BIFENAZATE

First draft prepared by
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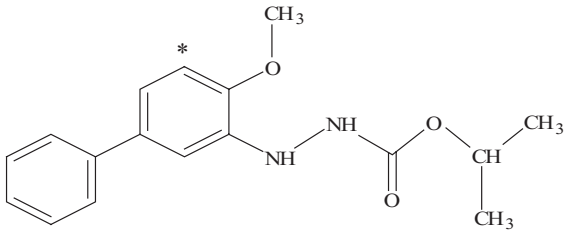
Explanation

Bifenazate is the International Organization of Standardization (ISO) approved name for *N*-(4-methoxy-biphenyl-3-yl) hydrazine-carboxylic acid isopropyl ester (International Union of Pure and Applied Chemistry). Bifenazate, a carbazate compound, is a new acaricide with a unique mode of action that is very selective for spider mites. It is intended for use on apples, pears, nectarines, peaches, plums, prunes, strawberries, grapes, hops and ornamentals.

Bifenazate was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) at the request of the Thirty-eighth Session of the Codex Committee on Pesticide Residues (CCPR). The Meeting has not previously evaluated bifenazate.

All pivotal studies with bifenazate were certified as complying with good laboratory practice (GLP).

Figure 1. Chemical structure of bifenazate



* Position of the bifenazate radiolabel used in the pharmacokinetic studies in rats

Evaluation for acceptable daily intake

1. Biochemical aspects: absorption, distribution, and excretion

Rats

The position of the radiolabel on bifenazate used in absorption, distribution, metabolism and excretion studies is shown in Figure 1.

In a dose range-finding study, groups of two male and two female fasted Sprague-Dawley rats were treated with [methoxyphenyl U-ring ^{14}C]bifenazate (lot No. CSL-94-516-73-20; purity, > 98%) in corn oil at 10 or 1000 mg/kg bw by gavage. Rats were housed individually in glass metabolism cages with food and water freely available. Dose preparations were analysed for chemical concentration and radiochemical purity by high pressure liquid chromatography (HPLC). Expired air from each animal passed through two trapping vessels that contained 40% (w/v) potassium hydroxide (KOH) and water (to trap carbon dioxide) and one trapping tube that contained Chromosorb (to trap radiolabelled volatile organic compounds). The trapping vessels were placed in a dry-ice bath. Expired air, urine and faeces were collected at intervals after dosing for 168 h, with cage washes done at the same intervals. At the end of the collection period, rats were killed and blood collected. Carcasses were weighed, homogenized and analysed. All samples were analysed for radioactivity by liquid scintillation counting (LSC).

The recovery of radiolabel was 93.77% and 100.85% of the administered dose of 10 and 1000 mg/kg bw, respectively. Radioactivity in the expired air was 0.21% and 0.6% of the 10 and 1000 mg/kg bw doses, respectively. Excretion via urine made up 12.4% of the dose at 10 mg/kg bw and 4.79% at 1000 mg/kg bw. Urinary excretion was 96% complete after 72 h at 10 mg/kg bw, and 61% complete after 72 h at 1000 mg/kg bw. Faecal excretion accounted for 68.9% of the administered dose at 10 mg/kg bw, and 91.6% at 1000 mg/kg bw. At 10 mg/kg bw, faecal excretion was 95% complete after 48 h, and at 1000 mg/kg, 61% complete at 72 h. Overall, more than 90% of excretion (via all routes) was complete after 72 h at 10 mg/kg bw, and after 96 h at 1000 mg/kg bw. At 168 h after dosing, blood accounted for 0.13% of the dose at 10 mg/kg bw and 0.14% at 1000 mg/kg bw. Concentrations in the carcass were 0.55% of the dose at 10 mg/kg bw and 0.33% of the dose at 1000 mg/kg bw (Andre & McClanahan, 1997).

In a series of experiments, groups of five male and five female Sprague-Dawley rats were given single oral doses of [methoxyphenyl U-ring ^{14}C]bifenazate (lot No. CSL-94-516-73-20; purity, > 98%) at 10 or 1000 mg/kg bw in corn oil. The administration volume was 10 ml/kg bw for oral dosing. Treated rats were housed individually in glass metabolism cages with food and water freely available. Urine and faeces were collected at intervals of 6, 12, 24, 48, 72, 96, 120, 144 and 168 h after dosing. Expired air was not collected since it was not shown to be a significant route of elimination in the pilot study. At the termination of the study, samples of brain, heart, lungs, liver, adrenals, kidneys, gastrointestinal tract and contents, bone (femur), bone marrow, muscle, mesenteric fat, mesenteric lymph nodes, testes, ovaries, seminal vesicle (uterus), urinary bladder, eyeballs, pituitary, mandibular salivary glands, spleen, pancreas, thyroid, thymus, whole blood, plasma and erythrocytes were collected and analysed for radioactivity. The remaining carcass and cage washings were also analysed for radioactivity.

Overall recovery of administered radioactivity was excellent and was approximately 93–98% for the single-dose, single highest-dose (Table 1), and biliary studies. Most of the dose was excreted in the faeces and to a lesser extent, in the urine. In rats at 10 mg/kg bw, approximately 24% and 66% of the administered dose was excreted in the urine and faeces, respectively. At the highest dose of 1000 mg/kg bw, greater amounts were excreted in the faeces (82.0–82.8%) with less excreted in the urine (7.9% and 9.4% for males and females, respectively). A small amount of radioactivity (up to 5.2% of the administered dose) was recovered in the cage wash. At 10 mg/kg bw, 90% of the urinary and faecal excretion was complete by 48 h after dosing (Table 2). At 1000 mg/kg bw, 90% of the urinary and faecal excretion was complete by 96 h after dosing (Table 3). At 1000 mg/kg bw, males excreted more radioactivity into urine and faeces than did females (77% vs 54%) by 48 h.

Table 1. Mean recovery of radioactivity (as a percentage of administered dose ± standard deviation) at 16 h after in rats given radiolabelled bifenazate as a single dose by oral gavage

Samples	Administered dose (mg/kg bw)[a]			
	10		1000	
	Males	Females	Males	Females
Faeces	66.1 ± 3.9	66.4 ± 3.0	82.0 ± 6.3	82.8 ± 3.3
Urine	24.3 ± 3.1	24.7 ± 4.0	7.9 ± 1.3	9.4 ± 1.0
Cage wash	3.3 ± 0.6	4.1 ± 1.2	3.3 ± 2.1	5.2 ± 1.3
Tissues	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Carcass	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
Total	94.3 ± 2.8	95.9 ± 4.0	93.6 ± 5.7	97.9 ± 2.1

From McClanahan (1998)

[a] n = 5 for all groups

Table 2. Mean cumulative excretion of radioactivity (as a percentage of dose) in urine and faeces of rats given radiolabelled bifenazate at a dose of 10 mg/kg bw by oral gavage

Time-point (h)	Males[a]				Females[a]			
	Urine	Faeces	Cage wash	Total	Urine	Faeces	Cage wash	Total
6	1.9	NS	0.7	2.6	4.7	NS	1.7	6.3
12	10.7	NS	1.7	12.5	1.8	NS	2.9	14.7
24	19.7	54.1	2.7	76.5	20.2	49.5	3.8	73.5
48	23.4	63.5	3.1	90.0	23.8	62.5	4.0	90.3
72	24.0	65.1	3.2	92.4	24.5	65.3	4.1	93.9
96	24.2	65.6	3.3	93.0	24.6	65.9	4.1	94.6
120	24.3	65.8	3.3	93.4	24.6	66.2	4.1	94.9
144	24.3	65.9	3.3	93.4	24.7	66.3	4.1	95.1
168	24.3	66.1	3.3	93.7	24.7	66.4	4.1	95.2

From McClanahan (1998)
NS, no sample.
[a] $n = 5$ for all groups.

Table 3. Mean cumulative excretion of radioactivity (as a percentage of administered dose) in urine and faeces of rats given radiolabelled bifenazate at a dose of 1000 mg/kg bw by oral gavage

Time-point (h)	Males[a]				Females[a]			
	Urine	Faeces	Cage wash	Total	Urine	Faeces	Cage wash	Total
6	0.3	NS	0.1	0.4	0.4	NS	0.3	0.7
12	0.8	NS	0.4	1.1	0.8	NS	0.8	1.6
24	2.4	47.0	1.6	51.1	2.1	32.3	1.8	42.2
48	5.5	68.9	2.6	76.9	4.6	45.5	3.7	53.9
72	7.2	78.2	3.0	88.4	7.9	74.6	4.8	87.3
96	7.7	81.0	3.1	91.7	8.9	80.5	5.1	94.5
120	7.8	81.7	3.2	92.7	9.2	82.3	5.2	96.7
144	7.9	81.9	3.3	93.0	9.3	82.7	5.2	97.2
168	7.9	82.0	3.3	93.2	9.4	82.8	5.2	97.4

From McClanahan (1998)
NS, no sample.
[a] $n = 5$ for all groups.

