kg bw per day NTBC	40 µg/kg bw per day NTBC
15	8.09	9.7	73.77	25.93	167.7
19	20.7	33.13	305.7	388.7	419.7

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione
Source: Blanck (2005)

Minimal to slight mottled kidney (bilateral) was observed in 2/3 animals at an NTBC dose of 40 µg/kg bw per day and in 1/3 animals at NTBC doses of 20 and 5 µg/kg bw per day. No other macroscopic findings were observed.

In conclusion, oral gavage administration of NTBC together with administration of diet containing 2% w/w L-tyrosine provoked tyrosinaemia in a dose-related manner and was associated with an increase in the incidence of rats with white area on both eyes compared with the control group (Blanck, 2005).

In a study conducted to assess the potential effects of increased plasma tyrosine concentration on selected organs (eye, liver, pancreas and thyroid gland) of rats, four groups were used, each consisting of 10 Wistar rats of each sex and treated for 28 days as follows: *Group 1*: Animals received the vehicle alone (demineralized water) by gavage and untreated diet throughout the study. This group acted as a control. *Group 2*: Animals received the vehicle alone (demineralized water) by gavage and L-tyrosine at 2% w/w in the diet throughout the study. *Group 3*: Animals received NTBC, an inhibitor of HPPD, a key enzyme of tyrosine catabolism, by gavage at a volume of 10 mL/kg bw and untreated diet throughout the study. *Group 4*: Animals received NTBC by gavage at a volume of 10 mL/kg bw

and L-tyrosine at 2% w/w in the diet throughout the study. Plasma tyrosine concentrations were measured, organ weights were determined and selected organs were examined by histopathology.

One female in the NTBC only group died prematurely due to an accidental trauma. In the NTBC + 2% L-tyrosine group, treatment-related clinical signs consisted of white area on the eye in 9/10 males between study days 23 and 26 on one or more occasions. Body weight, body weight gain and feed consumption were not affected by the treatment. In the NTBC + tyrosine group, at the end of the study, 9/10 male rats were noted with corneal oedema, all 10 males were noted with snowflake corneal opacity and 3/10 females were noted to have snowflake corneal opacity. These are considered to be treatment related, as they are linked with increased plasma tyrosine concentration. There were no treatment-related ophthalmological findings in either the NTBC only or the L-tyrosine only group. When measured on study day 29 or 30, after overnight fasting, plasma tyrosine concentrations were similar to those of controls in the tyrosine group and markedly increased relative to controls in both the NTBC and the NTBC + tyrosine groups (Table 29).

Table 29. Plasma tyrosine concentration after administration of NTBC or tyrosine or co-administration of NTBC + tyrosine in rats

Sex	Plasma tyrosine concentration (nmol/mL)			
	Control	Tyrosine	NTBC	NTBC + tyrosine
Males	70.17	77.71	1 302.05	1 477.45
Females	66.73	63.06	1 531.85	1 474.14

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione
Source: Blanck (2006a)

These results were not those that had been expected in advance of the study. Rather, it had been expected that the plasma tyrosine concentrations in the group administered NTBC at 10 µg/kg bw per day would be similar to those observed in the groups receiving either control diet or diet supplemented with 2% tyrosine. In the NTBC + tyrosine group, relative liver weight was significantly increased in both males and females relative to controls. At necropsy, in the NTBC + tyrosine group, 3/10 males were noted with eye opacity. There were no other treatment-related macroscopic findings. Findings that are considered to be treatment related were observed in the pancreas, thyroid and eye in the male and female rats in the NTBC + tyrosine group (Table 30). Findings in the pancreas were acinar atrophy/fibrosis and/or acinar degeneration/apoptosis of the exocrine pancreas and interstitial inflammation. In the thyroid gland, treatment-related findings were colloid alteration and potentially follicular cell hypertrophy based on increased severity in one animal in the NTBC + tyrosine group. In the eye, the treatment-related change was unilateral or bilateral keratitis.

In conclusion, administration of NTBC at 10 µg/kg bw per day plus 2% dietary tyrosine markedly increased plasma tyrosine concentrations and produced findings in the eye, pancreas and thyroid. These findings were not observed after administration of either tyrosine or NTBC alone, despite an increase in plasma tyrosine levels after gavage administration of NTBC alone at 10 µg/kg bw per day (Blanck, 2006a).

In a study conducted to assess the potential effects of increased plasma tyrosine concentration on selected organs (eye, kidney, liver, pancreas and thyroid gland) of rats, four groups were used, each consisting of five Wistar rats of each sex and treated for 28 days as follows: *Group 1*: Animals received the vehicle alone (demineralized water) by gavage and untreated diet throughout the study. This group acted as a control. *Group 2*: Animals received the vehicle alone (demineralized water) by gavage and L-tyrosine at 2% w/w in the diet throughout the study. *Group 3*: Animals received NTBC, an inhibitor of HPPD, a key enzyme of tyrosine catabolism, by gavage at a volume of 10 mL/kg bw and untreated diet throughout the study. *Group 4*: Animals received NTBC by gavage at a

Table 30. Histopathology of selected organs after administration of NTBC or tyrosine or co-administration of NTBC + tyrosine in rats

	Males				Females			
	Control	TYR	NTBC	NTBC + TYR	Control	TYR	NTBC	NTBC + TYR
No. examined	10	10	10	10	10	10	9	10
Pancreas								
Acinar atrophy/fibrosis: focal/multifocal	1	2	0	3	0	0	0	5
Acinar degeneration/ apoptosis: focal/ multifocal	0	1	0	2	0	3	0	5
Interstitial inflammation: focal/multifocal	0	1	0	3	0	0	0	5
Interstitial inflammation: diffuse	0	0	0	0	0	0	0	1
Thyroid								
Follicular cell hypertrophy: diffuse	2	3	1	3	0	1	0	0
Colloid alteration	0	1	1	6	0	0	0	0
Eye								
Keratitis: diffuse: unilateral and bilateral	1	0	0	9	0	0	0	1

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; TYR: L-tyrosine
Source: Blanck (2006a)

volume of 10 mL/kg bw and L-tyrosine at 2% w/w in the diet throughout the study. During the acclimatization phase, all animals were subjected to an ophthalmological examination; all animals were re-examined during week 4. Blood samples were collected for measurement of blood tyrosine levels at selected intervals. All animals were necropsied, and selected organs were weighed, collected, fixed and examined microscopically.

