BUPROFEZIN

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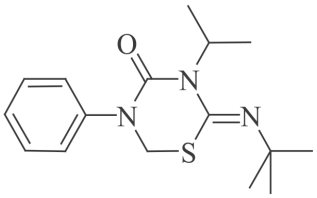
Explanation

Buprofezin is the ISO approved name for (*EZ*)-2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one (IUPAC), CAS No. 69327-76-0. Buprofezin is an insecticide that acts by the inhibition of chitin synthesis.

Buprofezin was previously evaluated by the JMPR in 1991 when an acceptable daily intake (ADI) of 0–0.01 mg/kg bw per day was established based on a no-observed-adverse-effect level (NOAEL) of 0.9 mg/kg bw per day identified in a 2-year study in rats and with a safety factor of 100. The JMPR in 1999 considered that the establishment of an acute reference dose (ARfD) was unnecessary. Buprofezin was re-evaluated by the present Meeting as part of the Codex Committee on Pesticide Residues (CCPR) periodic re-evaluation programme. New studies not previously evaluated by the Meeting included several studies of acute oral toxicity, irritation, sensitization and genotoxicity, metabolism studies in rats and a two-generation study in rats.

The more recent studies complied with good laboratory practice (GLP), but many of the older reported studies were performed before the widespread use of GLP.

Figure 1. Chemical structure of buprofezin



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

The absorption, metabolism, distribution and excretion of [phenyl-^{14}C]buprofezin (radiochemical purity, > 97%; specific activity, 0.074 or 0.833 GBq/mmol) were investigated in fasted male Sprague-Dawley rats given single oral doses of buprofezin at 10 or 100 mg/kg bw. Additionally, bile-duct cannulated rats were given buprofezin at a dose of 10 mg/kg bw. Neither a GLP nor a quality assurance statement was included.

Irrespective of the dose administered, excretion was complete within 2–3 days after administration (Table 1), with 22–25% found in the urine and 70–74% in the faeces. In the bile-duct cannulated rats, 33.2% of the administered dose was excreted in the bile.

Absorption peaked at 9 h after administration and excretion was biphasic with half-lives of 13 h and 60 h (Table 3). C_{max} was approximately proportional to the dose administered, suggesting that absorption was not saturated in this dose range. Within 2 h, buprofezin was widely distributed into the organs, with the highest concentrations of radiolabel measured in adipose tissue, kidney, liver and urinary bladder at most of the time-points between 2 h and 96 h (Table 4). At 10 mg/kg bw, in the liver only, more than 1 µg equivalents/g were found at 96 h. In the group at the lower dose, elimination from the thyroid was slower than from other organs. At the higher dose, concentrations of radiolabel in many organs were higher than proportional to the administered dose at several time-points but not at study termination. There was no evidence for accumulation of radiolabel in any organ. The results of the organ analysis were essentially confirmed by whole-body autoradiography performed at the same time-points as organ analysis.

The metabolism of buprofezin was studied in rat liver homogenates and in urine, faeces and bile in vivo. Hydroxylation with consecutive methylation of the phenyl ring, oxidation of sulfur with consecutive ring opening of the thiadiazinane ring and conjugation reactions with sulfate and

glucuronic acid were the main metabolic routes. Besides the conjugates, the metabolites buprofezin (BF1), buprofezin sulfoxide (BF10), isopropylphenylurea (BF12), 4-hydroxybuprofezin (BF2), dihydroxy-buprofezin (C), hydroxy-methoxy-buprofezin (BF27) and 2-hydroxyisopropylphenylurea (BF13) (only in vitro) were identified (Figure 2) (Sugimoto, 1982).

In a supplementary study, previously not-analysed polar metabolites detected in male rats given buprofezin at a dose of 10 or 100 mg/kg bw (Sugimoto, 1982) were further characterized. This lead to the identification of these metabolites as glucuronic acid and/or sulfuric acid conjugates of 4-aminophenol (BF22, K) (0.2% in urine at 10 mg/kg bw) and 4-hydroxyacetanilide (BF23) (4.1% of administered dose in the urine and 2.7% of the administered dose in the faeces at 10 mg/kg bw) (Figure 2) (Uchida, 1988).

Table 1. Cumulative excretion of radiolabel in male rats given a single oral dose of radiolabelled buprofezin

Dose (mg/kg bw)	Time after administration (days)	Recovery (% of administered dose)			
		Urine	Faeces	Expired	Total
10	0.5	11.87	24.11	0.05	36.03
	1	18.91	63.23	0.16	82.3
	2	21.28	72.46	0.19	93.93
	3	21.69	73.58	0.20	95.47
	4	21.91	73.95	0.21	96.07
100	0.5	12.10	15.14	0.07	27.31
	1	18.81	41.75	0.15	60.71
	2	24.00	67.01	0.19	91.20
	3	24.68	69.95	0.20	94.83
	4	25.20	70.45	0.21	95.86

From Sugimoto (1982)

Table 2. Recovery of radiolabel in the blood of male rats given a single oral dose of radiolabelled buprofezin

Time after administration (h)	Concentration of radiolabel (µg equivalents/g)	
	Dose (mg/kg bw)	
	10	100
0.5	0.122	0.976
1	0.169	1.476
2	0.268	2.594
4	0.485	6.154
6	0.941	9.442
9	1.164	13.818
12	1.080	12.553
15	0.933	10.142
24	0.576	5.930
48	0.375	4.084
96	0.245	2.472

From Sugimoto (1982)

Table 3. Blood pharmacokinetic parameters in male rats given a single oral dose of radiolabelled buprofezin

Dose (mg/kg bw)	t_{max} (h)	c_{max} (µg/ml)	$t_{1/2\ e(9–24h)}$ (h)	$t_{1/2\ e(24–96)}$ (h)
10	9	1.164	13	60
100	9	13.282	13	60

From Sugimoto (1982)

Table 4. Recovery of radiolabel from organs of male rats given a single oral dose of radiolabelled buprofezin

Organ/tissue	Dose(mg/kg bw)										Ratio of recovery, 100 mg/kg bw: 10 mg/kg bw				
	Concentration of radiolabel (µg equivalents/g or ml)														
	10					100									
	Time after administration (h)					Time after administration (h)					Time after administration (h)				
	2	5	9	24	96	2	5	9	24	96	2	5	9	24	96
Adipose	0.8	4.0	4.2	1.7	0.1	23.6	86.8	114.7	46.0	1.6	31	21	28	27	14
Adrenal	0.4	2.3	1.6	0.5	0.1	10.6	43.5	47.0	7.1	1.0	26	19	30	14	13
Blood	0.1	0.8	1.0	0.5	0.2	2.6	9.4	13.7	6.0	2.5	21	12	13	12	10
Bone	0.1	0.4	0.7	0.1	0.0	2.4	6.8	6.2	1.7	0.3	17	16	9	14	8
Brain	0.3	0.5	0.3	0.1	0.0	5.5	12.5	10.4	1.2	0.3	21	25	37	16	12
Eye ball	0.2	0.2	0.2	0.1	0.0	1.5	5.2	5.3	1.0	0.2	9	27	23	13	11
Heart	0.3	0.7	0.6	0.2	0.1	6.9	12.9	14.7	2.4	0.5	21	18	23	11	10
Intestine	NA	NA	NA	NA	0.3	NA	NA	NA	NA	3.8	NA	NA	NA	NA	11
Kidney	1.2	2.3	2.5	0.7	0.3	16.7	35.8	33.6	8.1	2.1	14	16	13	12	8
Liver	4.9	11.2	8.4	3.2	1.2	44.3	70.3	85.5	25.9	8.0	9	6	10	8	7
Lung	0.4	0.8	0.7	0.4	0.1	5.7	18.6	14.8	3.8	0.9	16	23	20	11	9
Muscle	0.2	0.3	0.4	0.1	0.0	3.7	9.6	9.1	1.5	0.4	21	28	24	17	16
Pancreas	0.6	1.2	1.3	0.3	0.0	15.1	32.8	31.4	6.4	0.4	24	28	25	21	16
Salivary gland	0.4	0.6	0.6	0.2	0.0	5.1	14.8	14.8	2.8	0.4	14	24	24	15	10
Spleen	0.3	0.6	0.6	0.3	0.1	6.9	32.8	13.3	3.0	0.6	23	51	22	12	12
Testis	0.2	0.4	0.3	0.1	0.0	3.7	8.6	8.2	1.0	0.3	23	21	25	11	11
Thymus	0.2	0.5	0.4	0.2	0.0	4.0	10.9	11.3	1.7	0.3	24	23	27	11	17
Thyroid	0.4	0.9	0.7	0.6	0.5	10.6	22.1	17.6	5.5	0.7	26	24	24	9	1
Urinary bladder	5.3	9.7	14.3	2.1	0.1	53.8	49.2	70.7	15.7	1.6	10	5	5	8	11

From Sugimoto (1982)

NA, not analysed.

The absorption, distribution, metabolism and excretion of [phenyl-^{14}C]buprofezin (radiochemical purity, > 97%; specific activity, 0.798 GBq/mmol) was investigated in male and female CD rats. Groups of five male and five female rats were given a single dose of radiolabelled buprofezin at 10 mg/kg bw or 100 mg/kg bw by oral gavage. Urine and faeces were collected at 6 h, 24 h and thereafter every 24 h until terminal sacrifice at 168 h. After sacrifice, organs were retained for radioactivity analysis. An additional group of three male and four female rats were bile-duct cannulated and given a single dose of radiolabelled buprofezin at 10 mg/kg bw. Urine, faeces and bile were collected for

24 h. A statement of GLP compliance was not included in the study report but a statement of quality assurance was provided.

At both doses, most of the radioactivity was excreted in the faeces within 48 h; urinary excretion accounted for 20–22% in males and 13–15% in females (Table 5). Excretion initially was slightly faster in males than in females and was completed after 168 h in males and females.

At both doses in males and females, the highest concentrations of radiolabel were found in blood cells, the thyroid and the liver (Table 6). Maximal concentrations in these organs were 0.36 µg equivalents/g at the lower dose and 2.3 µg equivalents/g at the higher dose. Less than 0.7% of the administered dose was recovered in the body at terminal sacrifice of rats at either dose.

In the bile-duct cannulated rats, only 2.6–5.5% of the administered dose was found in the urine (Table 7); this is about one quarter of the amount found in non-cannulated rats. In the bile, approximately 30–40% of the administered dose was excreted within 24 h. Therefore, there was evidence for enterohepatic recirculation of buprofezin. Since the concentration of radiolabel in the gastrointestinal tract of females was higher than in males, absorption in females might be slower than in males.

Thin-layer chromatography (TLC) of samples of urine and bile indicated a high degree of conjugation, while acid hydrolysis of faecal extracts revealed unchanged buprofezin (BF1), 4-hydroxy-buprofezin (BF2) and possibly dimethoxy-buprofezin (BF20, A-15) (Caley & Cameron, 1988).

Table 5. Recovery of radiolabel in rats given a single dose of radiolabelled buprofezin by oral gavage

Time interval	Concentration of radiolabel (mean % of administered dose)											
	Dose (mg/kg bw)											
	10						100					
	Urine		Faeces		Total		Urine		Faeces		Total	
	M	F	M	F	M	F	M	F	M	F	M	F
0–24 h	18.5	10.8	60.7	53.5	79.2	64.4	18.1	11.8	57.4	48.5	75.5	60.3
0–48 h	20.1	12.6	71.0	76.0	91.1	88.5	20.6	13.7	69.3	73.0	90.0	86.7
0–168 h	20.9	13.4	72.8	79.1	94.8	93.4	21.7	14.6	72.7	85.1	95.2	100.2

From Caley & Cameron (1988)

F, females; M, males

Table 7. Distribution of radiolabel in bile-duct cannulated rats given a single dose of radiolabelled buprofezin at 10 mg/kg bw by oral gavage

Tissue/body fluid	Recovery of radiolabel (% of administered dose)	
	Males	Females
Bile	29.8	38.2
Urine	5.5	2.6
Faeces	34.0	19.0
Gastrointestinal tract	16.6	27.5
Liver	1.0	1.5
Carcass	3.8	3.2
Total	90.9	94.5

From Caley & Cameron (1988)

Table 6. Recovery of radiolabel at 168 h from organs of rats given a single dose of radiolabelled buprofezin by oral gavage

Organ	Dose (mg/kg bw)							
	10				100			
	Males		Females		Males		Female	
	µg equivalents/g	% of dose	µg equivalents/g	% of dose	µg equivalents/g	% of dose	µg equivalents/g	% of dose
Bone	0.023	—	0.017	—	0.207	—	0.128	—
Fat	0.031	—	0.028	—	0.316	—	0.252	—
Skeletal muscle	0.024	—	0.017	—	0.229	—	0.205	—
Brain	0.014	0.001	0.016	0.001	0.228	0.002	0.190	0.001
Heart	0.032	0.002	0.030	0.001	0.531	0.002	0.443	0.001
Liver	0.313	0.182	0.360	0.151	2.198	0.128	2.053	0.091
Lung	0.048	0.004	0.054	0.003	0.528	0.003	0.547	0.005
Spleen	0.034	0.001	0.034	0.001	0.441	0.001	0.328	0.001
Kidney	0.068	0.007	0.074	0.007	0.618	0.007	0.532	0.004
Testes/ovaries	0.014	0.002	0.042	0.000	0.095	0.001	0.304	0.000
Uterus	—	—	0.032	0.001	—	—	0.283	0.001
Thyroid	0.155	0.000	0.359	0.000	1.831	0.000	2.342	0.001
Stomach + contents	0.010	0.003	0.013	0.002	0.071	0.003	0.070	0.001
Intestine + contents	0.027	0.033	0.036	0.034	0.230	0.029	0.195	0.018
Plasma	0.026	—	0.031	—	0.365	—	0.377	—
Blood cells	0.150	—	0.140	—	2.278	—	1.855	—

From Caley & Cameron (1988)

— Not calculable, according to the study report

In an accumulation study, two groups of 27 young male rats were given diets containing technical buprofezin (purity, 99%) at a concentration of 200 or 1000 ppm. At 2 and 4, days and 1, 2, 4, 8, 12, 16 and 24 weeks, three rats were sacrificed and buprofezin content of the organs was analysed. The limit of detection (LOD) in the organs was 0.1 ppm. No statements of compliance with GLP or quality assurance were provided.

At 200 ppm, buprofezin was found occasionally at very low concentrations that were slightly above the LOD. In adipose tissue from day 4 onwards, concentrations of buprofezin were up to approximately 1 ppm. In the groups at 1000 ppm, concentrations of buprofezin in the liver were up to approximately 1 ppm and in adipose tissue up to approximately 10 ppm. There was no evidence for accumulation of buprofezin, but since only buprofezin was analysed, no conclusion about the metabolite profiles in the tissues can be drawn (Sugaya & Uchida, 1980).

To study the metabolism of buprofezin in rats, five males were given a single oral dose of [phenyl-^{14}C]buprofezin (radiochemical purity, 99%; specific activity, 0.601 GBq/mmol) at 100 mg/kg bw. Urine and faeces were collected until terminal sacrifice at 72 h after dosing; metabolites in organs were analysed at study termination. The study complied with GLP.