After 168 h, total residues in the tissues and carcass were < 0.6% of the administered dose. The highest concentrations of radioactivity were found in the liver, kidney, whole blood, heart, spleen, erythrocytes and lungs (Table 4). There was no difference in tissue residue concentrations between males and females at 10 mg/kg bw. In animals at 1000 mg/kg bw, females had higher residue concentrations than males in the liver, kidney, heart, spleen and erythrocytes. The difference was most evident in erythrocytes and the spleen.

In a study of the time-course of distribution, three male and three female rats per time-point were given radiolabelled bifenazate as a single dose at 10 or 1000 mg/kg bw and sacrificed at 6, 24 h or 48 h after dosing (10 mg/kg bw) or at 18, 42 or 72 h after dosing (1000 mg/kg bw). The highest concentrations were typically seen in the liver, kidney, whole blood, heart, spleen and erythrocytes, while the lowest were in the adrenals, eyes, muscle, pituitary, bone marrow and seminal vesicles or uterus. At 10 mg/kg bw, concentrations in all tissues decreased over the observation period, and were generally lower in females than in males. At 1000 mg/kg bw, concentrations peaked at 42 h, with a slower decline from erythrocytes than from most other tissues. Spleen concentrations also increased for longer than in other tissues. The erythrocyte and spleen effects were more prominent in females than males. While initially concentrations were lower in females, concentrations at 72 h were either similar or higher in females than in males. No toxicologically significant differences in the tissue concentrations were apparent between the sexes at either 10 or 1000 mg/kg bw.

Table 4. Mean residues in selected tissues of rats (mg of bifenazate equivalents/kg and percentage of administered dose) in rats given a single dose of radiolabelled bifenazate by oral gavage

Tissue	Males[a]				Females[a]			
	10 mg/kg bw		1000 mg/kg bw		10 mg/kg bw		1000 mg/kg bw	
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Gastrointestinal tract + contents	0.029	0.04	1.771	0.03	0.028	0.04	3.451	0.05
Liver	0.400	0.21	11.116	0.06	0.421	0.21	18.037	0.10
Kidney	0.195	0.02	10.782	0.01	0.204	0.02	14.617	0.02
Whole blood	0.135	0.11	15.375	0.12	0.166	0.13	14.817	0.11
Heart	0.068	< 0.005	4.863	< 0.005	0.072	< 0.005	7.883	< 0.005
Spleen	0.056	< 0.005	25.255	0.01	0.072	< 0.005	68.243	0.04
Erythrocytes	0.209	0.07	28.926	0.09	0.270	0.08	47.163	0.13
Lung	0.067	< 0.005	4.490	< 0.005	0.089	0.01	6.080	< 0.005
Thyroid	0.013	ND	ND	ND	ND	ND	ND	ND
Total (all tissues)[b]	—	0.4	—	0.24	—	0.41	—	0.32
Residual carcass	0.02	0.20	1.94	0.17	0.03	0.24	2.89	0.18

From McClanahan (1998)
ND, not detected.
[a] $n = 5$ for all groups
[b] Not including erythrocytes, blood plasma, mesenteric fat, bone and muscle.

In another experiment, groups of three male and three female Sprague-Dawley rats were given radiolabelled bifenazate as a single dose at 10 or 1000 mg/kg bw to investigate biliary elimination and the time-course of tissue distribution. In bile-duct cannulated animals, bile was collected at 1, 2, 4, 6, 8, 10, 12, 16, 24, 48 and 72 h after dosing, urine at 6, 12, 24, 48 and 72 h after dosing and faeces at 24, 48 and 72 h after dosing. The gastrointestinal tract of these animals was excised and analysed for radioactivity at the end of the experiment.

In the bile-duct cannulation experiment at 10 mg/kg bw, approximately 7% of the administered dose was excreted in the faeces, 11% in the urine and 68–73% in the bile. Residual concentrations in the carcass were approximately 1.4% of the administered dose, and concentrations in the gastrointestinal tract were 0.17% in males and 0.81% in females. At 1000 mg/kg bw in males, 57% of the administered dose was excreted in the faeces, 3.4% in the urine and 26% in the bile, while in females 64.2% of the administered dose was excreted in the faeces, 1.4% in the urine and 21% in the bile. Approximately 1% of the administered dose remained in the carcass, with 5% in the gastrointestinal tract of males and 8% in the gastrointestinal tract in females. In bile-duct cannulated rats, cumulative excretion indicated that > 87% of the administered dose had been excreted by 72 h at both doses. The study author estimated that, based on urinary and biliary excretion at 10 mg/kg bw, approximately 85% of the administered dose was absorbed in males and 79% in females, while at 1000 mg/kg bw, approximately 29% of the administered dose was absorbed in males and 22% in females. Absorption at 1000 mg/kg bw appeared to have reached saturation; absorption in males was slightly higher than in females.

The pharmacokinetics of radiolabelled bifenazate were studied by determining the concentration of radioactivity in blood samples. Samples were taken from rats at 10 mg/kg bw at 0, 2.5, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h after dosing and from rats at 1000 mg/kg bw at 1, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, 96, 120 and 144 h after dosing.

The pharmacokinetic parameters were calculated using non-compartmental analysis (Table 5). The maximum plasma concentration (C_{max}) was reached earlier after a dose of 10 mg/kg bw (5–6 h) than after a dose of 1000 mg/kg bw (18–24 h). However, the elimination half-lives were slightly longer at 1000 mg/kg bw (12–16 h) than at 10 mg/kg bw (12–13 h). No apparent sex-related differences in the pharmacokinetics of bifenazate were observed. The relative systemic exposure to the test material by comparison of the plasma C_{max} and the area under the curve (AUC) shows dose–dependency and is consistent with saturation of absorption at 1000 mg/kg bw (McClanahan, 1998).

Table 5. Pharmacokinetic parameters based on total radiolabel in rats given radiolabelled bifenazate as a single dose by oral gavage

Parameter	Dose (mg/kg bw)			
	10		1000	
	Male	Female	Male	Female
T_{max}	5 h	6 h	18 h	24 h
C_{max}	6.37 mg/kg	5.58 mg/kg	119.0 mg/kg	71.35 mg/kg
AUC	121 µg·h/g	79 µg·h/g	5909 µg·h/g	4733 µg·h/g
$T_{½}$	11.5 h	13.3 h	12 h	15.6 h

From McClanahan (1998)
AUC, area under the curve of concentration–time.

In a repeat-dose pharmacokinetic study, groups of five male and five female Sprague-Dawley rats were treated with 14 consecutive daily oral doses of unlabelled bifenazate at 10 mg/kg bw per day, followed by a single oral dose of [methoxyphenyl U-ring ^{14}C]labelled bifenazate at a concentration of 10 mg/kg bw in corn oil. Urine and faeces were collected over dry ice at 24-h intervals after administration of the final dose, with sacrifice at 168 h; samples of selected tissues, carcass and cage washings were taken for analysis.

Approximately 90% of the administered dose was excreted. The extent of excretion was similar in males and females. At 168 h after dosing, approximately 34% and 30% of the administered dose was excreted in the urine of males and females respectively, with most (approximately 75%) being excreted in the first 24 h. Radioactivity recovered in the faeces accounted for approximately 54% and 57% of

the administered dose in males and females, respectively. Less than 0.5% of the administered dose remained in the body. Approximately 0.13% of the administered dose was found in the liver. There were no differences in tissue residue concentrations between males and females (Banijamali, 2001).

Samples of excreta collected for 96 h after dosing from the single-dose study were analysed to identify metabolites. Samples from three out of five animals per group were pooled by sex and subjected to extraction and chromatographic analysis. Excreta from three animals of each sex per dose from the biliary excretion study were pooled for the 72 h after dosing. The structures of the metabolites were determined by several methods, including enzymatic or chemical degradation. Excreta from the repeat-dose study collected at 24 h and 48 h after dosing (five animals of each sex) were pooled by sex and collection time, separately.