There were no mortalities during the study. Treatment-related clinical signs were limited to the NTBC + tyrosine group. These signs were white area on the eye in all males and one female between study days 24 and 30 and half-closed eyes in 4/5 males between study days 22 and 30 on one or more occasions. At NTBC at 10 µg/kg bw per day + 2% L-tyrosine, in females, mean body weight was reduced by between 2% and 5% during the second half of the study. Mean body weight gain per day was 2.1 g compared with 3.6 g in the controls between study days 8 and 15, resulting in an overall cumulative body weight gain reduction of 11% compared with the controls by study day 29. These effects were not statistically significant, but were considered to be biologically relevant. At an NTBC dose of 10 µg/kg bw per day and at 2% L-tyrosine, body weight evolution was unaffected by treatment in either sex. In females of the NTBC + tyrosine group, feed consumption was decreased throughout the study, with the greatest effect observed during week 2. There was no effect on feed consumption in any other group. Treatment-related ophthalmological findings were limited to the NTBC + tyrosine group and consisted of corneal oedema and snowflake corneal opacities in all five males, neovascularization of the cornea in one male, snowflake corneal opacity in one female and anterior synechia of the iris in another female.

In the NTBC + tyrosine group, a time-dependent increase in blood tyrosine was observed from the first measurement at study day 2, with a plateau reached at measurement on study day 21 (Table 31). In the NTBC group, blood tyrosine was increased relative to controls only on study day 29 or 30, prior to fasting. In the L-tyrosine group, blood tyrosine was increased relative to controls at all

time points. Blood tyrosine concentration was similar to that of controls in the L-tyrosine group and markedly elevated in both the NTBC and the NTBC + tyrosine groups.

Table 31. Plasma tyrosine concentration after administration of NTBC or L-tyrosine or co-administration of NTBC + tyrosine in rats

Day	Plasma tyrosine concentration (nmol/mL)							
	Males				Females			
	Control	TYR	NTBC	NTBC + TYR	Control	TYR	NTBC	NTBC + TYR
2	73.78	341.08	81.79	367.57	50.65	208.84	56.12	152.99
7	73.29	310.83	84.66	389.98	46.28	148.13	55.18	104.75
14	73.96	314.26	83.12	1 166.95	44.40	141.84	66.37	235.88
21	76.94	279.71	98.57	1 852.20	50.15	152.11	66.37	921.57
29/30	75.39	229.26	249.02	1 981.35	52.92	138.64	306.31	892.32
30/31	67.66	73.85	1 231.86	1 846.68	54.63	61.66	1 451.51	1 421.71

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; TYR: L-tyrosine
Source: Blanck (2006b)

In females in the NTBC + tyrosine group, relative liver weight was increased compared with control animals. There were no other differences in organ weight. Macroscopic examination revealed opacity of the eye in all males and in 1/5 females in the group treated with NTBC + tyrosine. All other gross pathologicdal changes were considered to be incidental and not treatment related.

Findings that are considered to be treatment related were observed in the pancreas, thyroid and eye in the male and female rats in the NTBC + tyrosine group (Table 32). Findings in the pancreas were diffuse interstitial mixed inflammation, along with acinar degeneration/apoptosis of the exocrine pancreas. In the thyroid gland, the only treatment-related finding was colloid alteration, which was observed only in the males. In the eye, the treatment-related change was bilateral keratitis. No pathological treatment-related effect was seen in the liver.

Table 32. Treatment-related histopathological findings after administration of NTBC or L-tyrosine or co-administration of NTBC + tyrosine in rats

	Males				Females			
	Control	TYR	NTBC	NTBC + TYR	Control	TYR	NTBC	NTBC + TYR
No. examined	5	5	5	5	5	5	5	5
Pancreas								
Acinar degeneration/ apoptosis: focal/ multifocal	1	1	1	3	0	0	1	5
Interstitial mixed cell inflammation: diffuse	0	0	0	2	0	0	1	1
Thyroid								
Follicular cell hypertrophy: diffuse	0	0	0	1	0	0	0	0
Colloid alteration	0	0	0	3	0	0	0	0
Eye								
Keratitis: diffuse: bilateral	0	0	0	5	0	0	0	1

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; TYR: L-tyrosine
Source: Blank (2006b)

The results of this study clearly show that sustained systemic tyrosinaemia provokes bilateral keratitis of the eye, focal or multifocal acinar degeneration or apoptosis of the exocrine pancreas, and minimal to slight colloid alteration of the thyroid follicles. These data taken together support the hypothesis that a threshold plasma tyrosine concentration exists below which tyrosine-related findings will not be observed (Blank, 2006b).

In another study, the objectives of which were to assess the relationship between the increase in blood tyrosine levels and potential effects on pregnancy and embryo/fetal development, pregnant Sprague-Dawley rats were administered NTBC by gavage at 10 µg/kg bw per day from GD 6 to GD 20 and L-tyrosine at 2% w/w in the diet from GD 6 to GD 21. Similar groups were co-administered vehicle alone (demineralized water) by gavage plus untreated diet (control group), NTBC by gavage plus untreated diet, or vehicle alone by gavage plus diet supplemented with L-tyrosine at the same dosage. Twenty-three pregnant rats were used per dose group.

There were no treatment-related mortalities or clinical signs during the course of the study. The pregnancy rate was unaffected by treatment with L-tyrosine at 2% in the diet, oral administration of NTBC at 10 µg/kg bw per day or co-administration of L-tyrosine at 2% in the diet plus NTBC at 10 µg/kg bw per day by gavage. Mean maternal body weight gains and mean maternal body weights in the group treated with L-tyrosine alone were similar to those of the control group (Table 33). There was a reduction of 58% (not statistically significant) in mean maternal body weight gain between GD 6 and GD 8 in the group treated with NTBC alone compared with the controls. Thereafter, mean maternal body weight gains were comparable to the control values, resulting in an overall similar body weight gain between GD 6 and GD 21. There was a reduction of 55% (not statistically significant) in mean maternal body weight gain between GD 6 and GD 8 in the group co-treated with L-tyrosine plus NTBC compared with the control group. Thereafter, mean maternal body weight gains in this treated group were similar to the control values, but the overall body weight gain between GD 6 and GD 21 was still slightly reduced by 6% (not statistically significant) in comparison with the control group. Mean maternal corrected body weight change in all treated groups was comparable to the controls. There was no treatment-related effect on feed consumption in any group.