Renal excretion was virtually complete within 48 h and accounted for 12.9% and faecal excretion for 79.1% of the administered dose; less than 0.8% remained in the body. The highest concentrations of residual radioactivity were found in kidney (1 µg equivalents/g), blood, thyroid and liver (7 µg equivalents/g).

Of the administered dose, 60.7% could be allocated to individual metabolites and no unidentified individual metabolite exceeded 3.7% (Table 8). The metabolic pathways include phenyl-ring hydroxylation, oxidation of the t-butyl group and thiadiazinane ring-opening with intensive conjugation of metabolites. Buprofezin and BF27 were identified after extraction of faeces and BF9, BF10, BF12, BF13 after hydrolysis of unextractable faecal metabolites. BF13 and BF23, BF28 conjugates were identified as urine residues after sulfatase cleavage. Figure 2 depicts the metabolic scheme in the rat (Huang & Smith, 1997).

In a supplemental metabolism study designed to identify early metabolites in the organs, a single oral dose of 100 mg/kg bw [phenyl-^{14}C]buprofezin (radiochemical purity, not specified; specific activity, 1.13 GBq/mmol) was given to 12 male Sprague Dawley rats. Groups of four rats were killed at 3, 6 and 72 h and metabolites in the liver, kidney, heart and plasma and the content of the gastrointestinal tract were identified. Faeces and urine were collected throughout the study and analysed for metabolites. To investigate immediate early metabolism, rat liver microsomes were incubated with 2 µmol/l of [phenyl-^{14}C]buprofezin and after 10 and 30 min, respectively, the metabolites were extracted and investigated. The study complied with GLP.

Apart from a range of previously identified rat metabolites, the liver homogenates were found to contain the metabolites tert-butylhydroxy-buprofezin (BF4), phenylbiuret (BF11) and thiobiuret (BF25) (Table 9). In the in-vivo study, newly identified metabolites included BF4 in liver kidney, heart and the gastrointestinal tract, BF11 was found in liver and kidney and BF25 in the liver only (Yoshizane, 2008).

Conjugates are not shown; structures in brackets were not found in rats but are proposed intermediates; BF26 was found in plants.

1.2 Effects on enzymes and other biochemical parameters

No information was available.

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with buprofezin are summarized in Table 1.

Table 8. Identification of metabolites in rats given a single oral dose of radiolabelled buprofezin

Metabolite	Recovery (% of administered dose)	
	Urine	Faeces
Buprofezin (BF1)	ND	45.4
Dione metabolite (BF9)	ND	< 0.1
Buprofezin sulfoxide (BF10)	ND	0.1
Isopropylphenylurea (BF12)	ND	0.1
4-Hydroxyisopropylphenylurea (BF13)	0.5	< 0.1
4-Hydroxyacetanilide (BF23)	2.5	ND
Hydroxy-methoxy-buprofezin (BF27)	ND	7.2
2-[3-Isopropyl-3-[methylsulfonylmethyl(phenyl)carbamoyl]ureido]-2-methylpropionic acid (BF28)	0.3	4.6

From Huang & Smith (1997)

ND, not detected

Table 9. Recovery of metabolites found in male rat liver microsomes in vitro[a] and in organs[b] of rats given a single oral dose of radiolabelled buprofezin

Metabolite	Recovery (% of total radioactive residue)				
	Microsomes	Liver	Kidney	Heart	Gastrointestinal tract
Buprofezin (BF1)	39.22	12.2	17.03	30.25	47.34
4-Hydroxybuprofezin (BF2)	28.22	8.02	8.25	14.6	0.09
Tert-butylhydroxy-buprofezin (BF4)	10.99	7.97	1.42	2.06	0.68
Dione metabolite (BF9)	0.04	0.11	ND	ND	ND
Phenylbiuret (BF11)	0.04	0.27	0.14	ND	ND
Isopropylphenylurea (BF12)	ND	0.56	ND	ND	0.03
Thiobiuret (BF25)	0.17	0.09	ND	ND	ND
2-Amino-2-methylpropyl-2-methylethy *l*-4-Phenyl-allophanate (BF26)	ND	ND.	ND	ND	ND
Hydroxy-methoxy-buprofezin (BF27)	ND	2.60	10.69	25.82	1.42
Others	8.05	8.82	13.76	14.2	2.34

From Yoshizane (2008)

ND, not detected

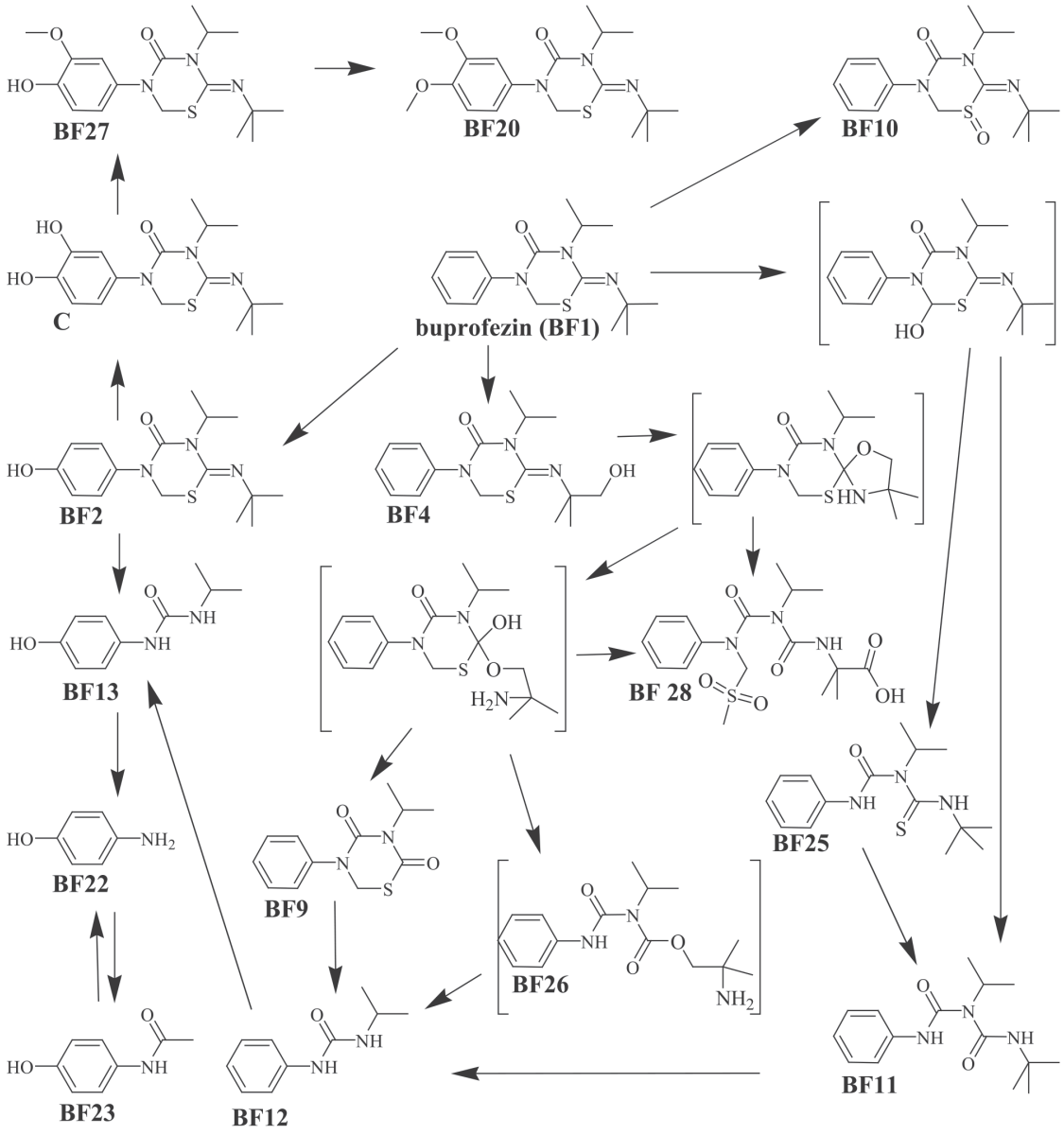
[a] After incubation with [phenyl-^{14}C]buprofezin for 10 min

[b] Rats killed and samples taken 3 h after a single oral dose of [phenyl-^{14}C]buprofezin at 100 mg/kg bw

Table 10. All identified rat metabolites of buprofezin

Metabolite		References
BF1	buprofezin	Sugimoto (1982); Caley & Cameron (1988); Huang & Smith (1997); Yoshizane (2008)
BF2	4-hydroxybuprofezin	Sugimoto (1982); Caley & Cameron (1988); Yoshizane (2008)
BF4	tert-butylhydroxy-buprofezin	Yoshizane (2008)
BF9	dione metabolite	Huang & Smith (1997); Yoshizane (2008)
BF10	buprofezin sulfoxide	Sugimoto (1982); Huang & Smith (1997)
BF11	phenylbiuret	Yoshizane (2008)
BF12	isopropylphenylurea	Sugimoto (1982); Huang & Smith (1997); Yoshizane (2008)
BF13	4-hydroxyisopropylphenylurea	Sugimoto (1982); Huang & Smith (1997)
BF20	dimethoxy buprofezin	Caley & Cameron (1988)
BF22	4-aminophenol	Uchida (1988)
BF23	4-hydroxyacetanilide	Uchida (1988); Huang & Smith (1997)
BF25	thiobiuret	Yoshizane (2008)
BF27	hydroxy-methoxy-buprofezin	Sugimoto (1982); Huang & Smith (1997); Yoshizane (2008)
BF28	2-[3-isopropyl-3-[methylsulfonylmethyl(phenyl) carbamoyl] ureido]-2-methylpropionic acid	Huang & Smith (1997)
C	dihydroxy buprofezin	Sugimoto (1982)

Figure 2. Proposed metabolic pathway of buprofezin in rats



(a) Oral administration

Groups of 10 male and 10 female ICR mice were given buprofezin (purity, > 99.5%) as a single oral dose at 2500, 5000 or 10000 mg/kg bw by gavage.

No mice died at any dose. Female mice at 2500 mg/kg bw and above and males at 10 000 mg/kg bw showed reduced locomotor activity within the first 2 days after dosing, and males also had loose stool (Ebino & Shirasu, 1981a).

Groups of 10 male Golden hamsters were given buprofezin (purity, > 99%) as a single dose at 7692 or 10000 mg/kg bw by oral gavage.

No hamsters died at either dose and no clinical signs of toxicity were observed (Tsuchiya & Sugimoto, 1979a).

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single dose at 1412, 1765, 2206, 2758 or 3447 mg/kg bw by oral gavage. In all treated groups, decreased locomotor movement and lacrymation were observed. At doses of ≥ 1765 mg/kg bw, duodenum ulceration and duodenum oedema were observed. At ≥ 1765 mg/kg bw, mortality was observed and the median lethal dose (LD_{50}) was calculated to be 2198 mg/kg bw in males and 2355 mg/kg bw in females (Ebino & Shirasu, 1981b).

Groups of five male and five female Sprague-Dawley rats were given buprofezin (purity, 99.6%) as a single dose at 1000, 1400, 1960, 2744 or 3842 mg/kg bw by oral gavage in the first of a two-part experiment. The study was performed according to GLP requirements.

Clinical signs in all treated groups consisted of decreased locomotor activity, orbital and nasal discharge within 3 to 6 h after dosing. Tremor, lacrymation and loose stool were also seen between 6 h and 1 day. One male at the highest dose died.

In the second part of the experiment, groups of five male and five female Sprague-Dawley rats were given a single dose of buprofezin at 2959, 3846, 5000, 6500 or 8450 mg/kg bw. The LD_{50} in this experiment was 3847 mg/kg bw in males and 2278 mg/kg bw in females (Komatsu, 1996).

Groups of 10 male and 10 female Sprague-Dawley rats were given buprofezin (purity, > 99%) as a single dose at 1021, 1429, 2000, 2800, 3920 or 5488 (males only) mg/kg bw by oral gavage.

Clinical signs in treated rats included decreased locomotor activity, lacrymation, urine incontinentia and diarrhoea and at necropsy duodenal ulcers were also found. The LD_{50} was 1635 mg/kg bw in males and 2015 mg/kg bw in females (Tsuchiya & Sugimoto, 1982).

Two male Japanese albino rabbits were given buprofezin (purity, > 99%) as a single dose at 5000 mg/kg bw by oral gavage.

No clinical signs of toxicity and no mortality were observed (Tsuchiya & Sugimoto, 1979b).

(b) Intraperitoneal administration

Groups of 10 male and 10 female ICR mice were given buprofezin (purity, > 99.5%) as a single intraperitoneal dose at 2500, 5000 or 10 000 mg/kg bw.

No mice died at any dose. Males at 5000 mg/kg bw and above and females at 10 000 mg/kg bw had swollen livers (Ebino & Shirasu, 1981a).

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single intraperitoneal dose of 2500, 5000 or 10 000 mg/kg bw.

No mortalities were observed. At all doses, male and female mice had swollen livers and kidneys and males also had lung petechia (small haemorrhagic spots) (Ebino & Shirasu, 1981b).

(c) Dermal application

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single dermal application at 1000, 2000 or 5000 mg/kg bw.

No clinical signs of toxicity or mortalities were observed (Ebino & Shirasu, 1981b).

(d) Subcutaneous application

Groups of 10 male and 10 female ICR mice were given buprofezin (purity, > 99.5%) as a single subcutaneous dose at 2500, 5000 or 10 000 mg/kg bw.

No mice died at any dose. Apart from an intermittent decrease in body weight in two males and eight females at 10 000 mg/kg bw, no treatment-related findings were recorded (Ebino & Shirasu, 1981a).

Groups of 10 male and 10 female Fischer rats were given buprofezin (purity, > 99.5%) as a single subcutaneous dose at 2500, 5000 or 10 000 mg/kg bw.

No mortalities or clinical signs of toxicity were observed (Ebino & Shirasu, 1981b).

(e) Exposure by inhalation

Groups of 10 male and 10 female Fischer rats were exposed for 4 h to buprofezin (purity, 99.6%) at a dose of 3.57 or 4.57 mg/l.

All rats showed blood-like red staining around the nose. One female rat in the group at the higher dose was found dead on day 2 (Tsuda, 1984).

(f) Dermal and ocular irritation

Six male New Zealand White rabbits were exposed dermally to 0.5 g of buprofezin (purity, 99.3%) in 0.5 ml of 0.9% saline for 4 h. The study was conducted according Japanese GLP requirements.

No clinical signs of toxicity and no mortalities were observed (Blaszcak, 1986b).

Six male New Zealand White rabbits were given 42.5 mg of ground buprofezin (purity, 99.3%) in the right eye for 24 h; the eye was then washed. The study was conducted according Japanese GLP requirements.

Within the first hour after application, signs of conjunctival irritation and iridial changes were observed. In five rabbits, clinical signs had resolved within 24 h and in one rabbit within 72 h (Blaszcak, 1986a).

(g) Dermal sensitization

In a maximization test in female Hartley Albino guinea-pigs, buprofezin showed an equivocal potential for skin sensitization. In the challenge phase of the study, very minor initial signs of a dermal response, which was greater than that shown by the concurrent controls but which did not reach score 1 of the applied scoring system, were recorded in 50% of the guinea-pigs. In the re-challenge phase, 30% of the guinea-pigs also showed very minor signs of dermal reaction (Blaszcak, 1987).