Table 6. Distribution of metabolites (as a percentage of administered radioactivity) after oral administration of radiolabelled bifenazate to bile-cannulated rats

Metabolites[a]	Single oral dose study				Bile-cannulation study			
	10 mg/kg bw		1000 mg/kg bw		10 mg/kg bw		1000 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine[b]								
Urine sample	25.38	23.92	8.47	9.40	11.30	10.61	3.36	1.42
Identified	20.76	18.59	7.44	5.81	4.08	4.58	1.81	0.84
D9569 conjugate	4.83	0.17	0.73	ND	0.43	0.30	0.36	0.13
D9569 sulfate	11.76	8.97	2.27	0.45	3.32	2.59	0.49	0.15
A1530 sulfate	4.17	9.45	4.44	5.36	0.34	1.70	0.96	0.56
Unidentified	4.54	5.23	0.98	3.59	6.66	5.66	1.33	0.20
Faeces[c]								
Faecal sample	64.18	66.25	84.22	79.31	7.40	7.94	56.72	64.59
Extractable	47.88	50.91	74.95	68.33	6.19	5.73	60.11	60.35
Identified	39.29	39.94	71.64	64.64	4.43	3.64	58.27	58.09
D2341 glucuronide	8.92	6.3	5.63	4.71	—	—	—	—
D9569	1.75	0.93	0.15	0.17	—	—	—	—
4-OH D2341	3.56	6.77	2.37	6.64	—	—	—	—
D9477	2.27	2.98	0.05	1.21	—	—	—	—
A1530	5.54	7.06	1.19	2.07	—	—	—	—
4-OH D3598	2.40	5.56	ND	0.23	—	—	—	—
D2341	7.21	4.82	61.26	47.88	2.70	1.32	57.03	56.11
D1989	3.49	0.51	0.11	0.12	1.08	1.37	0.41	1.44
D3598	4.15	5.01	0.88	1.61	0.66	0.95	0.83	0.10
Unidentified	8.46	10.81	3.06	3.22	1.43	1.32	ND	ND
Unextractable	14.61	14.92	10.20	11.61	1.66	2.05	2.59	1.66
Bile[d]								
Bile sample	—	—	—	—	71.86	66.94	25.67	20.67
Identified	—	—	—	—	58.91	56.60	21.69	17.12

D2341 glucuronide	—	—	—	—	12.12	9.23	13.40	8.95
D9569	—	—	—	—	7.56	6.89	1.41	1.56
4-OH D2341	—	—	—	—	2.36	3.33	1.36	0.88
D9477	—	—	—	—	16.89	18.56	2.81	2.82
A1530	—	—	—	—	19.98	17.00	2.12	2.50
4-OH D3598	—	—	—	—	ND	1.58	ND	ND
D2341	—	—	—	—	ND	ND	0.59	0.42
Unidentified	—	—	—	—	7.62	6.60	2.10	1.62
Other	—	—	—	—	5.32	3.74	1.88	1.93

From McClanahan (1998)

ND, not detected; —, not applicable.

[a] See Figures 2 and 3 for structural identity of metabolites.

[b] Sum of values for 6, 12, 24, 48, 72, and 96-h urine samples (average of three rats per dose per sex combination; as a percentage of the administered dose). Sum of values for 6, 12, 24, 48, and 72 h urine sample from bile-cannulated rats (average of three animals).

[c] Sum of values for 6, 12, 24, 48, 72, and 96-h urine sample (average of three out of five animals; as a percentage of the administered dose). Sum of values for 6, 12, 24, 48, and 72-h urine sample from bile-cannulated rats (average of three animals).

[d] Samples were pooled from 0–24 h collection at 10 mg/kg bw and from 0–72 h at 1000 mg/kg/bw. 'Other' consists of diffuse, low-level radioactivity, no discernible components.

The single-dose study showed that radiolabelled bifenazate was extensively metabolized in rats given a dose at 10 mg/kg bw in contrast to rats at 1000 mg/kg bw, in which metabolism was less extensive; a large proportion of the dose was excreted in the faeces as unchanged bifenazate (61.26% and 47.88% for males and females, respectively; Table 6). The major metabolites of bifenazate in the faeces and urine resulted from hydrazine oxidation, demethylation, ring hydroxylation, cleavage of the hydrazine-carboxylic acid portion of the molecule and conjugation with glucuronic acid and sulfate (see Figure 3).

A total of eight metabolites and parent bifenazate were identified in the faeces, while three metabolites were identified in urine. The parent compound, bifenazate, was identified in the faeces of rats at both the lowest and highest doses; it represented < 7.2% of the lowest dose in male and female rats, but > 48% of the highest dose. This difference reflects the fact that a significant amount of the compound was not systemically absorbed at the highest dose. The glucuronide metabolite of bifenazate, found in relatively consistent proportions in male and female rats at both doses, arose from conjugation of the hydrazine moiety of the parent compound. This metabolite, one of the first formed, was one of the precursors to subsequent metabolic attack. The metabolite 4-OH bifenazate was formed from hydroxylation of the aromatic ring of bifenazate glucuronide and was found in slightly higher concentrations in the faeces of females than of males. Further metabolism of 4-OH bifenazate with subsequent loss of the hydrazine moiety resulted in the glucuronide or sulfate conjugate, D9477. This metabolite represented < 3% of the radioactivity in the faeces of rats at 10 mg/kg bw and < 1.2% of the radioactivity in rats at the highest dose. D9477 was further demethylated to form the metabolite D9569 which appeared as both glucuronide and sulfate conjugates.

An alternate metabolic pathway identified in the faeces led to the formation of D3598 which arose from the oxidation of the hydrazine moiety. This metabolite represented approximately 5% of the administered dose in the faeces of male and female rats at 10 mg/kg bw but < 2% of the dose in rats at 1000 mg/kg bw. Scission of the hydrazine–carboxylic acid portion resulted in the formation of D1989. With further demethylation of D1989, the metabolite A1530 was formed, which was identified as both the free metabolite and as a glucuronide or sulfate conjugate. In general, the metabolic profiles for male and female rats at 10 mg/kg bw were relatively consistent, with only small differences in metabolite formation between the sexes. The metabolic profile in rats at 1000 mg/kg bw reflected

limited absorption of parent compound, with a large percentage of the dose excreted in the faeces as unchanged bifenazate (61.3% for males and 47.9% for females).

Three primary metabolites were identified in the urine, all resulting from the loss of the hydrazine–carboxylic acid portion and demethylation of the parent compound. These were identified as glucuronide or sulfate conjugates of D9569 and the sulfate conjugate A1530. Slight differences in the concentration of metabolites in the urine were identified with male rats at the lowest dose producing more of the glucuronide conjugate D9569 than females at the lowest dose, while female rats at the lowest dose produced slightly more of the A1530 sulfate conjugate. Lower relative concentrations of these metabolites were found in the urine of male and female rats at the highest dose, which may represent saturation of the metabolic pathways leading to their formation.

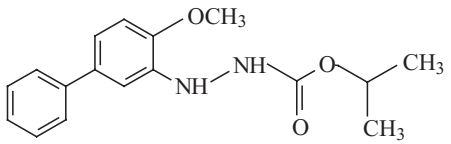
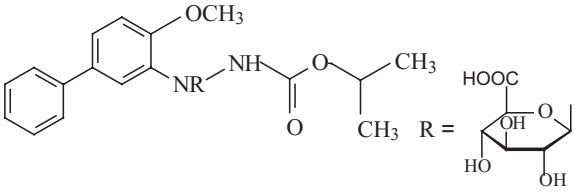
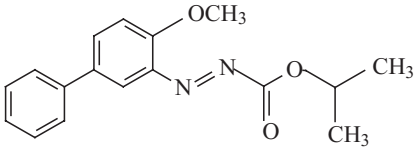
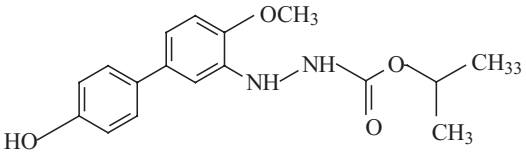
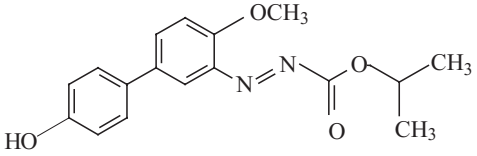
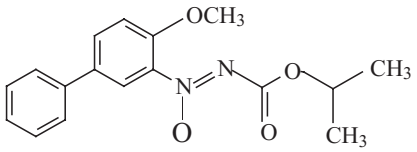
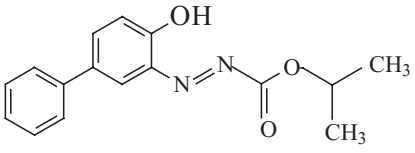
Six metabolites were identified in the bile of rats given bifenazate. These metabolites were also identified in the faeces and arose from the same metabolic pathways; however, variations in their concentrations between bile and faeces may represent further metabolism by gut bacteria. For example, the glucuronide metabolites of bifenazate, D9569, D9477, and A1530 were found in the bile of male and female rats at the lowest dose at significantly higher concentrations than in the faeces. No significant differences in the concentration of biliary metabolites in male and female rats at the lowest dose were identified. The glucuronide conjugate of parent bifenazate was the primary metabolite identified in the bile of male and female rats at the highest dose and was present in relatively the same concentrations as in rats at the lowest dose. However, the remaining metabolites were found in considerably lower concentration which suggests saturation of subsequent metabolic pathways leading to their formation (McClanahan (1998).