Table 33. Maternal body weight change, maternal plasma L-tyrosine level and fetal weight after administration of NTBC or L-tyrosine or co-administration of NTBC + tyrosine in rats

Parameter	Days	Control	TYR	NTBC	NTBC + TYR
Maternal body weight gain (g)	0–6	29.0	30.7	29.5	30.9
	6–8	6.6	4.7	2.8	3.0
	6–10	15.9	12.6	11.4	12.4
	6–14	33.9	32.4	30.9	30.7
	6–18	75.2	74.3	70.9	73.0
	6–21	123.7	124.1	120.4	116.1
Maternal corrected body weight change (g)	–	45.7	50.7	48.7	45.9
Maternal plasma tyrosine (nmol/mL)	–	46.04	216.4	388.6	2 888
Fetal weight, both sexes (g)	–	5.43	5.39	5.30	5.04**
Fetal weight, males (g)	–	5.56	5.54	5.43	5.17**
Fetal weight, females (g)	–	5.31	5.25	5.16	4.93**

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; TYR: L-tyrosine; **: $P < 0.01$
Source: Kennel (2006)

Maternal plasma tyrosine concentrations were increased in all groups, with the greatest increase observed in the NTBC + tyrosine group (Table 33). Mottled kidney of minimal to slight severity was observed in all treatment groups, but not in the control group. Four animals showed minimal unilateral or bilateral corneal opacity in the NTBC + tyrosine group; no corneal opacities were observed in other groups. There was no effect of treatment on liver weight in any group.

There were no effects of treatment on the numbers of live or dead fetuses, corpora lutea, early or late resorptions, or preimplantation or postimplantation losses.

Fetal weight was statistically significantly decreased in the NTBC + tyrosine group. There was also a slight, statistically non-significant decrease in fetal weight in the NTBC group (Table 33). There were no external findings observed that were considered to be treatment related. There was no effect of treatment on the number of runt fetuses observed. A limited number of skeletal structures was examined – namely, those that were observed to differ from controls in other studies conducted with HPPD inhibitors. In the NTBC + tyrosine group, there was a decrease in ossification of specific bones and an increase in the incidence of short 14th rib and extra ossification points on the 14th thoracic vertebrae (Table 34). All of these findings are classified as variants.

Table 34. Fetal skeletal observations after administration to dams of NTBC or L-tyrosine or co-administration of NTBC + tyrosine in rats

	Fetuses				Litters			
	Control	TYR	NTBC	NTBC + TYR	Control	TYR	NTBC	NTBC + TYR
No. examined	175	162	146	170	23	22	20	23
7th cervical centrum, unossified/normal cartilage	1	6	9	72	1	5	6	19
5th sternebra, incomplete ossification/normal cartilage	49	36	57	71	20	12	17	21
6th sternebra, incomplete ossification/normal cartilage	0	1	0	10	0	1	0	2
5th sternebra, unossified/ normal cartilage	1	2	1	12	1	2	1	6
14th thoracic rib (unilateral/bilateral), short	2	2	0	4	1	2	0	4
Extra ossification point (unilateral/bilateral) on 14th thoracic vertebra	2	8	5	20	2	5	4	11
Forepaw, 3rd and/or 4th phalanges, unossified/ normal cartilage	0	1	0	8	0	1	0	3
5th metacarpal, incomplete ossification/normal cartilage or unossified/ normal cartilage	7	7	6	21	5	4	5	8
1st metatarsal, unossified/ normal cartilage	1	4	2	6	1	3	2	4
Fewer than 9 sacrocaudal vertebrae ossified/normal cartilage	1	0	0	7	1	0	0	4

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; TYR: L-tyrosine
Source: Kennel (2006)

In conclusion, co-administration of L-tyrosine at 2% w/w in the diet between GD 6 and GD 21 plus NTBC by oral gavage at 10 µg/kg bw per day between GD 6 and GD 20 provoked a marked maternal tyrosinaemia, which caused a general delay of ossification in fetuses. Furthermore, the results observed with animals treated with only tyrosine or only NTBC, where maternal plasma tyrosine was not markedly increased and where there was little or no effect on fetal ossification, show that the effects of increased maternal plasma tyrosine on fetal ossification are threshold based. Marked increases in maternal plasma tyrosine concentration are required for fetal effects to be observed (Kennel, 2006).

To compare the metabolism of tyrosine in untreated and HPPD-inhibited conditions across species, hepatocyte preparations (Liverbeads™) from rat, mouse, rabbit, dog and human were incubated with tyrosine, in the absence or the presence of NTBC, a potent inhibitor of the HPPD enzyme. Incubation times ranged from 0 to 4 hours; at the end of the incubation, the concentrations of tyrosine and HPLA were measured.

Basal tyrosine levels were similar across the species and did not change during the incubation period, with the exception of the rabbit, which presented slightly lower basal levels. After addition of NTBC, tyrosine levels were similar across the species at all incubation times, with the rabbit displaying the lowest levels. After addition of enriched tyrosine medium, tyrosine levels were similar across the species and did not change during the incubation period, with the exception of the mouse. After addition of enriched tyrosine medium and NTBC, tyrosine levels were similar across the species at all incubation times (Table 35).

Table 35. Tyrosine concentration in incubation medium after incubation of hepatocytes with or without excess tyrosine and with or without NTBC

Condition	Time (h)	Tyrosine concentration (mg/L)				
		Rat	Dog	Rabbit	Mouse	Human
Basal	0	23.69	26.22	15.25	29.12	24.33
	2	25.93	26.47	17.95	20.75	23.03
	4	25.18	26.22	16.27	21.13	24.40
Basal + NTBC	0	23.82	26.25	15.35	28.48	24.42
	2	26.87	27.60	17.30	24.63	24.48
	4	27.60	27.48	16.85	29.25	27.33
Basal + TYR	0	82.17	77.18	74.45	69.45	76.07
	2	74.42	81.18	74.55	60.63	74.03
	4	74.78	82.60	78.13	54.02	74.47
Basal + TYR + NTBC	0	84.92	78.77	75.60	70.12	74.62
	2	79.07	82.77	76.05	68.52	76.73
	4	79.30	81.38	79.77	73.17	77.70

NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; TYR: L-tyrosine
Source: Totis (2006)

Basal HPLA was not detected in any species, with the exception of the mouse, at any time point. In the mouse Liverbeads™, basal levels of HPLA were barely detectable and did not increase during incubation times. After addition of NTBC to basal medium, HPLA was not observed in the rat, dog or rabbit Liverbeads™ incubations at any time point. In mouse and human incubations, however, HPLA concentration increased with time.