Buprofezin (purity, 99.9%) was tested in a local lymph node assay (LLNA) that complied with GLP requirements. Groups of five female CBA/JNCrj mice were given 25 µl of buprofezin at a concentration of 6.25%, 12.5% or 25.0% topically for three consecutive days.

Since stimulation was very low at all concentrations and did not follow a dose–response relationship the Meeting concluded that buprofezin gave negative results in the LLNA (Inagaki, 2006b).

2.2 Short-term studies of toxicity

Rats

Groups of five male and five female CD rats were exposed to buprofezin (purity, 99%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by dermal application for at least 6 h per day for 24 days. An additional five males and five females in the control group and at the highest dose served as reversibility groups. Rats were observed twice per day for clinical signs of toxicity, and body weights and feed consumption were recorded regularly. Haematology, clinical chemistry and urine analysis investigations were performed towards the end of the study. At terminal sacrifice, macroscopic examination of the organs was performed and selected organs were weighed and prepared for histology. The study complied with GLP.

In females at the highest dose, absolute and relative kidney weights were increased at study termination in the main study group but not in the reversibility group. In three males at the highest dose, focal necrosis with inflammatory infiltrate in the liver was observed; in all other groups of males or females, the incidence of this finding was one or zero. In males at the highest dose, there was more hyperkeratosis in the skin than in males in the control group.

Table 11. Acute toxicity of buprofezin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
Mouse	ICR	M/F	Oral	> 10 000	—	> 99.5	Ebino & Shirasu (1981a)
Mouse	ICR	M/F	Intraperitoneal	> 10 000	—	> 99.5	Ebino & Shirasu (1981a)
Mouse	ICR	M/F	Subcutaneous	> 10 000	—	> 99.5	Ebino & Shirasu (1981a)
Hamster	Golden-	M	Oral	> 10 000	—	> 99.0	Tsuchiya & Sugimoto (1979a)
Rat	Fischer	M	Oral	2 198	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	F	Oral	2 355	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	M/F	Subcutaneous	> 10 000	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	M/F	Intraperitoneal	> 10 000	—	> 99.5	Ebino & Shirasu (1981b)
Rat	Fischer	M/F	Dermal	> 10 000	—	> 99.5	Ebino & Shirasu (1981b)
Rat	SD	M	Oral	3 847	—	99.6	Komatsu (1996)
Rat	SD	F	Oral	2 278	—	99.6	Komatsu (1996)
Rat	SD	M	Oral	1 635	—	> 99.0	Tsuchiya & Sugimoto (1982)
Rat	SD	F	Oral	2 015	—	> 99.0	Tsuchiya & Sugimoto (1982)
Rabbit	Japanese albino	M	Oral	> 5 000	—	> 99.0	Tsuchiya & Sugimoto (1979b)
Rat	Fischer	M/F	Inhalation	—	> 4.57	99.6	Tsuda (1984)

F, female; M, male; SD, Sprague-Dawley

Since the findings in this study were not clearly treatment-related and of questionable toxicological relevance, the NOAEL was 1000 mg/kg bw per day (Willoughby, 1995).

Groups of 10 male and 10 female Jcl:SD rats were fed diets containing buprofezin (purity, > 99%) at a concentration of 0, 40, 200, 1000 or 5000 ppm (equal to 0, 3.4, 13.0, 68.5 and 316.2 mg/kg bw per day in males and 0, 4.1, 16.3, 81.8 and 362.2 mg/kg bw per day in females, respectively) for 13 weeks. The rats were observed twice per day for clinical signs of toxicity, and body weights were recorded weekly and feed intake was noted twice per week. Ophthalmological examinations were done at week 12 and urine analysis, haematology and clinical chemistry parameters were investigated at study termination. Organs were weighed and macroscopically and histologically examined. This study complied with GLP.

There were no deaths and no treatment-related clinical signs in any group. In males, feed intake was statistically significantly reduced at 200 ppm and above, beginning from week 2 at 200 ppm and from week 1 at higher doses. In females, feed intake was lower at 5000 ppm. In males and females at 5000 ppm, body weights were statistically significantly lower than those of the controls from the first week of treatment. At study termination, body weights were lower by 9% and 14% in males and females, respectively. In the urine of male rats, increased numbers of round epithelial cells were found at 1000 and 5000 ppm. In males, concentrations of haemoglobin, hematocrit (erythrocyte volume fraction) and erythrocyte and monocyte counts were statistically significantly reduced at 5000 ppm and activated partial thromboplastin time was prolonged. For females, all except rats at the highest dose and rats in the control group had increased erythrocyte counts and in all rats treated with buprofezin MCV and MCH were lowered. In females at the highest dose, activated partial thromboplastin time was prolonged and neutrophil counts were reduced at dietary concentrations of 200 ppm and above.

In males at 200 ppm and above and in females at 5000 ppm, glucose concentrations were statistically significantly reduced. Triglyceride concentrations were lowered at 5000 ppm in males and females, and cholesterol, phospholipids and blood urea nitrogen increased. In females at the highest dose, cholinesterase activity was slightly reduced. In females at 1000 ppm and above, α-and β-globulins were increased, as was albumin at 5000 ppm. Albumin and α- and β-globulins were increased in males at 5000 ppm and γ-globulins were lowered.

At 5000 ppm in males and females, thyroids and livers were enlarged and livers had a dark brownish appearance. In males at 5000 ppm, caeca were enlarged and kidneys appeared dark brownish in colour. Treatment-related organ-weight changes included increases in absolute and relative liver and thyroid weights and decreases in absolute and relative spleen weights in males and females at 5000 ppm (Table 12). Liver weights in females were also increased at 1000 ppm. In males and females at 5000 ppm, absolute heart and lung weights were statistically significantly decreased but not the relative weights, while relative brain weights were increased but not absolute brain weights.

In the livers of males and females at 5000 ppm, hepatocellular hypertrophy, enlarged hepatocellular nuclei and nucleoli, focal infiltration of small round cells and, additionally, in females single-cell and focal hepatocellular necrosis and focal infiltration of small round cells were observed (Table 13). The thyroids of males and females at dietary concentrations of 1000 ppm and above showed thickening and hyperplasia of follicular epithelial cells. In males at 200 ppm and above, fatty changes of the proximal tubular epithelium in the kidney were seen without a clear dose–response relationship.

The NOAEL was 40 ppm, equal to 3.4 mg/kg bw per day, on the basis of decreased feed intake in males and mild clinical chemistry changes at 200 ppm (Watanabe, 1986).

Table 12. Body weights, absolute organ weights and relative organ weights for rats given diets containing buprofezin for 13 weeks

Dietary concentration (ppm)	Body weight (g)	Organ weights					
		Liver		Spleen		Thyroid	
		Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Males							
0	536	16.702	3.11	0.958	0.18	0.021	0.0039
40	533	15.139	2.83*	0.869	0.16	0.023	0.0042
200	519	14.597*	2.82*	0.896	0.17	0.022	0.0042
1000	517	16.490	3.19	0.866	0.17	0.025	0.0048*
5000	484**	21.995**	4.54**	0.738**	0.15*	0.043**	0.0088**
Females							
0	272	7.825	2.88	0.549	0.20	0.016	0.0060
40	269	7.377	2.75	0.520	0.19	0.016	0.0058
200	266	7.467	2.80	0.511	0.19	0.016	0.0060
1000	258	8.270	3.22*	0.479	0.19	0.017	0.0067
5000	238**	10.539**	4.44**	0.413**	0.17**	0.038**	0.0159**

From Watanabe (1986)

* $p < 0.05$

** $p < 0.01$

Table 13. Histological changes in rats given diets containing buprofezin for 13 weeks

Dietary concentration (ppm)	Incidence of histological changes									
	Males (n = 10)					Females (n = 10)				
	0	40	200	1000	5000	0	40	200	1000	5000
Liver										
Single-cell necrosis	1	1	5	0	0	1	1	2	0	5
Focal necrosis	0	1	0	0	1	0	0	0	0	5
Hepatocellular hypertrophy	0	0	0	2	10	0	0	0	0	10
Enlargement of hepatocellular nuclei	0	0	0	0	10	0	0	0	2	8
Enlargement of hepatocellular nucleoli	0	0	0	0	7	0	0	0	1	7
Focal infiltration of small round cells	0	0	0	0	0	1	0	1	0	5
Fat-loaded hepatocytes	0	1	0	0	5	0	1	2	3	2
Kidney										
Fatty change of proximal tubular epithelium	3	4	6	7	8	8	2	7	4	2
Thyroid										
Thickening/hyperplasia of follicular epithelial cells	0	0	0	10	10	0	0	0	9	10

From Watanabe (1986)

Dogs

Groups of four male and four female beagle dogs were given capsules containing buprofezin (purity, 99%) at a dose of 0, 2, 10, 50 or 300 mg/kg bw per day for 13 weeks. Clinical signs, feed consumption and body-weight development were recorded regularly, haematology, blood chemistry and urine analysis were noted periodically and ophthalmology was performed towards the end of the study. After terminal sacrifice, organ weights were recorded and organs were examined macroscopically and histologically. The study complied with GLP.

Subdued behaviour was observed in one male at 50 mg/kg bw per day and in virtually all dogs at 300 mg/kg bw per day 1 h after treatment and persisted throughout the day. This observation was made predominantly in the early part of the first week of treatment, but also at other time-points throughout the study. During the course of the study, the incidence of this finding clearly decreased. Subdued behaviour was also seen occasionally in two females at 2 mg/kg bw per day. In virtually all dogs at 300 mg/kg bw per day, slight ataxia (unsteadiness on feet) was observed 1 h after treatment and persisted for about 5 h. This observation was made early in the first week of treatment resolving in females in the first week but persisting with a lower incidence throughout the first nine weeks in one male. Two males and two females at the highest dose showed abdominal distension usually 1 h after treatment, which usually persisted for 4 h. This effect was also seen in one male in week 2 of treatment.

Dogs at the highest dose had slightly lower feed intake when compared to the pre-treatment period and to that of the controls and body-weight gains were statistically significantly decreased (Table 14). Prothrombin time in females at the highest dose was statistically significantly extended and the activity of alkaline phosphatase (ALP) was increased in males at 50 mg/kg bw per day and in males and females at the highest dose (Table 15). At the highest dose, alanine aminotransferase (ALT) activity was also slightly but statistically significantly increased. In all males receiving buprofezin, absolute and relative liver weights were increased. The effect was statistically significant for males and females at 50 and 300 mg/kg bw per day (Table 16). In males at doses of 50 mg/kg bw per day and above, absolute and relative thyroid weights were increased and in females the relative weights of the thyroid were increased at the highest dose. Absolute and relative kidney weights were increased in males at the highest dose; only the relative kidney weight was increased statistically significantly in females. On histological examination, homogeneity of hepatocellular cytoplasm was seen in all dogs at 300 mg/kg bw per day and in three males and three females at 50 mg/kg bw per day. Additionally, in all dogs at 300 mg/kg bw per day and in one female at 50 mg/kg bw per day, eosinophilic bodies in the hepatocellular cytoplasm were found.

The NOAEL in this study was 10 mg/kg bw per day, on the basis of increased ALP activity and liver- and thyroid-weight increases at 50 mg/kg bw per day (Broadmeadow, 1986).

Table 14. Mean terminal body-weight gain in dogs given capsules containing buprofezin for 13 weeks

Dose (mg/kg bw per day)	Body-weight gains ± standard deviation (kg)	
	Males	Females
0	4.3 ± 0.5	3.8 ± 0.6
2	3.8 ± 0.4	3.4 ± 0.5
10	3.7 ± 0.6	3.2 ± 0.5
50	3.5 ± 0.4	3.4 ± 0.3
300	3.2 ± 0.7*	2.0 ± 1.4**

From Broadmeadow (1986)

* $p < 0.05$

** $p < 0.01$

Table 15. Mean activity of alkaline phosphatase in dogs given capsules containing buprofezin for 13 weeks

Dose (mg/kg bw per day)	Alkaline phosphatase activity ± standard deviation (U/l)					
	Males			Females		
	Week –1	Week 6	Week 12	Week –1	Week 6	Week 12
0	105 ± 18	76 ± 17	68 ± 17	98 ± 18	72 ± 16	57 ± 12
2	121 ± 19	90 ± 12	82 ± 6	119 ± 25	85 ± 17	92 ± 39
10	110 ± 18	94 ± 17	94 ± 15	108 ± 19	85 ± 12	83 ± 11
50	103 ± 11	113 ± 20*	129 ± 30*	107 ± 22	96 ± 17	97 ± 21
300	105 ± 24	232 ± 43**	293 ± 62**	101 ± 12	218 ± 41**	281 ± 129**

From Broadmeadow (1986)

* $p < 0.05$

** $p < 0.001$

Table 16. Relative organ weights in in dogs given capsules containing buprofezin for 13 weeks

Dose (mg/kg bw per day)	Body weight (kg)	Relative organ weight ± standard deviation (% of body weight)		
		Liver	Kidney	Thyroid (× 1000)
Males				
0	14.4	3.1 ± 0.2	0.41 ± 0.02	5.4 ± 1.6
2	13.7	3.4 ± 0.3	0.41 ± 0.02	6.1 ± 0.6
10	13.7	3.8 ± 0.4	0.44 ± 0.02	6.9 ± 1.3
50	13.6	4.3 ± 0.6*	0.45 ± 0.03	9.0 ± 0.7**
300	12.9	5.2 ± 0.7**	0.51 ± 0.04**	11.3 ± 0.6**
Females				
0	13.3	3.1 ± 0.2	0.43 ± 0.04	6.7 ± 1.3
2	12.9	3.0 ± 0.2	0.42 ± 0.02	7.6 ± 1.7
10	12.8	3.3 ± 0.1	0.43 ± 0.03	7.2 ± 0.9
50	13.2	3.7 ± 0.2*	0.42 ± 0.04	7.6 ± 0.5
300	11.6	5.0 ± 0.5**	0.53 ± 0.03*	9.2 ± 1.4*

From Broadmeadow (1986)

* $p < 0.01$

** $p < 0.001$

Groups of six male and six female beagle dogs received oral capsules containing buprofezin (purity, ≥ 99.3%) at a dose of 0, 2, 20 or 200 mg/kg bw per day for 2 years (107 weeks). Clinical signs of toxicity, feed consumption, body-weight development, ophthalmology, haematology, blood chemistry parameters and urine analysis were recorded regularly. After terminal sacrifice, organ weights were recorded and organs were examined macroscopically and histologically. A statement of quality assurance was included.