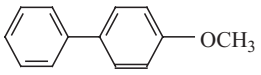
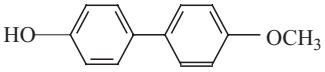
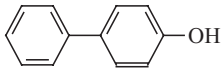
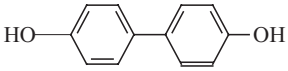
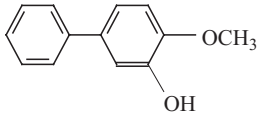
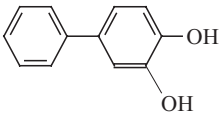
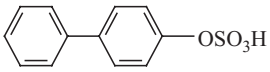
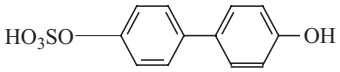
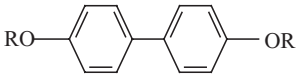
In a study of repeated doses, three major metabolites were identified, accounting for approximately 90% of the radioactivity in urine samples (22.49 and 21.27% of the administered dose identified in males and females, respectively). All components were sulfate conjugates of 4-hydroxybiphenyl or 4,4'-biphenol. In faeces, bifenazate accounted for 3.6% and 4.5% of the total radioactivity in faeces in males and females, respectively. The glucuronide conjugate of 4-hydroxybifenazate was also identified and accounted for 10.4 and 12.0% of the total radioactive residue in the faeces for males and females, respectively. The primary oxidation product of bifenazate, *N*-(4-methoxybiphenyl-3-yl)diazene-carboxylic acid isopropyl ester (D3598), was identified and accounted for 3.4% and 4.1% of the total radioactive residue in the faeces from males and females, respectively. 4-Hydroxy-D3598 was also identified in faeces, representing 13% and 13.8% of the radioactivity in the 0–24 h faeces samples for males and females, respectively. This metabolite was the only major component of the radioactivity in the 24–48 h faeces extracts. 4-Methoxybiphenyl was identified in faeces samples and accounted for 2.4% and 2.1% of the total radioactive residue in males and females, respectively. Bifenazate was also identified as the extra peak in the aqueous buffer extracts after lyophilization, accounting for 14.2% and 10.2% of the total radioactive residue in the faeces for males and females, respectively. The Meeting concluded that bifenazate had been formed as a result of hydrolysis of the glucuronide conjugate by the acidic buffer used (Banijamali, 2001).

2. Toxicological studies

Many of the studies in the toxicology data package provided were performed with bifenazate of 90–92% purity. Early lots of bifenazate contained an impurity coded F1879 or [1,1'-biphenyl]-3-sulfonic acid, 4-methoxy-2-(4-methoxy-[1,1'-biphenyl]-3-yl]hydrazide. This impurity was present at 5–7% weight depending on the lot; there were minor concentrations of several other impurities. In most dietary studies, the purity of the test article was taken into account in calculating the quantity to be administered. The purity of technical-grade bifenazate used in formulated products was stated to be approximately 96.7%.

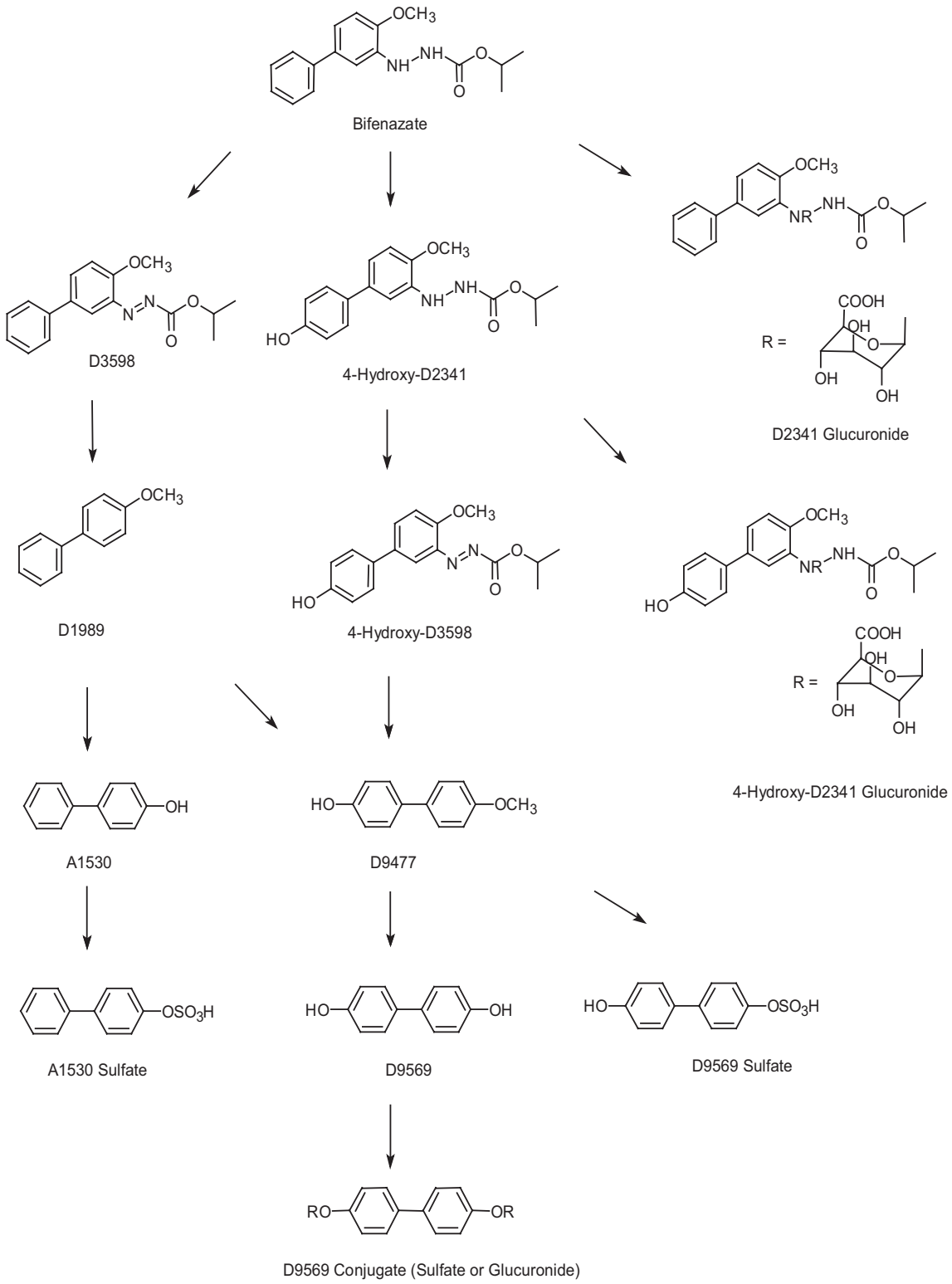
Figure 2. Chemical structures of bifenazate and its metabolites[a]

NAME/No.	STRUCTURE
Bifenazate/D2341	
D2341-Glucuronide	
Diazene/D3598	
4'-Hydroxy-D2341	
4'-Hydroxy-D3598	
D3598-N-Oxide	
Demethyl-D3598	

NAME/No.	STRUCTURE
D1989	
D9477	
A1530	
D9569	
D9963	
D9472	
A1530- Sulfate	
D9569-Monosulfate	
D9569-Sulfate or Glucuronide	

[a] The name and number assigned by the Crompton Corporation are given here.

Figure 3. Proposed metabolic pathway of bifenazate in rats



2.1 Acute toxicity

Results of studies of acute toxicity with bifenazate are summarized in Table 7.