In basal medium supplemented with tyrosine, HPLA was similarly observed only in mice. After the addition of NTBC to basal + L-tyrosine medium, HPLA was observed in the rat, rabbit,

mouse and human incubations. The concentration of HPLA increased with time in the mouse and human incubations, whereas in the rat, there was little or no increase in HPLA with time. In the rabbit, HPLA was observed only at the 4-hour time point. HPLA was not detected in dog Liverbeads™ incubations under any conditions (Table 36).

Table 36. HPLA concentration in incubation medium after incubation of hepatocytes with or without excess tyrosine and with or without NTBC

Condition	Time (h)	HPLA concentration (µg/mg protein)				
		Rat	Dog	Rabbit	Mouse	Human
Basal	0	< LOQ	< LOQ	< LOQ	0.15	< LOQ
	2	< LOQ	< LOQ	< LOQ	0.24	< LOQ
	4	< LOQ	< LOQ	< LOQ	0.25	< LOQ
Basal + NTBC	0	< LOQ	< LOQ	< LOQ	0.18	< LOQ
	2	< LOQ	< LOQ	< LOQ	0.42	0.33
	4	< LOQ	< LOQ	< LOQ	0.69	0.54
Basal + TYR	0	< LOQ	< LOQ	< LOQ	0.17	< LOQ
	2	< LOQ	< LOQ	< LOQ	0.20	< LOQ
	4	< LOQ	< LOQ	< LOQ	0.26	< LOQ
Basal + TYR + NTBC	0	< LOQ	< LOQ	< LOQ	0.12	< LOQ
	2	0.19	< LOQ	< LOQ	0.73	0.54
	4	0.23	< LOQ	0.36	1.31	1.08

LOQ: limit of quantification; NTBC: 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione; TYR: L-tyrosine
Source: Totis (2006)

In conclusion, the results of this study allowed the classification of species into two main groups: 1) human and mouse, which are able to produce HPLA and to use an alternative pathway for tyrosine catabolism when HPPD is inhibited; and 2) rabbit, dog and rat, for which this alternative pathway is much less efficient under "normal" and "stressed" conditions (Totis, 2006).

2.7 Studies on metabolites

(a) Metabolite RPA 202248: 2-Cyano-3-cyclopropyl-4-(2-methylsulfonyl-4-trifluoro-methylphenyl)propan-1,3-dione

Acute toxicity

In an acute oral toxicity study, groups of five male and five female Sprague-Dawley rats were orally administered RPA 202248 (purity > 99.0%) in 0.5% methyl cellulose and distilled water (20 mL/kg bw) at a dose level of 2000 or 5000 mg/kg bw. The animals were observed for mortality and clinical signs of toxicity for 15 days post-dosing. Two males and two females at 5000 mg/kg bw died by day 2; the clinical signs of toxicity observed in both sexes on day 1 included palpebral ptosis, piloerection, reduced motor activity, tremors (females) and coldness to touch (females).

Based on the results of this study, the acute oral LD_{50} for RPA 202248 was greater than 5000 mg/kg bw for both sexes (Bigot, 1995a).

In a second acute oral toxicity study, groups of five male and five female Sprague-Dawley rats received a single oral administration of RPA 202248 (purity 99.9%) in 0.5% aqueous methyl cellulose at a dose level of 2000, 2710, 3690 or 5000 mg/kg bw. Mortalities occurred by study day 3 at 5000 mg/kg bw (one male) and on study day 2 (two males, one female) at 3690 mg/kg bw. There

were no mortalities at either 2000 or 2710 mg/kg bw. The clinical signs of toxicity observed in both sexes, within a few hours of dosing, included piloerection at all dose levels and hunched posture in males at 2710 mg/kg bw as well as in both sexes at 3690 and 5000 mg/kg bw. Reduced motor activity was noted in both sexes at 3690 and 5000 mg/kg bw.

Based on the results of this study, the acute oral LD_{50} for RPA 202248 was greater than 5000 mg/kg bw in both sexes (Katchadourian, 1996).

Mutagenicity

In two independent microbial gene mutation assays, *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to RPA 202248 (purity 99.9%) at 250, 500, 1000, 2500 or 5000 µg/plate in the absence or presence of S9 activation. The confirmatory assay was conducted using the preincubation modification to the standard plate incorporation test. The S9 fraction was derived from Aroclor 1254–induced rat livers, and the test material was delivered to the test system in dimethyl sulfoxide (DMSO).

Minimal cytotoxicity was observed at 5000 µg/plate +S9 (plate incorporation method) or 5000 µg/plate −S9 (preincubation method). All strains responded to the mutagenic action of the appropriate positive control. There was, however, no evidence that RPA 202248 induced a mutagenic response in either trial (Percy, 1995).

(b) Metabolite RPA 203328: IFT-BA (2-mesyl-4-trifluoromethylbenzoic acid)

Acute toxicity

In an acute oral toxicity study, a group of five male and five female Sprague-Dawley rats was orally administered RPA 203328 (purity 99.7%) in 0.5% methyl cellulose and distilled water (20 mL/kg bw) at a dose level of 5000 mg/kg bw. The animals were observed for 15 days post-dosing. No mortalities were noted; the clinical signs of toxicity observed in two males and one female included dyspnoea, piloerection, soiled fur, mucoid faeces and increased salivation. The female also exhibited reduced motor activity, hunched posture and noisy breathing.

Based on the results of this study, the acute oral LD_{50} for RPA 203328 was greater than 5000 mg/kg bw for both sexes (Bigot, 1995b).

Short-term studies of toxicity

In a 14-day toxicity study, RPA 203328 (purity 100%) was administered by oral gavage to male and female Sprague-Dawley rats (five of each sex per dose) at a dose of 0, 30, 100, 300 or 1000 mg/kg bw per day. The control group received the vehicle alone (0.5% methyl cellulose) at the same dosing volume as the treated groups (10 mL/kg bw per day). Animals were observed daily for mortality and clinical signs for a period of 14 days. Body weight, feed consumption, haematology and clinical chemistry were monitored during the study, organs were weighed at necropsy and a gross pathological examination was carried out.