No treatment-related clinical signs of toxicity were observed. Terminal body-weight gain was low in males and females at the highest dose but statistically significant only in females (Table 17). This effect in males was attributable to decreased body-weight gains in the first 6 months of administration and in females in the second year of the study. A trend in absolute liver-weight increases was seen in males at the intermediate dose and above and in all females receiving buprofezin, becoming statistically significant in females at the intermediate dose and above. Relative liver weights were increased in all treated females and in males at the highest dose (Table 17). Relative thyroid weights were increased in males and females at the highest dose. An elongated activated partial thromboplastin time in males at the highest dose only at week 26 and at study termination was considered to occur by chance. In males and females at the intermediate and the highest dose, ALP activity was statistically significantly increased when compared to that in dogs in the control group (Table 18). An electrophoretic isoenzyme analysis showed that hepatic ALP isoenzyme contributed most to the increased ALP activity. In the second year of treatment, the activity of ALT was also increased in dogs with high ALP activity. Unchanged bromosulfonphthalein retention time in all dogs receiving buprofezin indicated normal liver function. In males and females at 200 mg/kg bw per day, fluctuations in thyroxine concentrations with a trend to lower concentrations was observed. Many male and female dogs at the intermediate dose and the highest dose, but none at the lowest dose or in the control group, were found to have slight bile-duct hyperplasia. Slight hepatocellular hypertrophy was found in males at the intermediate dose and at the highest dose. Four and thee females at the intermediate and the highest dose, respectively, had mammary hyperplasia while this change was only seen in one dog in the control group and one dog at the lowest dose.

The NOAEL was at 2 mg/kg bw per day on the basis of liver-weight increases and correlating histological changes and clinical chemistry changes at 20 mg/kg bw per day (Cummins, 1982).

Table 17. Mean terminal body-weight gain and organ weights[a] in dogs given capsules containing buprofezin for 2 years

Dose (mg/kg bw per day)	Body-weight gain (kg)	Liver weight		Thyroid weight	
		Absolute (g)	Relative (%)	Absolute (g)	Relative (% × 1000)
Males					
0	3.8 ± 1.7	422 ± 62	3.4 ± 0.7	1.01 ± 0.38	8.0 ± 2.5
2	3.7 ± 0.9	416 ± 63	3.4 ± 0.4	1.10 ± 0.25	8.9 ± 1.7
20	3.4 ± 0.7	489 ± 71	3.9 ± 0.5	1.19 ± 0.14	9.6 ± 1.1
200	2.5 ± 1.1	484 ± 47	4.3 ± 0.4**	1.30 ± 0.32	11.4 ± 2.2**
Females					
0	4.5 ± 1.4	367 ± 42	2.8 ± 0.1	0.94 ± 0.21	7.2 ± 1.0
2	3.9 ± 1.0	414 ± 42	3.4 ± 0.4*	1.05 ± 0.33	8.6 ± 2.5
20	4.2 ± 0.9	458 ± 76*	3.7 ± 0.3***	1.26 ± 0.41	10.2 ± 2.5
200	2.5 ± 1.2**	477 ± 90**	4.4 ± 0.6***	1.35 ± 0.45	12.4 ± 3.4**

From Cummins (1982)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] ± standard deviation

Table 18. Mean activity of alkaline phosphatase in dogs given capsules containing buprofezin for 2 years

Dose (mg/kg bw per day)	Alkaline phosphatase activity (U/l ± standard deviation)			
	Week –1	Week 4	Week 52	Week 102
Males				
0	111 ± 14	94 ± 16	72 ± 33	45 ± 10
2	137 ± 39	119 ± 28	83 ± 22	57 ± 19
20	121 ± 4	125 ± 17*	134 ± 44*	108 ± 27**
200	110 ± 14	240 ± 121**	362 ± 239**	310 ± 372**
Females				
0	102 ± 26	78 ± 17	57 ± 10	56 ± 16
2	100 ± 7	83 ± 14	87 ± 25	68 ± 26
20	132 ± 38	159 ± 49**	200 ± 70**	166 ± 46**
200	112 ± 23	210 ± 66**	404 ± 147**	288 ± 52**

From Cummins (1982)

* $p < 0.05$

** $p < 0.01$

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 80 male and 80 female ICR (Crj:CD-1) mice were fed diets containing buprofezin (purity, 99.5%) at a concentration of 0, 20, 200, 2000 or 5000 ppm (equal to 0, 1.8, 17.4, 190 and 481 mg/kg bw per day in males and 0, 1.9, 17.9, 191 and 493 mg/kg bw per day in females) for 2 years (104 weeks). Ten males and ten females were killed at 52 weeks for evaluation of toxicity. In all mice, clinical signs of toxicity, body-weight gains, feed and water consumption were regularly recorded. Haematological, clinical-chemistry and urine parameters were investigated at 52 weeks and at study termination. At study termination, organ weights were measured and histology on selected organs was performed. A statement of quality assurance was given.

There were no treatment-related clinical signs or mortalities in any of the treated groups. In males and females at 5000 ppm, body weights were slightly (females, 5–10%; and males, approximately 5%) but statistically significantly reduced from week 6 (males) and week 9 (females) onwards. A very slight trend to reduced body weight was also observed at 2000 ppm in males and females. These effects were not more statistically significant by the end of the study (Table 19). No changes in feed consumption in any group were observed.

Urine analysis showed a minor but statistically significant decrease (1–2%) in specific gravity at 52 and 104 weeks in males and females. In females at 2000 and 5000 ppm, more mice showed low urinary ketone-body levels. Haematology showed that mice at the highest dose at 104 weeks had statistically significantly increased platelet numbers (males, 19%; and females, 25%). In females at the highest dose at 52 weeks, but not at 104 weeks, erythrocytes, erythrocyte volume fraction and haemoglobin concentrations were statistically significantly reduced by 5–8%. Females in the groups at 2000 ppm and 5000 ppm at 52 weeks and males at 5000 ppm at 104 weeks had statistically significantly increased cholesterol concentrations.

At 52 weeks, absolute and relative liver weights were increased in males at 200 ppm and above and in females at 2000 ppm and above (Table 19). At 104 weeks, liver-weight increases were statistically significant only in males at the highest dose. Absolute and relative adrenal weights were also increased

in males at 52 weeks, but not at 104 weeks. In males and females at the highest dose, cloudy and dark-coloured livers and in males atrophic/softened testis were found with increased incidences. The only clearly treatment-related non-neoplastic histological changes were hypertrophic and hyperplastic alterations in the livers of males and females at 2000 ppm and above (Table 20). In female mice, the incidence of liver adenoma at 2000 and 5000 ppm was increased above the historical mean but was within the range for historical controls (historical controls: mean, 5.4%; range, 1.3–12.5%), but

Table 19. Liver-weight changes[a] in a 2-year study in mice given diets containing buprofezin

Dietary concentration (ppm)	52 weeks			104 weeks		
	Body weight (g)	Absolute liver weight (g)	Relative liver weight (%)	Body weight (g)	Absolute liver weight (g)	Relative liver weight (%)
Males						
0	51.5 ± 5.1	2.38 ± 0.24	4.65 ± 0.53	47.5 ± 7.6	3.32 ± 1.57	7.30 ± 4.00
20	51.8 ± 3.4	2.48 ± 0.33	4.79 ± 0.57	48.7 ± 5.9	3.69 ± 2.07	7.44 ± 3.74
200	54.5 ± 4.4	2.91 ± 0.44**	5.36 ± 0.80*	48.4 ± 7.3	3.35 ± 1.43	7.08 ± 3.33
2000	50.9 ± 4.3	2.80 ± 0.40*	5.50 ± 0.70*	48.0 ± 4.8	3.73 ± 1.67	7.82 ± 3.39
5000	49.9 ± 4.2	3.34 ± 0.70**	6.64 ± 0.94***	46.5 ± 6.2	4.40 ± 2.24*	9.57 ± 4.02*
Females						
0	50.1 ± 7.2	1.90 ± 0.29	3.83 ± 0.63	48.2 ± 9.2	2.44 ± 1.14	5.36 ± 3.54
20	50.9 ± 7.4	2.05 ± 0.25	4.13 ± 1.06	46.0 ± 9.6	2.04 ± 0.52	4.57 ± 1.41
200	49.3 ± 6.8	1.95 ± 0.31	3.98 ± 0.57	46.5 ± 7.0	2.22 ± 0.61	4.87 ± 1.70
2000	49.1 ± 4.7	2.28 ± 0.31*	4.64 ± 0.39**	46.4 ± 6.3	2.79 ± 1.08	6.02 ± 2.11
5000	46.3 ± 6.1	2.52 ± 0.35***	5.48 ± 0.72***	44.5 ± 4.9	2.74 ± 0.60	6.20 ± 1.41

From Yoshida (1983)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] ± standard deviation

Table 20. Incidence of non-neoplastic and neoplastic liver lesions in a 2-year study in mice given diets containing buprofezin

Liver lesions	Dietary concentration (ppm)									
	Males (n = 80)					Females (n = 80)				
	0	20	200	2000	5000	0	20	200	2000	5000
Non-neoplastic										
Centrilobular hepatocellular swelling	3	3	0	10*	13**	3	4	3	15**	12*
Diffuse hepatocellular swelling	9	9	10	14	42**	2	2	1	1	24**
Hepatocellular hyperplasia	12	17	17	13	27**	2	5	7	9*	8*
Neoplastic										
Adenoma	13	12	16	11	17	2	2	1	7	8*
Carcinoma	14	11	11	18	15	3	2	0	4	4

From Yoshida (1983)

* $p < 0.05$

** $p < 0.01$

there was no dose-related increase in incidence between 2000 and 5000 ppm. In these two groups, the incidence of liver carcinoma was also increased and was greater than the historical mean and also slightly above the range for historical controls (historical controls: mean, 1.0%; range, 0–3.8%). However, the incidence of carcinoma in concurrent controls was also at the upper bound of the range for historical controls and therefore there was no dose–response relationship in this study.

The NOAEL for toxicity was 200 ppm, equal to 17.4 mg/kg bw per day, on the basis of transiently increased liver-weight gains and histological changes in the liver at 2000 ppm in males and females. The NOAEL for carcinogenicity was 5000 ppm, equal to 481 mg/kg bw per day, the highest dose tested (Yoshida, 1983).

Rats

Groups of 55 male and 55 female Jcl:SD rats were fed diets containing buprofezin (purity, > 99.0%) at a concentration of 0, 5, 20, 200 or 2000 ppm (equal to 0, 0.26, 0.90, 8.71 or 89.46 mg/kg bw per day in males and 0, 0.33, 1.12, 11.19 or 114.71 mg/kg bw per day in females) for 2 years (104 weeks). Five males and five females per group were killed at 26 weeks, and ten males and ten females per group were killed at 52 weeks. Clinical signs of toxicity, body-weight gains and feed consumption were recorded regularly. Haematological, clinical-chemistry and urine parameters were investigated at 26, 52 weeks and at study termination. Phenolsulfonphthalein (PSP, phenol red) excretion into the urine after intramuscular administration and bromosulfonphthalein (BSP) excretion into serum after intravenous administration were analysed in rats in the control group and in the group at the highest dose before study termination. At weeks 26 and 52 for the interim sacrifice groups and at study termination, organ weights were recorded and histological investigations on selected organs were performed. The study complied with GLP.

No treatment-related changes in clinical parameters or mortality were seen in any group receiving buprofezin. Body weight in males and females at the highest dose were slightly lower than in controls, statistically significant only in the first 5 weeks of treatment in males and throughout the study with the exception of the period from month 7 to 13 in females, respectively. In males, bw were lower by 2–4% and in females by 5–10%. Feed intake was not significantly affected by treatment in any group. No treatment-related changes in haematological parameters and PSP and BSP excretion were found. In clinical chemistry, statistically significant and dose related decreases in serum glucose levels were seen in females at 20 ppm and above but not in males or females at any other time-point. In males at 2000 ppm, ALT and AST activity were consistently lower by 25–30% than in control, the effect being statistically significant at 6 and 24 months. At terminal sacrifice, the livers of a few males and females had a dark brownish appearance. Absolute and relative liver weights were increased in males and females at the highest dose at all time-points. Absolute and relative thyroid weights were increased in females at the highest dose at 26 and 52 weeks and, although not statistically significantly, at study termination (Table 21). In males and females at 2000 ppm, increased incidences of hepatocellular hypertrophy were seen at all sacrifice time-points (Table 22). This effect was accompanied by proliferation of the smooth endoplasmic reticulum in hepatocytes. Focal hepatocellular necrosis was also increased at 52 and 104 weeks in females, and in males and females at 2000 ppm. More hyperplastic hepatocellular nodules were found in males and females at study termination. At 2000 ppm, males and females exhibited very high incidences of thickened and/or hyperplastic thyroid follicular epithelia. Increased incidences of cardiac-muscle necrosis were seen in males and females at 2000 ppm group at 52 weeks. At study termination, the incidence of this effect was high in all groups, including controls. No dose-related increases in tumour incidences were observed (Watanabe et al., 1982).

After a change in diagnostic criteria and histopathology terminology in the late 1980s, the histology slides of the 2-year feeding study in rats (Watanabe et al., 1982) were re-examined in the

early 1990s (Todhunter & Goodman, 1995). Particularly affected by this criteria change were the liver lesions previously termed "hyperplastic nodules". Under the new classification standards, some of these lesion were re-classified as "hepatocellular adenoma" or "foci of cellular alteration". The study complied with GLP.

At 2000 ppm, males and females showed increased incidences of centrilobular hepatocellular hypertrophy and females also showed a slightly higher incidence of adenoma and eosinophilic foci (Table 23). The increase in liver adenoma was statistically not significant, not found in males and not accompanied by an increase in liver carcinoma it was judged accidentally. With regard to the thyroid, F-cell hypertrophy was seen in males at 200 ppm and in males and females at 2000 ppm. C-cell hyperplasia was increased statistically significantly at 2000 ppm in males and females (Table 24)

The NOAEL was 20 ppm, equal to 0.9 mg/kg bw per day, on the basis of slight increases in the incidence of thyroid F-cell hypertrophy at 200 ppm.

2.4 Genotoxicity

The genotoxic potential of buprofezin was investigated in a battery of studies of genotoxicity in vitro and two studies of micronucleus formation in mice in vivo (Table 29). All studies in vitro gave clearly negative results with and without metabolic activation.