Table 7. Results of studies of acute toxicity with bifenazate

Parameter	Species	Sex	Strain	Purity (%)	Results	Reference
Oral LD_{50}	Mice	Males and females	CD-1 [Crl: CD-1(1CR)BR]	90.4	> 5000 mg/kg bw[b]	Hoffman (1996g)[a]
Oral LD_{50}	Rat	Males and females	Sprague Dawley (Crl: CD BR)	90.4	> 5000 mg/kg bw	Hoffman (1996c)[a]
Dermal LD_{50}	Rat	Males and females	Sprague Dawley (Crl: CD BR)	90.4	> 5000 mg/kg bw	Hoffman (1996a)[a]
Inhalation LC_{50} (4 h nose only)	Rat	Males and females	Sprague Dawley (Crl: CD (SD) BR)	90.4	> 4400 mg/m^3 aerosol (4.4 mg/l)	Hoffman (1996b)[a]
Skin irritation	Rabbit	Males and females	New Zealand white (Hra: (NZW)SPF)	90.4	Minimal irritation	Hoffman (1996e)[a]
Eye irritation	Rabbit	Males and females	New Zealand white (Hra: (NZW)SPF)	90.4	Minimal irritation	Hoffman (1996f)[a]
Closed-patch skin sensitization—Buehler method	Guinea-pig	Males and females	Dunkin-Hartley Crl: (HA) BR	90.4	Not sensitizing	Hoffman (1996d)[a]
Skin sensitization—maximization method	Guinea-pig	Males and females	Dunkin-Hartley (Crj: Hartley)	91.4	Mild sensitization[c]	Ueda (1998)[a]
Skin sensitization—maximization method	Guinea-pig	Females	Dunkin-Hartley (Crj: Hartley)	97.9	Mild sensitization[d]	Rakhra & Donald (2001)[a]

LC_{50}, median lethal concentration; LD_{50}, median lethal dose.

[a] Study conducted under MAFF, OECD and United States Environmental Protection Agency (EPA) GLP standards.

[b] One out of five females died on day 8 after dosing, exhibited lacrimation, lethargy, irregular gait and laboured breathing.

[c] Eight out of twenty showed no sensitization; two out of ten had scores of 1 (scattered mild redness).

[d] Fifteen out of twenty had a score of 1 (discrete or patchy erythema); two out of twenty had a score of 2 (moderate or confluent erythema).

(a) Oral administration

Bifenazate (lot No. DS042795; purity, 90.4%) was given to groups of five male and five female young adult CD-1 by gavage (12.5% w/v in distilled water) as a single dose at 5000 mg/kg bw. Treated mice were subjected to gross necropsy at the end of a 14-day observation period. This study was conducted in accordance with GLP. One female died on day 8 after dosing. The female that died exhibited lacrimation, lethargy, irregular gait, and laboured breathing on day 7 after dosing. Decreased faecal volume was also noted for this female on days 2 through 5 and day 7 and for two other females on days 2 and 3 after dosing. There were no other mortalities, no treatment-related clinical signs of toxicity, and no necropsy findings or changes in body weight observed during the 14-day observation period. The median lethal dose (LD_{50}) was greater than 5000 mg/kg bw (Hoffman, 1996g).

Bifenazate (lot No. DS042795; purity, 90.4%) was given to groups of five male and five female young adult Sprague-Dawley rats by gavage (25% w/v in distilled water) as a single dose at 5000 mg/kg bw. Treated rats were subjected to gross necropsy at the end of a 14-day observation period. This study was conducted in accordance with GLP. There were no mortalities, no treatment-related clinical signs of toxicity, and no necropsy findings or changes in body weight observed during the 14-day observation period. The LD_{50} was greater than 5000 mg/kg bw (Hoffman, 1996c).

(b) Dermal administration

Groups of five male and five female young adult Sprague-Dawley (Crl:CD BR) rats were exposed for 24 h to bifenazate (lot No. DS042795; purity, 90.4%) at a dose of 5000 mg/kg bw moistened in 0.9% saline under an occlusive dressing. After 24 h, the occlusive dressing was removed and the treated site was wiped free of excess test material with dry gauze. Animals were observed for clinical signs and mortality for up to 14 days after dosing. This study complied with GLP. No deaths occurred. There were no treatment-related clinical signs or necropsy findings. All animals had normal body-weight gains. The dermal LD_{50} was greater than 5000 mg/kg bw (Hoffman, 1996a).

(c) Inhalation

Groups of five male and five female young adult Sprague-Dawley (Crl:CD BR) rats were exposed by head-nose only inhalation to bifenazate (lot No. DS042795; purity, 90.4%) as dust for 4 h at a nominal concentration of 5000 mg/m^3 (actual concentration, 4400 mg/m^3). Treated animals and controls were observed for 14 days and necropsied. This study complied with GLP. No mortalities occurred. No treatment-related effects were observed on body weights, except that one male and one female lost weight during the first week. Treatment-related clinical signs including moist rales, chromodacryorrhoea, and/or red/brown nasal discharge were noted in all rats after the exposure. All rats recovered by day 9. No abnormalities were noted at gross necropsy. The median lethal concentration (LC_{50}) was greater than 4400 mg/m^3 (Hoffman, 1996b).

(d) Dermal irritation

In a study of primary dermal irritation, groups of three male and three female young adult New Zealand White rabbits wereexposed for 4 h to 0.5 g of bifenazate (lot No. DS042795; purity, 90.4%) moistened with 0.5 ml of 0.9% physiological saline. Animals were then observed for 3 days. Irritation was scored by the Draize method at 30 min, 24 h, 48 h, and 72 h after removal of the dressing. This study complied with GLP. No mortalities were observed. Very slight erythema was noted in two out of six rabbits 30 min after patch removal, with resolution by 24 h. No oedema was noted on any rabbits. The primary irritation index was 0.1, i.e. bifenazate was minimally irritating to the eye under the conditions of this study (Hoffman, 1996e).

(e) Ocular irritation

In a study of primary ocular irritation, 0.1 ml of bifenazate (equivalent to 54 mg; lot No. DS042795; purity, 90.4%), was instilled into the conjunctival sac of one eye of groups of three male and three female young adult New Zealand White rabbits. Irritation was scored by the Draize method at 1 h, 24 h, 48 h, and 72 h after exposure. This study was conducted in accordance with GLP. No corneal opacity or iritis was found in the treated rabbits. All rabbits exhibited slight conjuctival irritation for up to 24 h after exposure. Bifenazate was considered minimally irritating to the eye under the conditions of this study (Hoffman, 1996f).

(f) Sensitization

In a study of dermal sensitization with bifenazate (lot No. DS042795; purity, 90.4%), 10 young male and female Dunkin Hartley guinea-pigs (Crl: (HA) BR) were tested using the Buehler method. For the induction and challenge phase, the test article was moistened with saline and applied to the skin of each animal in Hilltop chambers. For the induction phase, animals were treated dermally with the test article three times (once per week). The Hilltop chambers were left for 6 h. The challenge took place 2 weeks after the last induction. At the same time as the challenge phase, a control study of irritation was done, in which the same challenge procedure was used with naïve animals to verify that any reactions seen were not the result of irritation. The skin reactions were scored 24 h and 48 h after the induction and challenge exposures. This study complied with GLP. No deaths or abnormal signs were observed during the study. Body weights and body-weight gains were not affected by the treatment. No dermal irritation was observed at any time during the induction or the challenge phases. Bifenazate is not a skin sensitizer in guinea-pigs, as determined by the Buehler method (Hoffman, 1996d).

In a study of dermal sensitization with bifenazate (lot No. CPL 00492; purity, 91.4%), young female Dunkin-Hartley guinea pigs (Crj:Hartley) were tested using the Magnusson & Kligman maximization method. The test substance was mixed with white petrolatum. In this study, the test concentrations chosen were 2.5% for intradermal induction, 50% for topical induction, and 1% for the challenge. Skin reactions at the challenge sites were observed at 24 h and 48 h after removal of the patch. This study was conducted in accordance with GLP. Eighteen out of 20 animals in the treatment group exhibited reaction scores of 0 (no reaction) and two animals exhibited a score of 1 (scattered mild redness) to the challenge. This study indicated a mild potential for skin sensitization with bifenazate (Ueda, 1998).

In another study of dermal sensitization with bifenazate (batch No. SI 7231 lot 4; purity, 97.9%), young female Dunkin-Hartley guinea pigs (Crj:Hartley) were tested using the Magnusson & Kligman maximization method. The test substance was mixed with water. In this study, the test concentrations chosen were 6% for intradermal induction, 60% for topical induction, and 60% for the challenge. Skin reactions were observed 24 h after intradermal induction and topical application. Skin reactions were observed 24 h and 48 h after the challenge. This study was conducted in accordance with GLP. Discrete or patchy erythema was noted in all treated and control groups at 24 h after intradermal induction. No erythema was noted in any of the animals after topical application. After challenge with 60% bifenazate, positive responses were noted in 17 out of 20 animals. Discrete or patchy erythema was seen in 15 animals. Moderate or confluent erythema was noted in two animals. This study indicated a mild potential for skin sensitization with bifenazate (Rakhra & Donald, 2001).