There were no treatment-related mortalities in the study. The only treatment-related clinical sign was increased salivation from 300 mg/kg bw per day. Body weight and body weight gain were slightly decreased in males from 300 mg/kg bw per day, but with no effect in females. Feed consumption was unaffected by treatment. No treatment-related changes were observed at the ophthalmological examination. Increased red blood cell count, haemoglobin and haematocrit were noted in males at the top two doses (300 and 1000 mg/kg bw per day), but not in females. Cholesterol concentration was decreased in females at 1000 mg/kg bw per day, but not in males. There was no effect on organ weights in any dose group. Slight to moderate pale abnormal colour of the liver was the only gross finding noted and was seen in females of all treatment groups and in some males at 300 mg/kg bw per day.

The NOAEL for RPA 203328 in this 14-day gavage study was 30 mg/kg bw per day, based on increased salivation, slightly decreased body weight gains and changes in the haematology and clinical chemistry parameters seen at 300 mg/kg bw per day (Dange, 1994).

In a 28-day toxicity study, RPA 203328 (purity 99.7%) was administered in the diet to male and female Sprague-Dawley rats (10 of each sex per dose) at a concentration of 0, 150, 500, 5000 or 15 000 ppm (equal to 0, 11.14, 37.57, 377.0 and 1118 mg/kg bw per day for males and 0, 12.68, 42.70, 421.5 and 1268.7 mg/kg bw per day for females, respectively). Clinical signs, body weights and feed consumption were monitored. Blood and urine were collected near or at the end of the study for haematological, clinical chemical and urine analysis determinations. Ophthalmological examinations were conducted near the end of the study in the control and 15 000 ppm animals. At the end of the study, selected organs were weighed, and histopathological examinations were conducted.

Among males, a slightly lower urinary pH at 15 000 ppm and minimally higher urinary refractive index at 500 and 15 000 ppm were noted. In the absence of other adverse effects on other parameters, these changes were considered to be a normal physiological response to ingestion of an acidic compound. There were no mortalities, clinical signs or changes in body weight or body weight gain in either males or females. No treatment-related ophthalmological abnormalities were noted. No effects were observed on haematological or clinical chemistry parameters. There were no effects of administration of RPA 203328 over 28 days on organ weights or histopathological observations.

The NOAEL of this 28-day dietary study with RPA 203328 was 15 000 ppm (equal to 1118 mg/kg bw per day), the highest dose tested (Dange, 1995).

In a 90-day toxicity study, male and female Sprague-Dawley rats (10 of each sex per dose) were fed diets containing RPA 203328 (purity 99.0%) at a concentration of 0, 1200, 4800 or 12 000 ppm (equal to 0, 73.21, 306.1 and 768.9 mg/kg bw per day for males and 0, 93.10, 371.4 and 952.4 mg/kg bw per day for females, respectively). Clinical signs, body weights and feed consumption were monitored. Blood and urine were collected near or at the end of the study for haematological, clinical chemical and urine analysis determinations. In addition, grasping, righting, corneal, pupillary, auditory startle and head shaking reflexes were examined once during the acclimatization phase and during week 12. Ophthalmological examinations were conducted near the end of the study in the control and 12 000 ppm animals. At the end of the study, selected organs were weighed, and histopathological examinations were conducted.

There were no mortalities, clinical signs or changes in body weight or body weight gain in either males or females. No effects were observed on haematological or clinical chemistry parameters. From 4800 ppm, urine pH was increased in females, but in the absence of any other findings, the toxicological significance of this finding is unclear. No clearly treatment-related macroscopic organ changes were found at necropsy. At gross necropsy, dark or yellowish liver, marked lobular liver and/or dark kidneys were noted in some animals. In the absence of histological changes, these were not considered to be related to treatment. No treatment-related changes were noted at microscopic examinations.

The NOAEL for RPA 203328 in this 90-day dietary study was 12 000 ppm (equal to 768.9 mg/kg bw per day), the highest dose tested (Bigot, 1998).

Mutagenicity

The genotoxicity studies conducted on RPA 203328 (a metabolite of isoxaflutole) are summarized in Table 37.

Developmental toxicity

In a developmental toxicity study, RPA 203328 (purity 99.0%) was administered to 25 female CD rats by gavage in a volume of 10 mL/kg bw at a dose level of 0, 75, 250 or 750 mg/kg bw per day from GD 6 to GD 20, inclusive. The test material was suspended in 0.5% aqueous methyl cellulose. Females were weighed on days 0, 3, 6, 8, 10, 12, 14, 16, 18 and 21 of gestation. Feed consumption was recorded periodically throughout the study. At necropsy on GD 21, the gravid uterine weight was recorded, and the dams were evaluated for number of corpora lutea and number and status of

Table 37. Results of studies of genotoxicity with RPA 203328

Type of study	Organism/cell line	Dose range tested	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	100–5 000 µg/plate (in DMSO)	97.7	Negative ±S9 mix	Percy (1994)
Chromosomal aberration	Chinese hamster ovary cells	18.3–2 710 µg/mL (in DMSO)	99.0	Negative ±S9 mix	Murli (1998)
Gene mutation in mammalian cells	Chinese hamster ovary cells (HPRT)	5.3–2 700 µg/mL (in DMSO)	99.0	Negative ±S9 mix	Cifone (1998)
In vivo					
Mouse micronucleus	Male CD-1 mice	0, 500, 1 000 and 2 000 mg/kg bw (gavage in 0.5% methyl cellulose)	99.0	Negative	Curry (1998)

DMSO: dimethyl sulfoxide; HPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

implantations (resorptions, dead and live fetuses). Live fetuses were removed from the uteri, counted, weighed, sexed and examined externally. Approximately half of the live fetuses from each litter were fixed in Bouin's solution and subsequently dissected for internal examination. The remaining half were eviscerated, fixed in alcohol and stained with alizarin red S for skeletal examination.