One out of two studies of micronucleus formation in mice gave equivocal results and one gave negative results. In the first study, groups of six male and female BDF1 mice were given buprofezin at a dose of 0, 6400, 8000 or 10 000 mg/kg bw by gavage. In a first experiment, bone-marrow smears were taken from mice at 0 or 10 000 mg/kg bw at 12, 48 or 72 h after dosing, while in a second experiment bone-marrow smears were taken from mice at 0, 6400 and the 8000 mg/kg bw at 24 h

Table 21. Relative liver and thyroid weights in a 2-year study in rats fed diets containing buprofezin

Dietary concentration (ppm)	Relative (to body weight) weight [%]					
	26 weeks		52 weeks		104 weeks	
	Liver	Thyroid[a]	Liver	Thyroid[a]	Liver	Thyroid[a]
Males						
0	2.59 ± 0.25	3.65 ± 1.13	2.43 ± 0.24	4.17 ± 1.11	2.69 ± 069	5.18 ± 2.26
5	2.52 ± 0.11	3.82 ± 0.44	2.28 ± 0.15	3.70 ± 1.14	2.71 ± 0.45	4.79 ± 1.25
20	2.83 ± 0.16	3.60 ± 1.89	2.41 ± 0.14	3.73 ± 0.97	2.83 ± 0.88	4.88 ± 1.23
200	2.52 ± 0.19	3.34 ± 0.59	2.57 ± 0.18	3.94 ± 0.58	2.74 ± 0.66	4.99 ± 1.71
2000	3.13 ± 0.17**	4.59 ± 0.71	2.99 ± 0.24**	4.71 ± 1.38	3.33 ± 0.93*	10.13 ± 11.60
Females						
0	2.48 ± 0.18	4.75 ± 1.04	2.47 ± 0.17	5.20 ± 0.67	2.58 ± 0.43	5.52 ± 2.88
5	2.66 ± 0.34	5.02 ± 0.84	2.57 ± 0.29	5.04 ± 1.14	2.43 ± 0.27	4.75 ± 1.58
20	2.55 ± 0.17	5.67 ± 0.89	2.45 ± 0.16	5.43 ± 1.15	2.63 ± 0.45	5.41 ± 1.17
200	2.51 ± 0.13	5.14 ± 1.55	2.52 ± 0.18	6.20 ± 1.43	2.71 ± 0.35	5.13 ± 1.31
2000	3.16 ± 0.22**	8.06 ± 1.53**	3.31 ± 0.34**	6.69 ± 1.63*	3.22 ± 0.61**	6.97 ± 2.29

[a] × 1000

From Watanabe et al. (1982)

* $p < 0.05$

** $p < 0.01$

Table 22. Incidence of non-neoplastic lesions in a 2-year study in rats fed diets containing buprofezin

Non-neoplastic lesion	Incidence of lesion									
	Dietary concentration (ppm)									
	Males					Females				
	0	5	20	200	2000	0	5	20	200	2000
26 weeks										
No. of rats examined	5	5	5	5	5	5	5	5	5	5
Enlargement of hepatocytes	0	0	0	0	2	0	0	0	0	3
Thickening and/or hyperplasia of thyroid follicular epithelium	0	0	0	1	5	0	0	0	1	5
Necrosis of cardiac muscle	1	0	1	0	0	1	0	0	1	0
52 weeks										
No. of rats examined	10	10	10	10	10	10	10	10	9	10
Enlargement of hepatocytes	0	0	0	0	4	2	0	0	0	10
Focal necrosis of hepatocytes	0	1	0	1	1	0	0	0	2	4
Enlargement of hepatocellular nuclei	0	0	1	1	0	0	0	0	0	8
Thickening and/or hyperplasia of thyroid follicular epithelium	0	0	0	2	8	0	0	0	0	10
Necrosis of cardiac muscle	1	1	2	0	3	0	0	0	1	4
104 weeks										
No. of rats examined	20	14	19	17	24	19	18	18	15	13
Enlargement of hepatocytes	1	0	0	0	7	0	0	0	0	2
Focal necrosis of hepatocytes	1	3	7	0	6	2	1	5	2	4
Enlargement of hepatocellular nuclei	5	6	8	4	6	6	6	6	6	6
Hyperplastic nodules of hepatocytes	1	1	4	0	7	0	2	2	3	5
Thickening and/or hyperplasia of thyroid follicular epithelium	0	1	0	1	24	0	0	0	0	9
Proliferation of parafollicular cells	2	1	0	3	19	0	0	0	0	10
Necrosis of cardiac muscle	11	9	15	5	19	7	11	14	10	7

From Watanabe et al. (1982)

only. In a confirmatory experiment, bone-marrow smears were taken from mice at 0, 6400, 8000 or 10 000 mg/kg bw at 24 h after dosing. In a further experiment, groups of male and female mice received four doses of buprofezin at 0 or 10 000 mg/kg bw on four consecutive days and bone-marrow smears were taken 12, 24, 48 and 72 h after administration of the last dose.

The ratio of polychromatic erythrocytes (PCE) to total erythrocytes (TE) was only slightly decreased in the experiment with repeated doses. Therefore, buprofezin was not excessively toxic to the bone marrow.

A statistically significant increase in the frequency of micronucleated PCE (MN-PCE) was found in all males receiving buprofezin and in females at 10000 mg/kg bw in the first and second experiments (Table 26). In the confirmatory experiment and in the experiment with repeated doses for 4 days, no evidence for micronucleus formation was identified. In view of the minor increase in the frequency of MN-PCE and its non-reproducibility, the Meeting concluded that this finding was not treatment-related (Sasaki, 1983).

Table 23. Incidence of liver lesions at study termination in a 2-year study in rats fed diets containing buprofezin, as originally classified and after re-examination

Liver lesion	Incidence of liver lesions (%)									
	Dietary concentration (ppm)									
	Original report					Re-examination				
	0	5	20	200	2000	0	5	20	200	2000
Males										
No. of rats examined	40	40	40	40	40	39	37	39	40	40
Adenoma	0.0	0.0	0.0	0.0	0.0	2.6	2.7	7.7	0.0	10.0
Carcinoma	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	2.5
Adenoma/carcinoma combined	0.0	0.0	0.0	0.0	0.0	5.1	2.7	7.7	0.0	12.5
Hyperplastic nodules	5.0	2.5	12.5	2.5	17.5*	—	—	—	—	—
Hyperplastic foci	20.0	25.0	25.0	25.0	25.0	—	—	—	—	—
Centrilobular hypertrophy	—	—	—	—	—	0.0	0.0	0.0	0.0	27.5*
Diffuse hypertrophy	—	—	—	—	—	5.1	5.4	7.7	5.0	17.5*
Eosinophilic foci	—	—	—	—	—	20.5	13.5	12.8	15.0	17.5
Homogenous basophilic foci	—	—	—	—	—	2.5	5.4	12.8	2.5	0.0
Tigroid basophilic foci	—	—	—	—	—	7.7	24.3	7.7	2.5	10.0
Females										
No. of rats examined	39	40	40	40	40	39	39	40	40	39
Adenoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7
Carcinoma	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Adenoma/carcinoma combined	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7
Hyperplastic nodules	0.0	7.5	5.0	10.0*	15.0*	—	—	—	—	—
Hyperplastic foci	15.4	25.0	15.0	17.5	27.5*	—	—	—	—	—
Centrilobular hypertrophy	—	—	—	—	—	0.0	0.0	0.0	0.0	35.9*
Diffuse hypertrophy	—	—	—	—	—	12.8	2.6	7.5	10.0	15.4*
Eosinophilic foci	—	—	—	—	—	10.3	10.3	12.5	22.5	28.2
Homogenous basophilic foci	—	—	—	—	—	2.5	5.1	5.0	7.5	0.0
Tigroid basophilic foci	—	—	—	—	—	0.0	2.5	10.0	15.0*	5.1

From Watanabe et al. (1982); Todhunter & Goodman (1995)

* $p < 0.05$

Table 24. Incidence of thyroid lesions in rats fed diets containing buprofezin, according to the re-examination report at termination in a 2-year study

Thyroid lesion	Incidence of lesion (%)									
	Dietary concentration (ppm)									
	Males					Females				
	0	5	20	200	2000	0	5	20	200	2000
No. of rats examined	36	35	38	39	39	37	36	40	33	39
F-cell adenoma	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	2.6
F-cell carcinoma	0.0	0.0	0.0	2.6	0.0	2.7	0.0	0.0	0.0	2.6
Adenoma/carcinoma combined	0.0	0.0	0.0	2.6	2.6	2.7	0.0	0.0	0.0	5.1
F-cell hypertrophy	16.7	31.4	31.6	48.7*	64.1*	8.1	5.6	2.5	12.1	51.3*
F-cell hyperplasia	0.0	0.0	0.0	0.0	5.1	0.0	0.0	0.0	0.0	2.6
C-cell adenoma	8.3	5.7	5.3	2.6	0.0	5.4	2.8	0.0	3.0	0.0
C-cell carcinoma	0.0	0.0	2.6	2.6	5.1	0.0	0.0	0.0	0.0	0.0
Adenoma/carcinoma combined	8.3	5.7	7.9	5.1	5.1	5.4	2.8	0.0	3.0	0.0
C-cell hyperplasia	31.1	62.9	73.7	64.1	84.6*	59.5	55.6	60.0	66.7	82.1*

From Todhunter & Goodman (1995)

* $p < 0.05$

Table 25. Incidences of thyroid lesions in rats fed diets containing buprofezin, according to the original report at termination in 2-year study

Thyroid lesion	Incidence of lesion (%)									
	Dietary concentration (ppm)									
	Males					Females				
	0	5	20	200	2000	0	5	20	200	2000
No. of rats examined	40	40	40	40	39	39	39	40	40	40
Follicular epithelial thickening/hyperplasia	2.5	2.5	0.0	7.5	82*	0.0	0.0	0.0	2.5	43*

From Watanabe et al. (1982)

* $p < 0.05$

In the second study, groups of five male Slc/ICR mice were given two doses of buprofezin at 0, 500, 1000 or 2000 mg/kg bw by gavage on two consecutive days. Mitomycin and colchicine were used as positive controls for substances known to provoke micronucleation. In a confirmatory experiment, mice were given two doses of buprofezin at 0 or 2000 mg/kg bw by gavage on two consecutive days. Bone-marrow smears were prepared for staining with acridine orange 24 h after the last dose. Immature erythrocytes and micronuclei were identified as red and yellowish green fluorescence-emitting bodies, respectively. In a further experiment, the mechanism of DNA damage was investigated with an anti-kinetochore antibody (CREST) and DNA-propidium staining .

The ratio of immature erythrocytes to total erythrocytes was only slightly decreased at the highest dose in both experiments. The Meeting therefore concluded that buprofezin was not excessively toxic to the bone marrow. In both experiments, a statistically significant increase in micronucleated

Table 26. The frequency of micronucleated polychromatic erythrocytes as a percentage of polychromatic erythrocytes in bone-marrow smears from BDF1 mice given buprofezin by gavage

Dose (mg/kg bw)	Frequency of micronucleated polychromatic erythrocytes (% of polychromatic erythrocytes)							
	Time after dosing (h)							
	12	24	48	72	12	24	48	72
	Males				Females			
Single dose: first experiment								
0	0.07	0.12	0.23	0.18	0.17	0.10	0.15	0.18
10 000	0.18	0.27*	0.23	0.10	0.08	0.30*	0.20	0.10
Single dose: second experiment								
0	—	0.03	—	—	—	0.10	—	—
6 400	—	0.15*	—	—	—	0.13	—	—
8000	—	0.20**	—	—	—	0.08	—	—
Single dose: confirmatory experiment								
0	—	0.10	—	—	—	0.02	—	—
6 400	—	0.07	—	—	—	0.03	—	—
8 000	—	0.17	—	—	—	0.03	—	—
10 000	—	0.03	—	—	—	0.05	—	—
Repeated doses								
0	0.18	0.15	0.10	0.08	0.10	0.08	0.12	0.07
10 000	0.26	0.15	0.14	0.01	0.23	0.16	0.14	0.15

From Sasaki (1983)

* $p < 0.05$

** $p < 0.01$

Table 27. Frequency of micronucleated immature erythrocytes and kinetochore-positive micronuclei in Slc/ICR mice given two doses of buprofezin by gavage

Test substance	Dose (mg/kg bw)	Micronucleated immature erythrocytes (% of immature erythrocytes)		Kinetochore-positive micronuclei (% of all micronuclei)
		First experiment	Confirmatory experiment	
Buprofezin	0	0.15	0.11	24.0
	500	0.12	—	—
	1000	0.18	—	—
	2000	2.02**	0.34**	31.6
Mitomycin	3	6.95**	4.65**	14.6*
Colchicine	1	—	1.90**	61.2**

From Inagaki (2006c)

* $p < 0.05$

** $p < 0.01$

immature erythrocytes was observed at the highest dose. While the aneugenic substance colchicine significantly increased the percentage of the kinetochore-positive micronuclei, the highest dose of buprofezin showed a slight and statistically not significant increase in kinetochore-positive micronuclei (Inagaki, 2006c).

In a published study, Syrian hamster embryo cells were exposed to buprofezin at a concentration of 12.5–100 µmol/l and the induction of morphological transformation of colonies, DNA repair-synthesis and micronuclei were investigated. To separate a putative aneugenic potential from clastogenic potential, kinetochore-positive micronuclei were stained with an anti-kinetochore serum (CREST). As control substances for aneugenic or clastogenic effects, respectively, the known aneugenic compound diethylstilbestrol (DES) and the clastogenic compound nitroquinoline-*N*-oxide (NQO) were included. As control substance for morphological transformation of Syrian hamster embryo cell colonies, praziquantel (PRZ) was used. The cloning efficiency and survival of cells treated with buprofezin were slightly lowered at all doses in a non-dose-related manner. Treatment with buprofezin did not induce DNA repair-synthesis at any dose. A clear response to treatment with buprofezin was seen in the form of increased transformation frequency, increase in micronuclei and kinetochore-positive micronuclei was seen at the highest concentration of 100 µmol/l, equal to 30.5 µg/ml (Table 28) (Herrera et al., 1993).

In another published study, buprofezin was tested for its potential to induce chromosomal aberrations in male mice given a single oral dose of a 25% formulation. At doses of 1.4 and 2.8 g/kg yes bw (corresponding to active ingredient, 350 and 700 mg/kg bw), an increase in the frequency of abnormal metaphase chromosomes of up to twofold was seen. Because the test material was a formulation with unknown ingredients, the increase in metaphase abnormalities was not interpretable because it was not necessarily related to treatment with buprofezin (Fahmy & Abdalla, 1998).

In conclusion, buprofezin was tested for genotoxicity in an adequate range of studies in vitro and for micronucleus formation in vivo. In the submittestudies, there was no evidence for genotoxicity in vitro; however, in the published non-GLP study (Herrera et al., 1993), micronuclei were induced in cultured cells by an aneugenic mechanism, rather than by chromosomal breakage.

Table 28. Frequency of micronucleated immature erythrocytes and kinetochore-positive micronuclei in Syrian hamster embryo cells treated with buprofezin

Test substance	Concentration (µmol/l)	Transformation frequency (%)	Micronuclei per 2000 cells[a]	Kinetochore-positive micronuclei (%)
DMSO	0.1%	< 0.12	22.0 ± 4.6	12
Buprofezin	12.5	0.17	29.7 ± 6.7	57
	25	0.19	32.0 ± 6.6*	58
	50	0.18	31.3 ± 1.5**	50
	100	0.80	51.3 ± 5.5***	70
PRZ	50	0.18	—	—
NQO	1	—	64.7 ± 12.7***	22
DES	30	—	—	69

From Herrera et al. (1993)

DES, diethylstilbestrol; DMSO, dimethyl sulfoxide; NQO, nitroquinoline-*N*-oxide; PRZ, praziquantel

[a] Three independent experiments

* $p < 0.05$

** $p < 0.025$

** $p < 0.005$

Table 29. Results of studies of genotoxicity with buprofezin

End-point	Test system[a]	Concentration	Purity (%)	GLP	Result	Reference
In vitro						
Reverse mutation[b]	*S. typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 [c]	1.6–5000 µg/ml ± S9	99.8	Yes	Negative ± S9	Callander (1988)
Reverse mutation	*S. typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100 and *E. coli* (WP2 *hcr*$^-$)	10–5000 µg/ml ± S9	99.3	Not stated	Negative ± S9	Moriya (1980)
DNA damage	*Bacillus subtilis* (H17 and M45*rec*$^-$)	20–5000 µg/disc	99.3	Not stated	Negative	Moriya (1980)
Chromosome aberrations[d]	Chinese hamster lung cells (CHL); 6 h exposure [c]	64.1–77.9 µg/ml + S9 26.5–38.2 µg/ml – S9	99.9	Yes	Negative ± S9	Inagaki (2006a)
Forward mutation[b]	Mouse lymphoma cell line L5178 (TK$^{+/-}$)	17.8–100 µg/ml + S9 13.3–42.2 µg/ml – S9	99.8	Yes	Negative ± S9	Cross (1988)
DNA damage	Human lymphocytes	10–100 µg/ml ± S9	99.8	Yes	Negative ± S9	Howard & Richardson (1988)
Unscheduled DNA synthesis	Primary rat hepatocytes	10^{-2}–10 µmol/l	99.8	Yes	Negative	Trueman (1988)
In vivo						
Micronucleus formation	BDF1 mice	Single dose at 6400, 8000, or 10 000 mg/kg bw and four consecutive doses at 10 000 mg/kg bw	99.5	Not stated	Negative	Sasaki (1983)
Micronucleus formation	Slc/ICR mice (males)	Two doses at 500, 1000, or 2000 mg/kg bw for two consecutive days	99.9	Yes	Equivocal	Inagaki (2006c)

S9, 9000 × *g* supernatant from rat livers.