2.2 Short-term studies of toxicity

(a) Dietary studies

Mice

In a 28-day dietary study, groups of 10 male and 10 female Crl:CD-1(ICR)BR mice (Charles River Laboratories, Raleigh, North Carolina, USA) were fed bifenazate (lot No. DS042895; purity, 91%) at a dietary concentration of 0, 200, 1000 and 5000 ppm. The consumption of compound for males was measured to be 0, 33.9 or 154.8 mg/kg bw per day at 0, 200 and 1000 ppm, while in females it was 0, 46.7 and 180 mg/kg bw per day at 0, 200 and 1000 ppm. Mice were housed individually in controlled conditions with free access to food and water. During

the study, diets were analysed for homogeneity, stability and concentration. Mice were observed twice per day for mortality and moribundity, with a more detailed observation daily and a detailed physical examination weekly. Body weight and food consumption were measured weekly. An ophthalmoscopy examination was performed before dosing and in week 4. Blood and urine were collected at the end of the study. Haematological parameters examined were erythrocyte counts, total and differential leukocyte count, haemoglobin, erythrocyte volume fraction, platelet count and cell morphology. The clinical chemistry parameters examined were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, creatinine, glucose and blood urea nitrogen (BUN). The urine was examined and the standard test parameters specified were examined. All animals were examined post mortem. The weights of the adrenals, brain (with brainstem), kidneys, liver (with gallbladder) and testes with epididymides were measured at terminal sacrifice. All tissues in the standard test parameters were preserved. Histopathological examination was done of all tissues from controls and the group at the highest dose and on all animals found dead. The lung, liver, kidney and gross lesions from all groups were examined, and where abnormalities were found in the tissues of mice at 5000 ppm, tissues from all groups were examined. The study was conducted according to United States Environmental protection Agency (EPA) test guideline 82-1, according to GLP (US EPA, Japan MAFF, and OECD).

The diet was found to be of suitable homogeneity, concentration and stability throughout the test. Mortality was seen from 1000 ppm, with 0, 0, 2, 10 and 10 males and 0, 0, 10, 10 and 10 females dying at 0, 200, 1000, 2500 and 5000 ppm, respectively. Deaths were seen up to day 8 at 2500 and 5000 ppm, and from days 13 to 22 in females and on days 24 and 29 in males at 1000 ppm. Abnormal clinical signs at 1000 ppm and above included head tilt, hunched posture, ataxia, circling, hypoactivity, tremors, limited use of front and/or hind limbs, rough hair-coat, urine stains, and dyspnoea and polypnoea in females. Decreased body-weight gain was seen from 200 ppm, with males 36% lower and females 60% lower than controls at 200 ppm. At 1000 ppm, mice lost weight during the study. Food consumption was unaffected at 200 ppm, while at 1000 ppm it was decreased by 18 or 19%. There were no treatment-related abnormalities on ophthalmoscopic examination.

On haematological examination, erythrocyte count was decreased in males at 1000 ppm, with slight decreases at 200 ppm. Leukocyte and lymphocyte counts were decreased in females at 200 ppm, with non-dose-related decreases seen in males. These findings are presented in Table 8.

Table 8. Haematological effects in mice fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)				
	Males			Females	
	0	200	1000	0	200
Erythrocyte count (10^6/ml)	9.95	9.48	9.11*	9.79	9.70
Leukocyte count (10^6/ml)	4.8	4.3	4.3	4.9	3.1*
Lymphocyte count (10^6/ml)	3.4	3.1	3.2	3.9	2.2*

From Trutter (1997a)

* $p < 0.05$

On clinical chemistry examination, increased ALT activities were seen in males at 200 and 1000 ppm and in females at 200 ppm. Slightly increased alkaline activities were seen in males at 1000 ppm, while slightly increased BUN concentrations were seen in females at 200 ppm. These findings are presented in Table 9.

Table 9. Clinical chemistry effects in mice fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)				
	Males			Females	
	0	200	1000	0	200
Alanine aminotransferase (U/l)	34	50*	90*	41	72*
Alkaline phosphatase (U/l)	111	124	146	154	150
Blood urea nitrogen (mg/dl)	34	28	32	20	30*

From Trutter (1997a)
* $p < 0.05$

There were no abnormalities on urine analysis. On gross examination of mice that died during the study, enlarged livers were seen in eight females and eight males at 5000 ppm, while some mice had dark lungs with mottled areas. There were no abnormalities found on gross examination of animals at the terminal sacrifice. On measurement of organ weights, the absolute weight of the testes was decreased in males at 1000 ppm; however, as the weights relative to body weight were unaffected, this was considered to be caused by body-weight loss. Absolute kidney weights were decreased in males at 1000 ppm and females at 200 ppm, with weights relative to body weight decreased in males. Absolute liver weights were increased in males at 1000 ppm, with weights relative to body weight increased at 200 and 1000 ppm. Organ weights are presented in Table 10.

Table 10. Organ weight changes in mice fed diets containing bifenazate for 28 days

Organ weight	Dietary concentration (ppm)				
	Males			Females	
	0	200	1000	0	200
Absolute kidney weight (g)	0.54	0.52	0.41*	0.39	0.35*
Kidney weight relative to body weight (%)	1.907	1.933	1.757	1.732	1.782
Absolute liver weight (g)	1.31	1.38	1.55*	1.18	1.08
Liver weight relative to body weight (%)	4.611	5.092*	6.658*	5.229	5.458

From Trutter (1997a)
* $p < 0.05$

On histopathological examination, liver findings included centrilobular necrosis, hepatocyte hypertrophy, fatty change and pigment deposition, which were seen from 1000 ppm. Congestion in the spleen was seen mainly in animals dying during the study at 1000, 2500 or 5000 ppm, while lymphoid depletion and necrosis were also observed. Increased pigment in spleen was seen in females from 200 ppm, and in males at 1000 and 2500 ppm. Lymphoid depletion in the lymph nodes (both mesenteric and mandibular) was seen from 1000 ppm in females and 2500 ppm in males. The incidences of these findings are presented in Table 11.

Table 11. Histopathological findings in mice fed diets containing bifenazate for 28 days

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	200	1000	2500	5000	0	200	1000	2500	5000
Liver										
Centrilobular necrosis	0	0	0	4	3	0	0	0	2	3

Hepatocyte hypertrophy	0	0	5	3	4	0	0	1	0	2
Fatty change	0	0	0	0	2	0	0	0	0	5
Pigment deposition	0	0	8	0	0	0	0	0	0	0
Spleen										
Congestion	0	0	3	5	7	0	0	7	7	7
Lymphoid depletion	0	0	3	7	9	0	0	9	10	10
Lymphoid necrosis	0	0	0	2	4	0	0	3	7	8
Increased pigment	0	0	8	3	0	0	5	8	1	0
Lymph nodes										
Mesenteric lymph node – lymphoid depletion	0	0	2	5	8	0	0	5	7	9
Mandibular lymph node – lymphoid depletion	0	0	0	4	8	0	0	5	6	6

From Trutter (1997a)

In this study, effects were seen in females at the lowest dose tested of 200 ppm, equal to 46.7 mg/kg bw per day (Trutter, 1997a).

In a 90-day study of oral toxicity, groups of 10 male and 10 female Crl:CD-1(ICR)BR mice were given diets containing bifenazate (lot No. DS042895; purity, 92.4%) at a concentration of 0, 50, 100, or 150 ppm (equal to 0, 8, 16.2 or 24 mg/kg bw per day in males and 0, 10.3, 21.7 or 32.9 mg/kg bw per day in females). Diets were prepared weekly and refrigerated. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice per day for signs of toxicity and mortality, with detailed cage-side observations done once daily. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at the end of the study. Blood was collected from all animals at terminal sacrifice for measurement of haematological and clinical parameters. Urine analysis was performed on all animals at terminal sacrifice. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Tissues were collected for histological examination from the control mice and mice at the highest concentration (150 ppm) and the lungs, liver, kidney, and spleen, and any gross lesions examined histopathologically. This study was conducted in accordance with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The test substance concentration analysis indicated that the measured concentrations ranged between 98.7% and 104% of the target concentrations.