At 250 and 750 mg/kg bw per day, 6 and 18 females (24% and 72%), respectively, had at least one occurrence of transient salivation upon treatment. In some animals dosed at 750 mg/kg bw per day, this was associated with red nasal discharge within a few minutes following administration. These observations disappeared approximately 1 hour after treatment and were probably linked with the acidic nature of the test substance. Statistically significantly reduced body weight changes occurred in the 750 mg/kg bw per day group during the interval GDs 8–10 and in the 250 mg/kg bw per day group during the interval GDs 10–14. Body weight parameters at 75 mg/kg bw per day were comparable to those of the control (Table 38). Mean feed consumption was significantly reduced for the entire treatment period at 750 mg/kg bw per day and from GDs 8 to 21 at 250 mg/kg bw per day. Feed consumption at 75 mg/kg bw per day was comparable to that of the control.

Table 38. Maternal body weight and body weight change in a rat teratology study with RPA 203328

	Day	0 mg/kg bw per day	75 mg/kg bw per day	250 mg/kg bw per day	750 mg/kg bw per day
Maternal body weight (g)	0	270.5	270.1	267.2	266.6
	6	306.8	305.7	300.4	301.0
	8	314.9	311.4	306.1	305.6
	10	325.3	321.5	313.4	309.2
	14	346.8	342.0	329.4	325.2
	18	390.7	385.1	372.0	367.3
	21	445.8	439.9	423.7	415.7
Corrected body weight change (g)		68.2	63.9	46.8**	43.1**

**: $P < 0.01$

Source: Repetto-Larsay (1999)

There was no effect of administration of RPA 203328 on gestation rate, implantation rate, the number of viable young, sex ratio or fetal weight. On examination of the fetuses, there was no effect of treatment on external, visceral or skeletal observations.

Based on the decreased body weight gain and feed consumption from 250 mg/kg bw per day, the maternal NOAEL was 75 mg/kg bw per day. The fetal NOAEL was 750 mg/kg bw per day, the highest dose tested (Repetto-Larsay, 1999).

Based on the available data, the Meeting concluded that there are no toxicological concerns for RPA 203328; therefore, the Meeting concluded that it should not be included in the residue definition. Isoxaflutole benzamide (2-mesyl-4-trifluoromethyl benzamide), a metabolite found in glyphosate-tolerant soya bean, is not likely to be of toxicological concern based on its structural similarity to RPA 203328; therefore, the Meeting concluded that it should not be included in the residue definition. For another metabolite in soya bean, 4-trifluoromethylbenzoic acid, the Meeting concluded that it should not be included in the residue definition due to its anticipated low toxicity based on the toxicity of benzoic acid.

The diketonitrile metabolite RPA 202248, RPA 205834 (2-aminomethylene-1-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)-propane-1,3-dione) and RPA 207048 (1-cyclopropyl-2-hydroxymethylene-3-(2-mesyl-4-trifluoromethylphenyl)-propane-1,3-dione) are structurally similar to the parent compound, and their toxicity is expected to be similar to that of the parent compound. Therefore, in the absence of limited to no data, the Meeting concluded that these three metabolites should be included in the residue definition.

3. Observations in humans

Employees working in isoxaflutole manufacturing plants have been monitored since 1997. Regular medical examinations are done every 1–5 years, depending on age and job tasks. The examinations include blood count, fasting blood sugar, liver enzymes, blood fat, blood pressure, audiometry and lung function testing and also address lifestyle factors such as nutrition. Biomonitoring is also included. No adverse effects were reported in about 58–63 employees working in the isoxaflutole manufacturing plant (Shipp, 2012).

Comments

Biochemical aspects

Following oral gavage dosing of rats, isoxaflutole was rapidly absorbed: about 70% after low dose (1 mg/kg bw) administration and about 40% after high dose (100 mg/kg bw) administration. The maximum concentrations in blood (C_{max}) were achieved between 0.5 and 1 hour post-dosing. Only about 1.4–4.3% of the dose was recovered in the tissues (e.g. kidney, liver, blood and plasma) 7 days after dosing. The elimination of the radioactivity associated with [^{14}C]isoxaflutole following oral administration was rapid, with the majority (80%) of the radioactivity being eliminated within 48 hours at the high dose level and within 24 hours at the low dose level. The urine was the major route of elimination for the low-dose groups (about 69–74% of the dose), whereas faeces was the major route of elimination for the high-dose group (about 55–63% of the dose). Isoxaflutole and/or its metabolites have a mean β-phase elimination half-life of about 60 hours, irrespective of the dose level. Up to nine radiolabelled components were found in the urine, and up to 11 in the faeces. The major component identified in urine, faeces and liver was a diketonitrile (RPA 202248, or 3-cyclopropyl-2-[2-mesyl-4-trifluoromethylbenzoyl]-3-oxopropane nitrile), followed by RPA 203328 (2-mesyl-4-trifluoromethylbenzoic acid). Unchanged isoxaflutole was detected primarily in faeces in the high-dose animals. There were no sex differences in absorption, distribution or metabolism.

Toxicological data

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw, and the acute dermal LD_{50} in both rats and rabbits was greater than 2000 mg/kg bw. The acute inhalation LC_{50} in rats was greater than the maximum achievable concentration of 5.23 mg/L air. Isoxaflutole was non-irritating to rabbit skin and minimally irritating to rabbit eyes. It was not a skin sensitizer in guinea-pigs, as determined by the Buehler method and the Magnusson and Kligman test.

The liver was the primary target organ in mice, rats and dogs in repeated-dose toxicity studies. Thyroid, kidney and the haematopoietic system were also target organs in dogs and rats. Corneal opacity was observed in repeated-dose toxicity studies in rats, but not in mice or dogs.

In a 28-day toxicity study in mice using dietary concentrations of 0, 175, 700, 2800 and 7000 ppm (equal to 0, 29.4, 120.7, 474.6 and 1140.1 mg/kg bw per day for males and 0, 34.7, 142.9, 534.4 and 1347.4 mg/kg bw per day for females, respectively), the NOAEL was 175 ppm (equal to 29.4 mg/kg bw per day), based on increases in liver enzymes (ALAT and ASAT), clinical chemistry changes (decreased bilirubin and creatinine levels) and increased liver weight at 700 ppm (equal to 120.7 mg/kg bw per day). In the absence of any other significant findings at 175 ppm, the increased liver weights observed at this dose were considered a minor adaptive change.

In a 90-day toxicity study in mice using dietary concentrations of 0, 50, 1000 and 2000 ppm (equal to 0, 7.6, 170.0 and 324.1 mg/kg bw per day for males and 0, 8.7, 181.2 and 376.2 mg/kg bw per day for females, respectively), the NOAEL of 50 ppm (equal to 7.6 mg/kg bw per day) was based on increased ALAT and ASAT activities, increased absolute and relative liver weights and increased incidence of periacinar hepatocytic hypertrophy at 1000 ppm (equal to 170.0 mg/kg bw per day).