[a] Positive controls were included in all tests for genotoxicity.

[b] Tests were conducted in duplicate.

[c] Precipitation at the highest concentration.

[d] Negative results also after exposure over 1.5–3.0 cell cycle lengths –S9 at 7.73–21.4 µg/ml.

In the experiments on micronucleus induction in immature erythrocytes of bone marrow of mice in vivo, conflicting results were obtained. One study reported statistically significantly increased incidences in two experiments, but the numerical results were very different and were nd ot fully supported by the equivocal results of an earlier study in which the doses administered were five times higher. Furthermore, the suggestion of an aneugenic effect in vitro in the published study was not confirmed in vivo.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 30 male and 30 female Wistar-Imachi rats (age 3 weeks) were fed diets containing buprofezin (purity, 99.1%) at a concentration of 0, 10, 100 or 1000 ppm, equal to 0, 0.6–0.8, 6.0–8.1 or 62.5–86.5 mg/kg bw per day in males and 0, 0.8–0.9, 7.9–8.9 or 82.6–85.2 mg/kg bw per day in females. All rats were exposed throughout the study, beginning 13 weeks before mating. After mating, dams were allowed to produce two litters (F_1a and F_1b). For developmental investigations, ten dams per group underwent caesarian section on day 21 of gestation. To produce F_1 parents, the remaining dams were allowed to rear F_1b pups. F_2a and F_2b generations were treated with buprofezin in the same way as the F_1a and F_1b generations. Feed and water intake and body weights were recorded weekly and clinical signs were recorded daily. The reproductive parameters fertility, duration of gestation, litter size, live and dead pups and postnatal development of pups were recorded. Upon caesarian section, implantation and pup developmental parameters were investigated. All rats in this study underwent a gross pathology examination. A statement of quality assurance was provided.

No clinical signs of toxicity attributable to treatment with burofezin were observed at any dose. F_1 and F_2 but not F_0 males and females at 10, 100 or 1000 ppm had statistically significantly lowered body weight in the first weeks of treatment (up to approximately 17% in males at the highest dose and approximately 10% in females, respectively). Only in males at 1000 ppm did this effect persist throughout the study, although with a clear trend for recovery. At all other doses, the low body weights returned to normal values within 2–6 weeks. F_0 and F_1 females had also slightly lower body weights at days 0 or 4 of lactation at all doses. Thereafter, body weights increased to normal. Feed and water intake was not affected by treatment.

Fertility parameters were not affected in males or females receiving buprofezin. The viability index at postnatal day 4 was lower in the F_1a generation at 10 and 1000 ppm, but comparable to values for controls in the other generations. The body-weight gains in male and female F_1a, F_1b, F_2a and F_2b pups decreased statistically significantly from postnatal day 4 to postnatal day 21 in the group at 1000 ppm when compared with values for the controls. At postnatal day 21, mean group body weights in this group were 10–18% lower than values for the controls**.** The incidences of skeletal and visceral variations and malformations were similar in all groups. With the exception of F_0 males, absolute and relative liver weights were increased in males and females at the highest dose in the F_0, F_1 and F_2 generations.

The NOAEL for parental toxicity was 100 ppm, equal to 6.0–8.1 mg/kg bw per day, on the basis of decreased body-weight gain in males at 1000 ppm. The NOAEL for reproductive effects was 1000 ppm, equal to 62.5–86.5 mg/kg bw per day, the highest dose tested. The NOAEL for pup development was 100 ppm, equal to 6.0–8.1 mg/kg bw per day, on the basis of reduced pup body-weight gain during lactation at 1000 ppm (Takeshima, 1982).

In a supplemental study, groups of 25 male and 25 female Wistar-Imachi rats (age 3 weeks) were fed diets containing buprofezin (purity, 99.1%) at a concentration of 0, 10, 100 or 1000 ppm,

equal to 0, 0.6, 6.4 or 65.6 mg/kg bw per day in males and 0, 0.9, 8.9 or 82.8 mg/kg bw per day in females All rats were exposed throughout the study, beginning 13 weeks before mating. The design of this study was the same as that of the previous study (Takeshima, 1982), except that only one generation (F_1a and F_1b) was produced and no caesarian sections for fetal examinations were performed.

No treatment-related effects on body-weight development, feed intake, clinical condition or fertility were observed. As in the first study, body-weight gains of male and female F_1a and F_1b pups decreased statistically significantly from postnatal day 4 to postnatal day 21 in the group at 1000 ppm when compared with values for the controls. At postnatal day 21, mean group body weights were 12–17% lower than those of the controls. Relative liver weights in male and females of the F_0 generation were slightly increased.

The NOAEL for parental and reproductive effects was 1000 ppm, equal to 65.6 mg/kg bw per day, the highest dose tested. The NOAEL for pup development was 100 ppm, equal to 6.4 mg/kg bw per day, on the basis of reduced pup body-weight gain during lactation at 1000 ppm (Takeshima, 1985).

In a third study of reproductive toxicity, groups of 26 male and 26 female Wistar-Imachi rats (age 3 weeks) were fed diets containing buprofezin (purity, 99.0%) at a concentration of 0, 10, 100 or 1000 ppm, equal to 0, 0.64–0.75, 6.46–7.42 or 66.0–73.97 mg/kg bw per day in males and 0, 0.92–1.02, 9.21–10.2 or 93.11–99.57 mg/kg bw per day in females. All rats were exposed throughout the study, beginning 10 weeks before mating. Only one filial generation in F_1 and F_2 were produced. Feed intake and bodyweights were recorded weekly and clinical signs were recorded daily. Fertility, duration of gestation, litter size, live and dead pups and postnatal development of pups were recorded. All rats underwent a gross pathology examination and selected organs were examined histologically. The study complied with GLP.

There were no treatment-related effects on clinical parameters, body weights, feed intake, fertility or litter data at any dose. Pup body-weight gains of males and females decreased statistically significantly from postnatal day 7 to postnatal day 21 in the group at 1000 ppm. At postnatal day 21, mean group body weights were approximately 10% lower in rats at 1000 ppm than in the control group. Statistically significant increases in absolute and relative organ weights at 1000 ppm were found in the kidney of males of both generations (Table 30). Other organ-weight changes in males and females were either only significant as absolute or relative weights or found in one generation only and therefore judged not to be relevant. There were no histological correlates accompanying the slight organ-weight changes.

The NOAEL for parental toxicity was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of very slight organ-weight changes. The NOAEL for reproductive effects was 1000 ppm, equal to 66.0 mg/kg bw per day, the highest dose tested. The NOAEL for offspring toxicity was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of reduced pup body-weight gain at 1000 ppm during lactation (Toyohara, 1997).

(b) Developmental toxicity

Rats

Groups of 22 one-to-one paired and pregnant Sprague Dawley CD rats received buprofezin (purity, 99.0%) at a dose of 0, 50, 200 or 800 mg/kg bw per day in 2% gum arabic by oral gavage on days 6–15 of gestation. The rats were examined daily for clinical condition and mortalities, and body weight, feed intake and water consumption were recorded regularly. On day 20 of gestation, fetuses were delivered by caesarean section and uterine examination was performed. Fetuses were examined for external, skeletal and visceral abnormalities. The study complied with GLP.

Table 30. Organ weight changes in parental rats given diets containing buprofezin in a study of reproductive toxicity

Organ	Weight	Organ-weight change (% of values for controls)							
		Males				Females			
		0	10	100	1000	0	10	100	1000
F_0 generation									
Liver	Absolute	100.0	110.6*	103.8	114.9**	100.0	96.0	98.3	111.3**
	Relative	100.0	106.5*	101.8	110.6**	100.0	94.4	95.1	106.9
Kidney	Absolute	100.0	107.1*	105.6	111.7**	100.0	107.5**	104.5	104.1
	Relative	100.0	103.6	103.9	108.2**	100.0	105.7**	101.3	99.7
Adrenals	Absolute	100.0	109.6*	101.9	109.6*	100.0	101.6	106.3	115.6**
	Relative	100.0	107.1	100.0	107.1	100.0	99.5	103.2	110.4*
F_1 generation									
Liver	Absolute	100.0	107.4*	104.2	107.9*	100.0	98.4	95.1	102.2
	Relative	100.0	103.6	99.8	103.4	100.0	99.7	93.7	98.8
Kidney	Absolute	100.0	106.8**	108.6**	110.2**	100.0	100.7	98.2	94.7
	Relative	100.0	102.8	103.9	105.7**	100.0	102.4	96.8	92.2*
Adrenals	Absolute	100.0	105.1	105.1	111.9**	100.0	98.7	98.7	107.9*
	Relative	100.0	101.8	100.9	108.0	100.0	100.8	97.9	105.0

From Toyohara (1997)

* $p < 0.05$

** $p < 0.01$

Thirteen rats at the highest dose showed several signs of ill-health: loose faeces, lethargy, hunched posture, thin appearance, piloerection and partially closed eyelids. The first appearance of these effects was on day 10 of gestation. One rat in this group was killed in extremis with gastrointestinal disturbances. No treatment-related clinical signs were seen in rats at the other doses.

From day 7 to day 10 of gestation, females at the highest dose did not gain body weight and thereafter they gained body-weight at approximately at the same rate as in the other groups but remained consistently 8–9% lower in weight than the controls (Table 31). From day 7 of gestation onwards, feed intake was statistically significantly reduced and water intake increased in the group at the highest dose. Increased water consumption was also observed in rats at 200 mg/kg bw. On final necropsy, two dams in the group at the highest dose (but none in the other groups) had bilateral hydroureter. No other treatment-related changes were observed in dams.

Four dams in the group at the highest dose had total litter losses, presumably as early postimplantation losses. If these rats were not included, the postimplantation losses were still high and fetal body weights were statistically significantly low when compared with those of the controls (Table 32). In the group at the highest dose, there were more fetuses with a space between body-wall and organs, subcutaneous oedema and retarded ossification (Table 33). The incidence of retarded ossification was also slightly increased at 200 mg/kg bw per day.

The NOAEL for both maternal and fetal toxicity was 200 mg/kg bw per day on the basis of decreased body-weight gains in dams, total litter losses and delayed fetal development as shown by retarded ossification and low fetal body weight at 800 mg/kg bw per day (Tesh et al., 1987).

Table 31. Group mean body weights during gestation in a study of developmental toxicity in rats given buprofezin by gavage

Dose (mg/kg bw per day)	Mean body weights (g)											
	Day of gestation											
	0	7	8	9	10	11	12	14	15	16	18	20
0	211	244	250	254	260	267	272	285	294	305	334	363
50	211	248	251	256	261	268	273	286	297	309	337	366
200	212	248	253	259	266	272	278	290	298	308	332	362
800	211	245	245*	245*	244*	249*	254*	267*	274*	283*	304*	331*

From Tesh et al. (1987)

* $p < 0.001$

Table 32. Litter data for dams killed on day 20 of gestation in a study of developmental toxicity in rats given buprofezin by gavage

Dose (mg/kg bw per day)	No. of pregnant rats	Total litter losses	No. of live fetuses	No. of resorptions	Implantation losses (%)		Fetal weight (g)
					Pre-	Post-	
0	22	0	13.9 ± 1.7	0.7 ± 0.9	7.0	5.0	3.40 ± 0.06
50	22	0	14.1 ± 2.1	0.9 ± 0.9	7.6	5.8	3.41 ± 0.06
200	21	0	13.7 ± 2.1	1.0 ± 1.0	6.1	7.1	3.34 ± 0.06
800	21	4*	10.5 ± 5.7*	4.5 ± 2.1**	9.9 [a]	30.2**	—
800 [b]	17	0	12.9 ± 2.5	2.2 ± 1.5	10.1	14.7*	3.04 ± 0.07***

From Tesh et al. (1987)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] Derived from 18 litters.

[b] Derived from surviving rats bearing live fetuses.

Table 33. Incidence of fetal findings in a study of developmental toxicity in rats given buprofezin by gavage

Dose (mg/kg bw per day)	No. of litters examined	No. of fetuses	Incidence of finding (%)				
			Body-wall organ space	Subcutaneous oedema	Incomplete ossification		First thoracic vertebral centrum absent
					Interparietal bone	Sternebrae (third)	
0	22	153	1.3	15.1	39.9	5.2	1.3
50	22	159	0.7	9.9	42.1	6.9	1.3
200	21	143	2.8	21.5	60.8**[b]	14.0	0.7
800	17	112	18.5*[b]	45.4**[a]	74.1***[b]	25.9***[a]	10.7*[a]

From Tesh et al. (1987)

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

[a] Outside range for historical controls.

[b] Within range for historical controls.

Rabbits

Groups of 17 artificially inseminated and pregnant New Zealand White rabbits received buprofezin (purity, 99.0%) at a dose of 0, 10, 50 or 250 mg/kg bw per day in 2% gum arabic by oral gavage on days 6–19 of gestation. The rabbits were examined daily for clinical condition and mortalities, and body weight, feed intake and water consumption were recorded regularly. On day 29 of gestation, fetuses were delivered by caesarean section and uterine examination was performed. Fetuses were examined for external, skeletal and visceral abnormalities. The study complied with GLP.

In the group at 10 mg/kg bw per day, one rabbit died and another was killed in extremis. The reasons were not treatment-related gastrointestinal and respiratory tract disturbances. In the first 4 days of treatment, rabbits at the highest dose lost weight; thereafter they gained body weight at approximately at the same rate as did rabbits in the other groups but remained consistently 4–7% lighter than the controls (Table 34). In this group, feed intake on days 6–12 of gestation was approximately 35% lower than that of the controls; thereafter it increased to more than that of the controls. Water intake was not affected.

One rabbit at 50 mg/kg bw per day aborted on day 25 of gestation and two rabbits at 250 mg/kg bw per day showed total litter losses. Since this was within the range for historical controls (two litter losses in 1 out of 28 historical control groups, and one litter loss in 3 out of 28 historical control groups) a link with treatment is questionable. No other litter parameters were affected by treatment (Table 35). One fetus in rabbits at the intermediate dose and one in rabbits at the highest dose had agenesis of one kidney. A supplemental position paper provided by the company including additional historical data shows that this finding is occasionally induced by untreated carrier males (Yoshizane, 2008). Two fetuses in the group at the highest dose had enlarged aortic arches. No other dose-related effects on fetuses were observed (Table 36).