No deaths occurred during the study. No compound-related clinical signs or ophthalmoscopic observations were observed in any of the test groups. Body weight, body-weight change, food consumption, and efficiency of food use showed no evidence of any compound-related changes. There were no treatment-related changes on haematological, clinical chemistry or urine analysis examination. On gross examination post mortem, two males at 150 ppm and one female at 100 ppm had pale livers. The liver weight relative to body weight was increased in males at 100 and 150 ppm and in all groups of treated females (not in a dose-related manner), while the absolute liver weight was increased in females at 150 ppm. However, as these findings were not associated with changes in liver enzyme activities or histopathological changes, they were not considered to be treatment-related. Microscopic examination of tissues showed an increased incidence and/or severity of pigment (morphologically compatible with haemosiderin) in the spleens of mice at 100 and 150 ppm (1, 1, 3, 5 males and 8, 9, 10, 10 females were affected at 0, 50, 100 and 150 ppm, respectively).

The degree of this finding was minimal in the males at 100 ppm, minimal to slight in the females at 100 ppm and males at 150 ppm, and slight to moderate in the females at 150 ppm.

The no-observed-adverse-effect level (NOAEL) was 50 ppm (equal to 8.0 mg/kg bw per day in males and 10.3 mg/kg bw per day in females). The lowest-observed-adverse-effect level (LOAEL) was 100 ppm, equal to 16.2 mg/kg bw per day in males and 21.7 mg/kg bw per day in females, for an increased incidence and severity of pigment deposition in the spleen (Trutter, 1997b).

Rats

In a 28-day dietary study, groups of 10 male and 10 female Sprague-Dawley derived [Crl CD(BR)] rats (Charles River Laboratories, Raleigh, North Carolina, USA) were housed individually in controlled conditions with free access to food and water and fed diets containing bifenazate (lot no. DS042895; purity, 91%) at a concentration of 0, 500, 1000, 5000 or 10 000 ppm. The achieved doses in males were 0, 33.3, 66.4, 319.4 and 410.4 mg/kg bw per day at 0, 500, 1000, 5000 and 10 000 ppm, respectively, and in females were 0, 35.3, 81.6 and 396.5 mg/kg bw per day at 0, 500, 1000 and 5000 ppm, respectively. The achieved dose for females at 10 000 ppm could not be calculated owing to high rates of early mortality. The diet was sampled at intervals throughout the study, and the homogeneity, stability and concentration assessed. Rats were observed twice per day for mortality and moribundity, with a more detailed cage-side observation done daily. Rats were given a detailed physical examination weekly. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and in week 4. Blood and urine samples were collected at the end of the study. Haematological examinations included erythrocyte count, total and differential leukocyte count, erythrocyte volume fraction, haemoglobin and platelet counts. Clinical chemistry examinations included ALT, AST, alkaline phosphatase, albumin, globulin, total protein, glucose, bilirubin, creatinine, BUN, calcium, phosphorus, potassium, sodium and chloride concentrations. The urine was analysed for the standard test parameters. All animals dying during the study, and all rats killed at the terminal sacrifice were examined for gross abnormalities. At the terminal sacrifice, the weights of the adrenals, brain and brainstem, kidneys, liver and testes with epididymides were measured. All standard tissues were collected and preserved. These tissues were examined in control rats and those at the highest dose and for all animals found dead. The lung, liver, kidney, brain with brainstem, spleen, thymus, mesenteric and mandibular lymph nodes, sternal and femoral bone marrow, seminal vesicles and mandibular salivary gland from all animals were examined. The study was conducted according to US EPA test guideline 82-1, according to GLP (US EPA, Japan MAFF, and OECD).

The diet was found to be of acceptable homogeneity, stability and concentration throughout the study. Deaths were seen in males at 10 000 ppm (9 out of 10 rats), and in females at 5000 ppm (six of 10) and 10 000 ppm (10 out of 10). Deaths were seen from day 14. No abnormal clinical signs were seen at 500 or 1000 ppm, and were only seen from week 2 in females at the highest dose or week 3 in males at the intermediate and highest doses. At 5000 and 10 000 ppm, common signs included ataxia, hypersensitivity to touch, pale appearance, rough hair-coat, hunched posture and cold to the touch. Less frequent signs included dyspnoea, abnormal respiratory sounds, anorexia, prostration, head tilt, partial closure of eyes, circling and tremors. There were no ophthalmological abnormalities at the end of the study. Body-weight gain and food consumption were decreased in all treated groups.

On haematological examination, decreased erythrocyte count, erythrocyte volume fraction and haemoglobin concentration were seen in males and females at 500 ppm and above. While results were obtained from the surviving male at 10 000 ppm, these were not included in Table 12 as they are of limited significance.

Table 12. Haematological abnormalities in rats fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	500	1000	5000	0	500	1000	5000
Erythrocyte count (10^9/ml)	7.66	7.44	7.05*	6.35*	7.71	6.93*	6.34*	5.99*
Haemoglobin (g/dl)	15.4	14.4	14.6*	13.8*	16.0	14.3*	13.6*	13.2*
Erythrocyte volume fraction	0.420	0.417	0.408	0.386*	0.430	0.396*	0.372*	0.368*

From Trutter (1997c)
* $p < 0.05$

On clinical chemistry examination, BUN concentrations were increased in females at 5000 ppm. AST activities were increased in females at 5000 ppm and the male at 10 000 ppm. Alkaline phosphatase activities were decreased in all treated groups, which may be related to decreased bone alkaline phosphatase activities associated with decreased growth and lower body-weight gain. These findings are presented in Table 13.

Table 13. Clinical chemistry abnormalities in rats fed diets containing bifenazate for 28 days

Parameter	Dietary concentration (ppm)								
	Males					Females			
	0	500	1000	5000	10 000[a]	0	500	1000	5000
Blood urea nitrogen (mg/dl)	14	13	13	17	21	13	15	15	32*
Aspartate aminotrasferase (U/l)	75	79	69	90	136	71	71	66	109*
Alkaline phosphatase (U/l)	169	142*	133*	104*	106	104	77*	83	62*

From Trutter (1997c)
* $p < 0.05$
[a] There was only one survivor in this group.

There were no abnormalities detected on urine analysis. On gross examination post mortem of rats dying during the study, dark livers and dark areas in the stomach were seen in males at 10 000 ppm and females at 5000 and 10 000 ppm. At terminal sacrifice, no gross abnormalities were seen. Increased adrenal and liver weights relative to body weight were seen at 1000 ppm and above. Absolute and relative adrenal and liver weights are presented in Table 14.

Table 14. Organ-weight changes in rats fed diets containing bifenazate for 28 days

Organ weight changes	Dietary concentration (ppm)							
	Males				Females			
	0	500	1000	5000	0	500	1000	5000
Adrenal weight (g)	0.062	0.055	0.064	0.064	0.077	0.066*	0.073	0.059*
Adrenal weight relative to body weight (%)	0.015	0.014	0.020*	0.025*	0.032	0.030	0.038*	0.043*
Liver weight (g)	12.01	12.61	10.62	11.19	7.48	7.16	8.06	7.05
Liver weight relative to body weight (%)	2.91	3.13	3.24*	4.57*	3.13	3.24	4.15*	5.15*

From Trutter (1997c)
* $p < 0.05$

On histopathological examination, liver and brain changes were seen in rats at 5000 and 10 000 ppm. Splenic congestion and pigment deposition were seen in all treated rats, while lymphoid depletion was seen at 1000 ppm and above. Lymphoid depletion was seen in the mesenteric and mandibular lymph nodes and thymus at 10 000 ppm in males, and 5000 and 10 000 ppm in females. The histopathological changes are presented in detail in Table 15.

Table 15. Histopathological findings in rats fed diets containing bifenazate for 28 days

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	500	1000	5000	10 000	0	500	1000	5000	10 000
Liver										
Single-cell necrosis	0	0	0	1	10	0	0	0	3	10
Oval cell hyperplasia	0	0	0	0	9	0	0	0	0	0
Atrophy	0	0	0	0	6	0	0	0	3	4
Pigment deposition	0	0	0	1	10	0	0	0	5	8
Brain										
Vacuolization	0	0	0	6	9	0	0	0	7	8
Spleen										
Congestion	0	10	10	10	10	0	10	10	10	10
Lymphoid depletion	0	0	10	10	10	0	0	10	10	10
Increased pigment	0	10	10	10	10	0	10	10	10	10
Mesenteric lymph node										
Lymphoid depletion	0	0	0	0	9	0	0	0	7	10
Lymphoid necrosis	0	0	0	0	1	0	0	1	5	5
Mandibular lymph node										
Lymphoid depletion	0	0	0	0	8	0	0	0	0	7
Lymphoid necrosis	3	0	2	2	5	2	0	3	5	3
Thymus										
Lymphoid depletion	0	0	0	1	9	0	0	0	6	10
Lymphoid necrosis	0	0	0	1	9	2	0	2	7	8

From Trutter (1997c)

In this study, effects were seen at the lowest dietary concentration tested of 500 ppm, equal to 33.3 mg/kg bw per day in males and 35.3 mg/kg bw per day in females (Trutter, 1997c).