In a 6-week toxicity study in rats given diets providing doses of 0, 25, 100, 400 and 1000 mg/kg bw per day, the LOAEL was 25 mg/kg bw per day, based on corneal opacities and effects on the liver observed at all doses. Most of the corneal opacities were resolved by the 2nd week of the reversibility period. In a 90-day dietary toxicity study in rats at doses of 0, 1.0, 3.0, 10 and 100 mg/kg bw per day, the NOAEL was 3.0 mg/kg bw per day, based on haematological changes, corneal opacity and liver toxicity observed at 10 mg/kg bw per day.

In a 1-year toxicity study in dogs using dietary concentrations of 0, 240, 1200, 12 000 and 30 000 ppm (equal to 0, 8.56, 44.81, 453 and 1265 mg/kg bw per day for males and 0, 8.41, 45.33, 498 and 1254 mg/kg bw per day for females, respectively), the NOAEL was 1200 ppm (equal to 44.81 mg/kg bw per day), based on reduced weight gains, increased liver weight, histopathological findings in the liver and changes in haematological and clinical chemistry parameters at 12 000 ppm (equal to 453 mg/kg bw per day).

In a 78-week study of toxicity and carcinogenicity in mice using dietary concentrations of 0, 25, 500 and 7000 ppm (equal to 0, 3.2, 64.4 and 977.3 mg/kg bw per day for males and 0, 4.0, 77.9 and 1161.1 mg/kg bw per day for females, respectively), the NOAEL was 25 ppm (equal to 3.2 mg/kg bw per day), based on liver effects seen at 500 ppm (equal to 64.4 mg/kg bw per day). The NOAEL for carcinogenicity was 500 ppm (equal to 64.4 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas and carcinomas in both sexes at 7000 ppm (equal to 977.3 mg/kg bw per day).

In a 2-year chronic toxicity and carcinogenicity study in rats given diets providing doses of 0, 0.5, 2, 20 and 500 mg/kg bw per day, the NOAEL was 2 mg/kg bw per day, based on liver, thyroid, ocular and nervous system toxicity in males and liver toxicity in females seen at 20 mg/kg bw per day. An increased incidence of adenomas and carcinomas of the liver was found in male and female rats at 500 mg/kg bw per day. In male rats, an increase of thyroid follicular cell adenomas was also observed at 500 mg/kg bw per day.

A 14-day dietary study in mice and rats indicated a marked increase in microsomal enzyme induction (increased PROD and BROD activities) and increased liver weights. There was no peroxisome proliferation. The data were inadequate to elucidate the precursor events leading to tumour formation and dose concordance for hepatocellular adenomas and carcinomas in mice and

rats. In a 14-day oral gavage study in rats, isoxaflutole was found to decrease T_4 levels, with little or no change in T_3 levels, and an increased systemic clearance of ^{125}I-labelled T_4 was observed. The results of these mechanistic studies were suggestive of the induction of microsomal enzymes and tumour formation, but failed to establish the mode of action.

The Meeting concluded that isoxaflutole is carcinogenic in mice and rats.

Special studies conducted to evaluate the corneal opacity seen in rats suggest that the lesion may be linked to the inhibition of the enzyme HPPD in the catabolic pathway of tyrosine. The studies have shown that if HPPD is inhibited, alternative pathways may be utilized to remove excess tyrosine, and species specificity may be linked to the differences in activity of these alternative pathways. The results of the comparative metabolism study in mice and rats suggest that the elimination of tyrosine as HPLA and HPAA is more efficient in the mouse than in the rat, with twice as much of the administered dose of [^{14}C]tyrosine observed in mouse urine as in rat urine. The results of special studies indicate that rats are more sensitive than mice, dogs and humans to tyrosinaemia.

Isoxaflutole was tested for genotoxicity in vitro and in vivo in an adequate range of assays. No genotoxicity was observed.

The Meeting concluded that isoxaflutole is unlikely to be genotoxic.

On the basis of the absence of genotoxicity and other available toxicological information, the Meeting concluded that the mode of action for the increased incidences of hepatocellular adenomas and carcinomas in both male and female mice and rats and the increased incidence of thyroid follicular cell adenomas in male rats, while not completely understood, is likely to involve a threshold. Therefore, the Meeting concluded that isoxaflutole is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats given diets providing doses of 0, 0.5, 2, 20 and 500 mg/kg bw per day, the NOAEL for parental systemic toxicity and offspring toxicity was 2 mg/kg bw per day. The parental systemic toxicity LOAEL of 20 mg/kg bw per day was based on increased liver weights, liver hypertrophy and vacuolation. The offspring toxicity LOAEL of 20 mg/kg bw per day was based on decreased pup weights and reduced pup viability. The NOAEL for reproductive toxicity was 500 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rats that tested doses of 0, 10, 100 and 500 mg/kg bw per day, the maternal NOAEL was 100 mg/kg bw per day, based on decreased body weight gain observed at 500 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on decreased fetal weight and delayed ossification observed at 100 mg/kg bw per day.

In a developmental toxicity study in rabbits that tested doses of 0, 5, 20 and 100 mg/kg bw per day, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased maternal body weight, decreased feed consumption and increased numbers of resorptions seen at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 20 mg/kg bw per day, based on slightly delayed development of the fetuses, decreased fetal weights and delayed ossification at 100 mg/kg bw per day.

The Meeting concluded that isoxaflutole is not teratogenic in rats or rabbits.

In an oral acute neurotoxicity study in rats that tested doses of 0, 125, 500 and 2000 mg/kg bw, no evidence of neurotoxicity or systemic toxicity was observed at doses up to 2000 mg/kg bw. In a 90-day neurotoxicity study in rats given diets providing doses of 0, 25, 250 and 750 mg/kg bw per day, no neurotoxicity was observed at doses up to 750 mg/kg bw per day. A NOAEL for systemic toxicity was not identified, as only limited parameters were evaluated in this study.