The NOAEL for maternal toxicity was 50 mg/kg bw per day on the basis of reduced body-weight gain at 250 mg/kg bw per day. The NOAEL for fetal toxicity was 50 mg/kg bw per day on the basis of an increased incidence of enlarged aortic arches in fetuses at 250 mg/kg bw per day (Tesh et al., 1986).

2.6 Special studies

(a) Thyroid function

In rats, the thyroids of males and females given buprofezin at dietary concentrations of 1000 ppm and above in a 90-day feeding study (Watanabe, 1986) and at 2000 ppm in a 104-week feeding study (Watanabe et al., 1982; Todhunter & Goodman, 1995) showed thickening and hyperplasia of follicular epithelial cells. In a mechanistic study to elucidate the possible thyroidal mode of action of buprofezin, male SD rats, ddY mice, Hartley guinea-pigs and Japanese White rabbits were exposed to buprofezin (purity, 99%) and to propylthiouracil (PTU), which served as an anti-thyroidal positive control. In the animals tested, serum concentrations of tri-iodothyronine (T3) and thyroxin (T4) and protein-bound iodine (PBI) were measured. Additionally, thyroid weights were recorded and thyroid peroxidase (TP) activity was measured and pituitaries were investigated histologically. The number of animals used in the study was not given.

In test 1, groups of rats were given buprofezin at a dose of 0 or 500 mg/kg bw per day by gavage for 1, 2, 4, or 7 consecutive days; the control group was probably treated only once. In test 2, groups of rats were given buprofezin at a dose of 0, 100, 300, 500, 1000 mg/kg bw per day by gavage for 7 consecutive days.

Serum T3 concentrations were decreased after the administration of four doses of buprofezin at 500 mg/kg bw and T4 was decreased after two doses (Table 37). T3 and T4 concentrations were decreased after dosing at 300 mg/kg bw per day for 7 days (Table 38), although the decrease in T3 did not follow a dose–response relationship.

Table 34. Group mean body weights during gestation in a study of developmental toxicity in rabbits given buprofezin by gavage

Dose (mg/kg bw per day)	Mean body weight (kg)										
	Day of gestation										
	0	6	8	10	12	14	16	18	20	24	28
0	4.00	4.12	4.13	4.19	4.21	4.28	4.35	4.36	4.37	4.44	4.47
10	3.96	4.10	4.11	4.16	4.19	4.26	4.31	4.32	4.34	4.40	4.45
50	3.96	4.09	4.12	4.16	4.19	4.25	4.31	4.31	4.32	4.42	4.43
250	3.97	4.06	3.91	3.90	3.94	4.04	4.10	4.13	4.15	4.28	4.28

From Tesh et al. (1986)

Table 35. Litter data for dams killed on day 29 of gestation in a study of developmental toxicity in rabbits given buprofezin by gavage

Dose (mg/kg bw per day)	No. of pregnant animals	Abortion and total litter resorption	No. of live fetuses	No. of resorptions	Implantation losses (%)		Fetal weight (g)
					Pre-	Post-	
0	16	0	9.0 ± 2.0	0.6 ± 0.8	13.6	5.9	42.0 ± 1.8
10	14	0	7.9 ± 2.6	0.4 ± 0.6	19.0	4.3	44.0 ± 1.8
50	17	1[a]	8.5 ± 1.7	1.0 ± 1.0	12.1	10.5	41.8 ± 1.9
250	14	2[b]	7.8 ± 2.2	0.6 ± 0.8	14.4	6.9	41.2 ± 2.0

From Tesh et al. (1986)

[a] Abortion.

[b] Total litter resorption.

Table 36. Incidences of fetal observations in a study of developmental toxicity in rabbits given buprofezin by gavage

Fetal observation	Dose (mg/kg bw per day)				Historical controls[b]	
	0	10	50	250		
					Mean (%)	Range (%)
No. of fetuses examined	144	110	136	94		
Litters	16	14	16	12		
Aortic arch enlarged	0 (0%)	0 (0%)	0 (0%)	2 (2.1%)[a]	0.01	0–0.9
Agenesis of one kidney	0 (0%)	0 (0%)	1 (0.7%)	1 (1.1%)	0.02	0–1.1

From Tesh et al. (1986)

[a] Two litters affected.

[b] Data for 92 studies, 9385 fetuses.

Table 37. Serum concentrations of T3 and T4 in rats given buprofezin at 0 or 500 mg/kg bw per day for up to 7 days (test 1)

Day of treatment	Concentration (ng/dl)	
	T3	T4
Negative control[a]	99.0 ± 19.7	3.80 ± 1.33
1	110.4 ± 7.5	2.40 ± 0.30
2	84.6 ± 16.9	1.50 ± 0.41
4	65.6 ± 11.3	0.85 ± 0.65
7	60.6 ± 5.9	0.58 ± 0.37

From Konaka & Nokata (1989)

T3, triiodothyronine; T4, thyroxin.

[a] Probably treated once only at 0 mg/kg bw per day.

Table 38. Serum concentrations of T3 and T4 in rats given buprofezin at varying doses for 7 days (test 2)

Dose (mg/kg bw per day)	Concentration (ng/dl)	
	T3	T4
0	99.0 ± 12.6	2.86 ± 0.57
100	90.2 ± 10.7	2.54 ± 0.70
300	65.8 ± 7.6	1.16 ± 0.42
500	60.0 ± 16.9	0.41 ± 0.26
1000	68.8 ± 13.1	0.20 ± 0.23

From Konaka & Nokata (1989)

T3, triiodothyronine; T4, thyroxin.

Table 39. Serum concentrations of T3 and T4 in rats given diets containing buprofezin at varying doses or PTU for 1, 3 or 6 months (test 3)

Test substance	Dietary concentration (ppm)	Months of treatment		
		1	3	6
Concentration of T3 (ng/dl)				
Buprofezin	0	88.8 ± 19.1	114.8 ± 33.4	116.2 ± 14.1
	1000	79.0 ± 16.1	127.1 ± 25.1	120.0 ± 21.4
	5000	56.9 ± 11.1	138.4 ± 37.9	122.0 ± 6.3
PTU	200	17.0 ± 10.8	29.9 ± 11.8	20.5 ± 14.1
Concentration of T4 (µg/dl)				
Buprofezin	0	4.01 ± 0.62	4.46 ± 0.91	2.99 ± 0.63
	1000	3.93 ± 0.51	3.98 ± 0.43	2.90 ± 0.53
	5000	1.53 ± 0.51	2.43 ± 0.94	2.51 ± 0.03
PTU	200	ND	ND	0.51 ± 0.17

From Konaka & Nokata (1989)

ND, not detected; PTU, propylthiouracil; T3, triiodothyronine; T4, thyroxin.

In test 3, groups of rats were fed diets containing buprofezin at a concentration of 0, 1000 or 5000 ppm or PTU at 200 ppm for 1, 3 or 6 months. Serum concentrations of T3 were only slightly low after 1 month of treatment at 5000 ppm. Serum concentrations of T4 were low in the group at 5000 ppm, with a clear trend for recovery to close-to-normal values as the study progressed (Table 39).

In test 4, groups of rats were given buprofezin at a dose of 0 or 500 mg/kg bw per day or PTU at a dose of 30 mg/kg bw per day by gavage for 15, 30 or 60 consecutive days. After study termination, thyroid weights, serum concentrations of T4 and TP activity were measured and pituitaries were examined histologically.

Relative (and absolute) thyroid weights increased in rats treated with buprofezin or PTU during the study (Table 40). Serum concentrations of T4 were initially decreased in rats treated with buprofezin or PTU but showed a tendency to increase during the study. TP activities were initially increased in rats treated with buprofezin or PTU, then recovered to normal levels at 30 days and increased again thereafter. The pituitaries of rats treated with buprofezin or PTU showed a higher grade of vacuolation than did those of rats in the control group.

To study the direct effects of buprofezin on TP activity, TP activity was measured in vitro by incubation with buprofezin at a concentration of up to approximately 10 µmol/l or similar concentrations of the known TP inhibitors, potassium cyanide (KCN) and PTU. While KCN and PTU completely inhibited TP, full TP activity was observed with buprofezin.

Furthermore, species differences in buprofezin-related changes in serum concentrations of T4 were investigated. In a first test, the dose-related decrease in T4 was shown to correlate with protein-bound iodine (PBI) in rats treated with buprofezin and BPI was also measured as a surrogate for T4 in mice, hamster and guinea-pigs. Repeated doses of buprofezin at 300 or 500 mg/kg bw per day (mice, up to 1000 mg/kg bw) did not significantly change PBI concentrations in these species. In rabbits treated with buprofezin at a dose of 300 or 1000 mg/kg bw per day, a decrease in PBI concentrations was seen from day 2 (Konaka & Nokata, 1989).

The Meeting concluded that buprofezin at a dietary concentration of 1000 ppm (equivalent to a dose of 68.5 mg/kg bw per day in the short-term feeding study by Watanabe, 1986) did not affect serum concentrations of T3 and T4 in rats treated for up to 6 months. Dosing with buprofezin at 500 mg/kg bw per day by gavage for 15–60 days caused increased thyroid weights and decreased serum concentrations of T4 but did not change TP activity significantly. While these effects of bruprofezin are comparable to those of PTU, although with a clearly lower potency, buprofezin did not (in contrast to PTU) show any direct inhibitory effect on TP in vitro.

Table 40. Changes in relative weight of the thyroid, serum concentration of T4 and TP activity (test 4) in rats given buprofezin or PTU by gavage

Test substance	Change (% of control values)								
	Relative thyroid weight			T4 concentration			TP activity		
	Day 15	Day 30	Day 60	Day 15	Day 30	Day 60	Day 15	Day 30	Day 60
Negative control	100	100	100	100	100	100	100	100	100
Buprofezin	129	268	289	11	32	56	161	105	144
PTU	591	1017	1477	5	8	23	227	115	143

From Konaka & Nokata (1989)

PTU, propylthiouracil; T4, thyroxin; TP, thyroid peroxidase.

(b) Induction of ulcers

Groups of 10 male and 10 female F344/DuCrj rats were given buprofezin (purity, 99.5%) as a single dose at 0, 613, 1036, 1751, 2959, or 5000 mg/kg bw by gavage. The study complied with GLP.

At all doses, clinical signs of intoxication were observed and there was high mortality at the two highest doses. On histological examination, induction of duodenal ulcers was observed in males at 1751 mg/kg bw and in females at 2959 mg/kg bw (Ueda, 1985).

(c) Studies on metabolites

(i) BF4, a rat metabolite

The acute oral toxicity of the rat metabolite *tert*-butylhydroxy-buprofezin (BF4) (purity, 99.8%) was investigated in groups of three female Slc:SD rats given a single dose at 300 or 2000 mg/kg bw. The study complied with GLP. The chemical of BF4 is depicted in Figure 2.

No rats died in the group at 300 mg/kg bw, but all rats in the group at 2000 mg/kg bw died within 24 h (Horiuchi, 2004b).

The mutagenic potential of BF4 (purity, 99.8%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 15.4–1250 µg/plate – S9 and 61.7–5000 µg/plate + S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2004b).

(ii) BF11, a rat metabolite

The acute oral toxicity of the rat metabolite phenylbiuret (BF11) (purity, 96.9%) was investigated in groups of three female F344/DuCrlCrlj rats given a single dose at 300 or 2000 mg/kg bw. The study complied with GLP. The chemical structure of BF11 is depicted in Figure 2.

No rats died at any dose within 14 days (Nagai, 2008a).

The mutagenic potential of BF11 (purity, 96.9%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 15.4–1250 µg/plate – S9 and 15.4–1250 µg/plate + S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2008a).

(iii) BF25, a rat metabolite

The acute oral toxicity of the rat metabolite thiobiuret (BF25) (purity, 92.1%) was investigated in groups of three female F344/DuCrlCrlj rats given a single dose at 300 or 2000 mg/kg bw. The study complied with GLP. The chemical structure of BF25 is depicted in Figure 2.

No rats died in the group receiving the lower dose within 14 days, but all rats in the group receiving the higher dose died within 3 days (Nagai, 2008b).

The mutagenic potential of BF25 (purity, 92.1%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 1.29–5000 µg/plate – S9 and 1.29–1250 µg/plate + S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2008b).

(iv) BF26, a plant metabolite

The acute oral toxicity of the plant metabolite BF26 (purity, 95.6%) was investigated in groups of three female Slc:SD rats given a single dose at 50 or 300 mg/kg bw. The study complied with GLP. The chemical structure of BF26 is depicted in Figure 2.

No rats died in the group at 50 mg/kg bw but all rats in the group at 300 mg/kg bw died within 15 min (Horiuchi, 2004a).

The mutagenic potential of BF26 (purity, 95.6%) was investigated in *S. typhimurium* strains TA100, TA1535, TA98, TA1537 and in *E. coli* WP2 *uvrA* treated at concentrations of 61.7–5000 µg/plate ± S9. The study complied with GLP.

No increase in the frequency of revertant colonies was observed at any dose (Inagaki, 2004a).

(v) Structure/activity relationship

The software Derek for Windows® (Lhasa Ltd, UK) was used to identify possible structural alerts in the metabolites BF11, BF26 and 1-*tert*-butyl-3-isopropyl-5-phenyl-2-thiobiuret. No structural alerts were found (Glomski, 2007).

3. Observations in humans

In a medical surveillance report on personnel involved with the synthesis and manufacture of buprofezin between December 1986 and January 1989, no effects attributable to buprofezin were reported (Nokata, 1990).

Comments

Biochemical aspects

Studies with [phenyl-^{14}C]buprofezin showed that the radiolabel was absorbed with a C_{max} at 9 h and was rapidly excreted (> 60% in 24 h and > 80% in 48 h) in male and female rats given doses of 10 and 100 mg/kg bw. In males and females, urinary (22–25%) and faecal (70–74%) cumulative excretion at 10 and 100 mg/kg bw was similar after 4 days. In a study in bile-duct cannulated rats, oral absorption after 24 h was low (40–45%) in males and females; of the administered dose, 30–38% was found in bile, 3–6% in the urine and about 5% in the liver and carcass (not including the gastrointestinal tract). The difference in urinary excretion between bile-duct cannulated and non-cannulated rats suggests that buprofezin excreted in the bile undergoes gastrointestinal re-circulation. The radiolabel was distributed within 2 h to the organs and tissues and after 7 days the highest concentrations were found in erythrocytes, the thyroid and the liver. The total amount of radiolabel recovered in the body accounted for less than 0.7% of the administered dose.

In a 24-week feeding study, no evidence for accumulation was observed. The metabolism of buprofezin was studied in rat liver homogenates and in vivo. Hydroxylation and subsequent methylation of the phenyl ring, oxidation of sulfur with subsequent ring-opening of the thiadiazinane ring and conjugation reactions with sulfate and glucuronic acid were the main metabolic routes. Buprofezin, 4-hydroxybuprofezin (BF2), *tert*-butylhydroxy-buprofezin (BF4), dione metabolite (BF9), buprofezin sulfoxide (BF10), phenylbiuret (BF11), isopropylphenylurea (BF12), 4-hydroxyisopropylphenylurea (BF13), dimethoxy buprofezin (BF20), 4-aminophenol (BF22), 4-hydroxyacetanilide (BF23), thiobiuret (BF25), hydroxy-methoxy-buprofezin (BF27), 2-[3-isopropyl-3-[methylsulfonylmethyl, (phenyl)carbamoyl]ureido]-2-methylpropionic acid (BF28) and dihydroxy buprofezin (C) were identified in the metabolism study in rats.

The results suggested that there are no significant differences between males and females in toxicokinetic parameters and metabolic profiles over a dose range of 10 to 100 mg/kg bw.

Toxicological data

Buprofezin was of low to moderate toxicity when administered orally, with an LD_{50} of 1635–3847 mg/kg bw in rats, LD_{50} > 5000 mg/kg bw in rabbits and LD_{50} > 10 000 mg/kg bw in mice and hamsters. By the dermal, subcutaneous and intraperitoneal routes, the LD_{50}s were > 10 000 mg/kg bw in mice and rats, and the inhalation LC_{50} was > 4.57 mg/L. In rabbits, buprofezin was not irritating to the skin and only very slightly irritating to the eye. In a Magnussen & Kligman maximization test in guinea-pigs, buprofezin gave equivocal results suggesting a very slight potential for delayed contact hypersensitivity, while the results of a local lymph-node assay with buprofezin in mice were negative.

In short-term studies in rats and dogs, the main effects were liver-weight increases accompanied by histological changes; in dogs, behaviour was also affected.

In a 13-week feeding study in rats, the feed intake in males at 200 ppm and above and in females at 5000 ppm was low after 1 or 2 weeks, resulting in lower body weights in the groups at 5000 ppm at study termination. At 200 ppm and above, slight changes in clinical chemistry parameters, including decreased glucose and triglyceride concentrations and increased cholesterol, phospholipid, urea nitrogen and albumin and globulin concentrations were observed. In males and females at 5000 ppm, liver and thyroid weights were increased and spleen weights were decreased. The increases in liver weight were accompanied by hypertrophic and necrotic changes and, in the thyroid, by hypertrophic and hyperplastic changes. The NOAEL was 40 ppm, equal to 3.4 mg/kg bw per day, on the basis of changes in clinical chemistry parameters in rats at 200 ppm.

In a 13-week study in dogs fed capsules containing buprofezin, transiently subdued behaviour was observed 1 h after dosing at 50 mg/kg bw per day and above. This observation was predominantly made in the first few days of treatment, but also at other time-points throughout the study, although with a lower incidence. At 300 mg/kg bw per day, slight ataxia was shown by virtually all dogs 1 h after dosing and persisting for about 5 h. This effect was seen in females only in the first few days of the study, but persisted for 9 weeks in one male. Male and female dogs at the highest dose had significantly lowered body-weight gains, increased liver, kidney and thyroid weights and two- to three-fold increases in the activity of ALP. Increased liver weights were also seen in males and females at 50 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day.

In a 2-year study in dogs given capsules containing buprofezin, which was performed before the 13-week study, no behavioural effects were reported at up to the highest dose of 200 mg/kg bw per day. Increased liver weights were seen in all females receiving buprofezin and in males at 200 mg/kg bw per day. Thyroid weights were high in males and females at 200 mg/kg bw per day. At 20 mg/kg bw per day and above, ALP activity was significantly increased from week 4 onwards and higher incidences of hepatocellular hypertrophy, bile-duct and mammary hyperplasia were found. The NOAEL was 2 mg/kg bw per day.

Since it was not clear whether the observation scheme used in the 2-year study could have detected putative behavioural changes 1 h after treatment, an overall NOAEL for behavioural changes could not be identified. The overall NOAEL for systemic toxicity in the 13-week and the 2-year studies in dogs was 10 mg/kg bw per day on the basis of hepatocellular hypertrophy and bile-duct and mammary hyperplasia at 20 mg/kg bw per day in the 2-year study in dogs.

The long-term toxicity and carcinogenicity of buprofezin has been investigated in mice and rats. The liver was identified as the main target of toxicity.

In the 2-year study in mice, body weights in males and females were slightly (5–10% in females and about 5% in males) but statistically significantly reduced in the group at the highest dose at

5000 ppm from week 6 (males) and week 9 (females) onwards. A very slight trend towards reduced body weight was also observed at 2000 ppm in males and females. At the highest dose of 5000 ppm, males and females had higher platelet counts at study termination and females had transiently lower erythrocyte counts and lower concentrations of haemoglobin. In males and females, liver weights were increased at 2000 ppm and above at 52 weeks; liver weights were statistically significantly increased at study termination only in males at 5000 ppm. Histologically, higher incidences of hepatocellular hypertrophy were seen at 2000 ppm and above. Hyperplastic changes were increased in the livers of males at 5000 ppm and of females at 200 ppm and above, without a clear dose–response relationship in females. In females at 2000 or 5000 ppm, slightly increased incidences of liver adenoma were close to the upper bound of the range for historical controls but without a dose–response relationship. The NOAEL for toxicity was 200 ppm, equal to 17.4 mg/kg bw per day, on the basis of hepatocellular hypertrophy at 2000 ppm. The NOAEL for carcinogenicity was 5000 ppm, equal to 481 mg/kg bw per day, the highest dose tested.

In the 2-year study in rats, terminal body weights were decreased in females at the highest dose of 2000 ppm. Males at 2000 ppm showed lowered activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Males and females at 2000 ppm had elevated liver weights at 26, 52 and 104 weeks. In the first year of the study, rats had higher thyroid weights that were statistically significant only in females. Treatment-related histological changes were restricted to the liver and the thyroid. Since the criteria for histopathological diagnosis had substantially changed since the release of the study report in 1982, the original histology slides for livers and thyroids were re-examined in 1995. At 2000 ppm, males and females had higher incidences for centrilobular hepatocellular and diffuse hypertrophy and females also had more eosinophilic foci than did the controls. A slight and statistically not significant increase in the incidence of liver adenoma was observed at 2000 ppm in females but not in males. Males at 200 ppm and males and females at 2000 ppm showed higher incidences of thyroid F-cell hypertrophy. The NOAEL for toxicity was 20 ppm, equal to 0.9 mg/kg bw per day, on the basis of higher incidences of thyroid F-cell hypertrophy at 200 ppm and the NOAEL for carcinogenicity was 2000 ppm, equal to 89.46 mg/kg bw per day, the highest dose tested.

Buprofezin was not carcinogenic in mice and rats.

Buprofezin was tested for genotoxicity in an adequate range of studies in vitro and for induction of micronucleus formation in vivo. In the submitted studies, there was no evidence for genotoxicity in vitro; however, in a published non-GLP study, micronucleus formation was induced in cultured cells by an aneugenic mechanism, rather than by chromosomal breakage. In assays for micronucleus formation in immature erythrocytes of mouse bone marrow in vivo, conflicting results have been obtained. One study reported statistically significantly increased incidences in two experiments, but the numerical results were very different and were not fully supported by equivocal results from an earlier study in which the administered doses were five times higher. Furthermore, the suggestion of an aneugenic effect in vitro in the published study was not confirmed in vivo.

The Meeting concluded that there was equivocal evidence that buprofezin might be genotoxic.

On the basis of clearly negative results in assays for genotoxicity in vitro and equivocal results in assays for genotoxicity in vivo and the absence of carcinogenicity, the Meeting concluded that buprofezin is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of buprofezin has been investigated in two two-generation studies and one one-generation study in rats. In none of the three studies were there any effects on the fertility of males or females or on reproductive performance. In one study, minor increases in liver and kidney weights, without histological correlates, were seen in parental males at 1000 ppm. Pup body weights at birth were not affected by treatment with buprofezin in any of the three studies, but pup body-weight gains were lower at 1000 ppm from postnatal day 4 to postnatal day 21. At postnatal day 21, the body weights of pups at 1000 ppm were 10–18% lower than those of the controls. The NOAEL for

parental toxicity was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of very slight changes in organ weights. The NOAEL for reproductive effects was 1000 ppm, equal to 66.0 mg/kg bw per day, the highest dose tested. The NOAEL for effects in offspring was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of reduced pup body-weight gain during lactation at 1000 ppm.

Developmental toxicity with buprofezin had been investigated in rats and rabbits. In rats at the highest dose of 800 mg/kg bw per day, clinical signs of intoxication were observed from day 10 of gestation onwards, dams had lower body-weight gains and lower feed intake from day 7 of gestation onwards and four total litter losses occurred. Additionally, postimplantation losses were increased and fetal body weights were low in this group. At this, the highest dose, there were also more fetuses with a space between body-wall and organs, subcutaneous oedema and retarded ossification. The NOAEL for maternal toxicity and fetal toxicity was 200 mg/kg bw per day on the basis of lower body-weight gains and litter losses in dams and retarded ossification in fetuses at 800 mg/kg bw per day.

In the study of developmental toxicity in artificially inseminated rabbit dams given pooled semen, body-weight gain and feed intake were lowered from the first days of treatment in the group given the highest dose of 250 mg/kg bw per day. One rabbit at 50 mg/kg bw per day aborted and two rabbits at 250 mg/kg bw per day showed total litter losses. Since the frequency of total litter loss was within the range for historical controls, a relationship to treatment is questionable. One fetus from the group at 50 mg/kg bw per day and one fetus from the group at 250 mg/kg bw per day showed unilateral agenesis of one kidney. Because this finding is occasionally observed and might be related to carrier males, its toxicological significance is questionable. Additionally, enlarged aortic arches were observed in one fetus in each of two litters of the group at the highest dose. Although the incidence of this finding was very low it was above the range for historical controls and was thus considered to be treatment-related. The NOAEL for maternal and fetal toxicity was 50 mg/kg bw per day on the basis of lowered body-weight gain and feed intake in dams and increased incidence of enlarged aortic arches in fetuses.

The Meeting concluded that buprofezin was developmentally toxic only at doses that were maternally toxic and did not induce structural changes in fetuses.

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

The Meeting considered that buprofezin is not neurotoxic on the basis of the available data.

In mechanistic studies on thyroid function in rats, buprofezin at a dose of 1000 ppm, equivalent to 68.5 mg/kg bw per day, did not affect serum concentrations of triiodothyronine (T3) and thyroxine (T4). At higher doses, T4 was lowered at the beginning of dosing only and recovered thereafter. At doses of 500 mg/kg bw per day administered by gavage for 15–60 days, thyroid weights increased and concentrations of T4 decreased, but the activity of thyroid peroxidase did not change markedly. The Meeting concluded that the mechanistic studies did not explain the thyroid changes in studies in rats and dogs.

In studies with the rat metabolite BF4, no mortalities were observed in rats given a single oral dose at 300 mg/kg bw, but all rats died at 2000 mg/kg bw. BF4 gave negative results in the Ames test. In studies with the rat metabolite BF11, no mortalities were observed in rats given a single oral dose at 2000 mg/kg bw. BF11 gave negative results in the Ames test. In studies with the rat metabolite BF25, no mortalities were observed in rats given a single oral dose at 300 mg/kg bw, but all rats died at 2000 mg/kg bw. BF25 gave negative results in the Ames test. The plant metabolite BF26 did not induce mortalities in rats given a single oral dose at 50 mg/kg bw, but all rats died at 300 mg/kg bw. BF26 and BF4 gave negative results in the Ames test.

No health effects related to exposure were reported among personnel involved in the synthesis and manufacture of buprofezin.

Toxicological evaluation

The Meeting established an ADI of 0–0.009 mg/kg bw based on a NOAEL of 0.9 mg/kg bw per day in the 2-year study in rats, identified on the basis of increases in the incidence of thyroid F-cell hypertrophy at 8.71 mg/kg bw per day. A safety factor of 100 was applied. The difference between the current ADI and the previous ADI of 0.01 mg/kg bw per day is due to rounding of the figures; both ADIs were based on the same NOAEL from the same study.

The Meeting established an ARfD of 0.5 mg/kg bw based on a NOAEL of 50 mg/kg bw identified on the basis of ataxia at 300 mg/kg bw per day in a 13-week feeding study in dogs. A safety factor of 100 was applied. This ARfD would also be protective against the finding of enlarged aortic arches in rabbit fetuses, although this effect is unlikely to be the result of a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	200 ppm, equal to 17.4 mg/kg bw per day	2000 ppm, equal to 190 mg/kg bw per day
		Carcinogenicity	5000 ppm, equal to 481 mg/kg bw per day[c]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 0.9 mg/kg bw per day	200 ppm, equal to 8.71 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 89.46 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a,d]	Reproductive toxicity	1000 ppm, equal to 66.0 mg/kg bw per day[c]	—
		Parental toxicity	100 ppm, equal to 6.46 mg/kg bw per day	1000 ppm, equal to 66.0 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 6.46 mg/kg bw per day	1000 ppm, equal to 66.0 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	200 mg/kg bw per day	800 mg/kg bw per day
		Embryo and fetal toxicity	200 mg/kg bw per day	800 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
Dog	13-week and 2-year study of toxicity[b, e]	Toxicity	10 mg/kg bw per day	20 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

[d] The results for three studies were combined.

[e] The results for two studies were combined.

Estimate of acceptable daily intake for humans

0–0.009 mg/kg bw

Estimate of acute reference dose

0.5 mg/kg bw

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

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

Critical end-points for setting guidance values for exposure to buprofezin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 40–45%
Dermal absorption	—
Distribution	Extensive, highest levels in erythrocytes, thyroid, liver
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Rapid, > 80% within 48 h, mainly via bile
Metabolism in animals	Extensive, primarily via oxidations, thiadiazinane ring opening and conjugation
Toxicologically significant compounds (animals, plants and environment)	Buprofezin, rat metabolite BF25, plant metabolite BF26
Acute toxicity	
Rat, LD_{50}, oral	1635–3847 mg/kg bw
Rat, LD_{50}, dermal	> 10 000 mg/kg bw
Rat, LC_{50}, inhalation	4.57 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization (test method used)	Not a sensitizer (Magnusson & Kligman and local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Feed intake, clinical chemistry (rat) liver weight increase with histological changes (dog)
Lowest relevant oral NOAEL	3.4 mg/kg bw per day (13-week study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day, highest dose tested (24-day study in rats)
Lowest relevant inhalation NOAEL	No data
Genotoxicity	
	Equivocal evidence of genotoxicity
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increases in liver weight with histological changes (mouse, rat) and thyroid changes (rat)
Lowest relevant NOAEL	20 ppm, equal to 0.9 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects; reduced body-weight gain in pups during lactation
Lowest relevant reproductive NOAEL	1000 ppm, equal to 66 mg/kg bw per day (rat)

Lowest relevant offspring NOAEL	100 ppm; equal to 6.46 mg/kg bw per day (rat)
Developmental target/critical effect	Enlarged aortic arches (rabbit), retarded ossification (rat)
Lowest relevant developmental NOAEL	50 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	
	No evidence in conventional studies
Other toxicological studies	
	Rat metabolites BF4 and BF25 and plant metabolite BF26 had moderate acute oral toxicity; the rat metabolite BF11 was of low acute oral toxicity. All metabolites were not genotoxic.
Medical data	
	Medical surveillance of workers in a plant producing buprofezin did not reveal any adverse health effects.

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.009 mg/kg bw	Rat, 2-year study	100
ARfD	0.5 mg/kg bw	Dog, 13-week study	100

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