In a developmental neurotoxicity study in rats that tested gavage doses of 0, 5, 25 and 250 mg/kg bw per day, the maternal NOAEL was 25 mg/kg bw per day, based on decreased maternal body weight, body weight gain and feed consumption at 250 mg/kg bw per day. The offspring toxicity NOAEL was 25 mg/kg bw per day, based on decreased pup survival, body weight and body weight

gain at 250 mg/kg bw per day. In the absence of any neurotoxic findings, the NOAEL for neurotoxicity in the rat was 250 mg/kg bw per day, the highest dose tested.

The Meeting concluded that isoxaflutole is not neurotoxic.

Toxicological data on metabolites and/or degradates

The acute oral LD_{50} of metabolite RPA 202248, a major metabolite of urine, faeces and liver, was greater than 5000 mg/kg bw. The metabolite was not genotoxic in the Ames test.

Metabolite RPA 203328, detected in urine and faeces, was extensively studied. The acute oral LD_{50} in rats was greater than 5000 mg/kg bw. RPA 203328 was not genotoxic in a range of in vivo and in vitro genotoxicity assays. In a 14-day gavage toxicity study in rats, the NOAEL for RPA 203328 was 30 mg/kg bw per day, based on increased salivation, slightly decreased body weight gains and changes in the haematology and clinical chemistry parameters seen at 300 mg/kg bw per day. Dietary 28-day and 90-day toxicity studies in rats were conducted for RPA 203328 at doses up to 15 000 ppm (equal to 1118 mg/kg bw per day) and 12 000 ppm (equal to 769 mg/kg bw per day), respectively. No evidence of systemic toxicity was observed in these studies. No evidence of teratogenicity or developmental toxicity in rats was observed in a developmental toxicity study for RPA 203328 at doses up to 750 mg/kg bw per day.

Human data

In reports on employees working in isoxaflutole manufacturing plants, no adverse health effects were reported.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.02 mg/kg bw on the basis of a NOAEL of 2 mg/kg bw per day in a 2-year dietary study of toxicity and carcinogenicity in rats, on the basis of liver, thyroid, ocular and nervous system toxicity in males and liver toxicity in females at 20 mg/kg bw per day. A safety factor of 100 was applied. This ADI is supported by a NOAEL for parental systemic toxicity and offspring toxicity of 2 mg/kg bw per day in a dietary two-generation reproductive toxicity study in rats, based on increased liver weights, liver hypertrophy, vacuolation, decreased pup weights and reduced pup viability observed at 20 mg/kg bw per day. The ADI provides a margin of exposure of at least 25 000 relative to the LOAEL for liver and thyroid tumours in rats and at least 48 000 relative to the LOAEL for the liver tumour response in mice. Thus, the Meeting considered that isoxaflutole is not likely to pose a carcinogenic risk to humans from the diet.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for isoxaflutole in view of its low acute toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment of isoxaflutole

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day study of toxicity[a]	Toxicity	50 ppm, equal to 7.6 mg/kg bw per day	1 000 ppm, equal to 170 mg/kg bw per day
	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	25 ppm, equal to 3.2 mg/kg bw per day	500 ppm, equal to 64.4 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 64.4 mg/kg bw per day	7 000 ppm, equal to 977 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity study[b]	Toxicity	2 000 mg/kg bw[c]	–
	Ninety-day study of toxicity[a]	Toxicity	3 mg/kg bw per day	10 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	2 mg/kg bw per day	20 mg/kg bw per day
		Carcinogenicity	20 mg/kg bw per day	500 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	500 mg/kg bw per day[c]	–
		Parental toxicity	2 mg/kg bw per day	20 mg/kg bw per day
		Offspring toxicity	2 mg/kg bw per day	20 mg/kg bw per day
	Developmental toxicity study[b]	Maternal toxicity	100 mg/kg bw per day	500 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	100 mg/kg bw per day
Rabbit	Developmental toxicity study[b]	Maternal toxicity	20 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	20 mg/kg bw per day	100 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	1 200 ppm, equal to 44.8 mg/kg bw per day	12 000 ppm, equal to 453 mg/kg bw per day

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level
[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.

Estimate of acceptable daily intake

0–0.02 mg/kg bw

Estimate of acute reference dose

Unnecessary

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 isoxaflutole

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 40–70%, depending on dose
Dermal absorption	Low, < 4.5%
Distribution	Widely distributed (highest levels in kidney and liver)
Potential for accumulation	None
Rate and extent of excretion	Rapid and complete, about 80% in urine and faeces in 24 h in rats
Metabolism in animals	Extensive; saturated at high doses
Toxicologically significant compounds in animals, plants and the environment	Isoxaflutole, RPA 202248[a], RPA 205834[a], RPA 207048[a]

Acute toxicity	
Rat, LD_{50}, oral	> 5 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.23 mg/L (whole-body exposure)
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Minimally irritating
Guinea-pig, dermal sensitization	Non-sensitizing (Buehler method and Magnusson-Kligman test)
Short-term studies of toxicity	
Target/critical effect	Eye, liver and red blood cells
Lowest relevant oral NOAEL	3 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver and thyroid
Lowest relevant oral NOAEL	2 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans from the diet
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	Pup viability and pup weights
Lowest relevant parental NOAEL	2 mg/kg bw per day
Lowest relevant offspring NOAEL	2 mg/kg bw per day
Lowest relevant reproductive NOAEL	500 mg/kg bw per day, the highest dose tested
Developmental toxicity	
Developmental target/critical effect	Delayed ossification, decreased fetal weights
Lowest maternal NOAEL	20 mg/kg bw per day (rabbit)
Lowest embryo/fetal NOAEL	10 mg/kg bw per day (rat)
Neurotoxicity	
Acute and subchronic neurotoxicity	Not neurotoxic
Other toxicological studies	
Studies on metabolites	Rat, LD_{50}, oral: > 5 000 mg/kg bw (RPA 203348 and RPA 203328)
	Lowest relevant short-term NOAEL: 769 mg/kg bw per day (RPA 203328)
	Not genotoxic (RPA 202248 and RPA 203328)
	No developmental toxicity (RPA 203328) at doses up to 750 mg/kg bw per day, highest dose tested
Medical data	
	No adverse effects

LC_{50}: median lethal concentration; LD_{50}: median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level

[a] Based on structural similarity to the parent compound.

Summary

	Value	Study	Safety factor
ADI	0–0.02 mg/kg bw	Two-year study of toxicity and carcinogenicity in rats	100
ARfD	Unnecessary	–	–

ADI: acceptable daily intake; ARfD: acute reference dose

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