In a 90-day study of oral toxicity, groups of 10 male and 10 female Sprague-Dawley rats (Crl: CD BR) were given diets containing bifenazate (lot No. DS042895; purity, 92.4%) at a concentration of 0, 40, 200, or 400 ppm, equal to 0, 2.7, 13.8 or 27.7 mg/kg bw per day in males and 0, 3.2, 16.3 or 32.6 mg/kg bw per day in females. Diets were prepared weekly, refrigerated and protected from light. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for signs of toxicity and mortality, with detailed cage-side observations done once daily and a physical examination done weekly. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at the end of the study. Blood was collected from all animals at terminal sacrifice for measurement of haematological and clinical parameters. Urine analysis was performed on all animals at terminal sacrifice. A neurobehavioural assessment, using a functional observation battery, was done during weeks 8

and 13. The assessment used home-cage/hand-held observations and open field observations, as well as assessments of reflexes. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Tissues were collected for histological examination from the control rats and from those at the highest dose (400 ppm). At 40 and 200 ppm, the lungs, liver, kidney, spleen, adrenal cortex and any gross lesions were examined histopathologically. This study complied with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. Measured test concentrations ranged between 102% and 105% of the target concentrations. No deaths or adverse clinical signs occurred. The neurological examination did not yield any treatment-related findings. No treatment-related ophthalmoscopic findings were observed nor were there any significant changes in urine analysis parameters. Body-weight gain was decreased by 19% and 28% in females at 200 and 400 ppm respectively, and by 26% in males at 400 ppm. Food consumption was decreased by 8% and 10% (statistically non-significant) in females at 200 and 400 ppm, respectively, and by 12% ($p < 0.05$) in males at 400 ppm. Overall food conversion efficiency was similar for all treatment groups compared to controls, except for decreased values in males at 400 ppm (16%) and females at 200 and 400 ppm (13% and 20%, respectively). Small but statistically significant and dose-related decreases in erythrocyte counts and haemoglobin values were seen in males at 400 ppm and in females at 200 and 400 ppm; these changes were considered to be toxicologically significant since they correlated with increased spleen weight and histopathological changes. Erythrocyte volume fraction values were also decreased in females at 400 ppm. Increased cholesterol was observed in all groups of treated females, and total protein was decreased in males at 200 and 400 ppm; however, these changes were considered incidental as they were of low magnitude and did not show any clear dose–response relationship. On gross examination post mortem, one male out of ten at 400 ppm had a pale kidney with a dilated pelvis. There were no other gross changes that were considered to be treatment-related. Spleen and kidney weights relative to body weight were increased in males at 400 ppm and in females at 200 and 400 ppm. Absolute liver weight was decreased in males at 400 ppm, which may be related to decreased body-weight gain; however, liver weight was increased in females at 200 and 400 ppm. Absolute and relative adrenal weights were increased in females at 400 ppm. On histopathological examination, kidney tubular cortical mineralization was seen in three of 10 females at 400 ppm, compared with one out of ten controls. A range of liver changes were seen, with centrilobular hepatocyte hypertrophy (minimal to moderate) at 200 and 400 ppm, and vacuolation, midzonal hypertrophy, extramedullary haematopoiesis and pigmented Kupffer cells (minimal) at 400 ppm. In males, individual cell necrosis (minimal to moderate) was seen at 200 and 400 ppm. In the spleen, increased pigment was seen at 200 and 400 ppm in males, with extramedullary haematopoiesis seen in females at 200 and 400 ppm and in all groups of treated males. However, at 40 ppm in males, the extramedullary haematopoiesis was only minimal, and this was not considered to be treatment-related. Vacuolation of the zona fasciculata of the adrenal cortex was seen in males at 200 and 400 ppm.

The NOAEL was 40 ppm, equal to 2.7 and 3.2 mg/kg bw per day in males and females, respectively. The LOAEL was 200 ppm, equal to 13.8 and 16.3 mg/kg bw per day in males and females, respectively, for increased centrilobullar hypertrophy, liver cell necrosis, vacuolation of the zona fasciculate of the adrenal cortex and extramedullary haematopoiesis in males and decreases in body-weight gains, decreases in food consumption and food efficiency, decreases in erythrocyte counts, decreases in haemoglobin, increases in absolute liver weights, increases in relative spleen and kidney weight, and histopathogical findings in liver in females (Trutter, 1997d).

Dogs

In a 90-day study of oral toxicity, groups of four male and four female beagle dogs were given diets containing bifenazate (lot No. PP159981B; purity, 92.4%) at a dose of 0, 40, 400, or 1000 ppm

(equal to 0, 0.9, 10.4 or 25 mg/kg bw per day in males and 0, 1.3, 10.7 or 28.2 mg/kg bw per day in females). Diets were prepared weekly and stored at room temperature away from light. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for morbidity or mortality, with clinical signs checked daily and a more through examination done weekly. A detailed physical examination was performed before commencement of treatment and at 1, 2, and 3 months. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at the end of the study. Blood was collected from all animals before dosing, and at 1, 2, and 3 months of dosing for measurement of haematological and clinical parameters. Urine analysis was performed on all animals before dosing, and at 1, 2, and 3 months of dosing. At the end of the study, a complete postmortem was done. The adrenals, brain, kidneys, liver, pituitary, thyroid and parathyroid, heart, spleen and testes/ovaries were weighed. The organs specified were examined microscopically. This study complied with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The test substance concentration analysis indicated that the measured concentration ranged between 98% and 100% of the target concentrations. No deaths occurred during the study. No treatment-related clinical signs, ophthalmoscopic findings, or abnormalities on the physical examination were observed. Slight decreases in body-weight gain were observed in males and females at 1000 ppm. No statistically significant changes in weekly food consumption were reported for any treatment group compared with controls. Erythrocyte count, haemoglobin and erythrocyte volume fraction values were decreased at 400 and 1000 ppm in males and females, with a compensatory increase in reticulocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), anisocytosis and platelet counts. Alkaline phosphatase and cholesterol values were increased in males at 1000 ppm after 3 months of treatment. A decrease in the percentage of protein peak 4 (not defined) on electrophoresis was noted in males and females at 1000 ppm and in females at 400 ppm. However, total protein, albumin, and globulin values were not altered. Brown coloration of the urine was seen in males at 400 ppm (one out of four animals) and at 1000 ppm (three out of four).

Increases in urinary bilirubin were also seen in males at 1000 ppm; the author suggested that this increase could be due to alteration in erythrocytic parameters. Absolute and relative liver weights were increased in males and females at 400 and 1000 ppm. No treatment-related gross pathological findings were observed in any organ. Centrilobular or diffuse hepatocellular hypertrophy was observed in females at 400 ppm and in males and females at 1000 ppm. In addition, brown pigment in Kupffer cells was seen in males and females at 400 and 1000 ppm.

The NOAEL was 40 ppm, equal to 0.9 mg/kg bw per day in males and 1.3 mg/kg bw per day in females. The LOAEL was 400 ppm, equal to 10.4 mg/kg bw per day in males and 10.7 mg/kg bw per day in females, for alterations in haematological parameters (decreases in erythrocytes, haemoglobin, and erythrocyte volume fraction, increases in reticulocytes, MCV, MCH, anisocytosis and platelets), a decrease in protein peak 4 (females only), brown-coloured urine (males only), increases in absolute and relative liver weights, hepatocellular hypertrophy (females only), and brown pigment in Kupffer cells.

The diet containing bifenazate at the lowest concentration (40 ppm) was determined to be unstable at the storage conditions used at the beginning of the study. Unfortunately, this was not discovered until week 5. Consequently, animals at the lowest dose were ingesting less compound than anticipated during the first 5 weeks. This deficiency was not insignificant, but it is noted that a NOAEL of 40 ppm was found in a 1-year study of toxicity (Goldenthal, 1997).

In a 1-year study of oral toxicity, groups of five male and five female beagle dogs were given diets containing bifenazate (lot No. PP159981B; purity, 92.4%) at a concentration of 0, 40, 400, or 1000 ppm, equal to 0, 1.01, 8.95 or 23.94 mg/kg bw per day in males and 0, 1.05, 10.42 or 29.19 mg/kg bw per day in females. Diets were prepared weekly and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were

inspected twice daily for morbidity or mortality and clinical signs. Detailed clinical examinations were performed weekly. A complete physical examination was performed on each dog before study initiation and during months 3, 6, 9, and 12. Body weight and food consumption were measured weekly. An ophthalmoscopic examination was done before dosing and at 6 and 12 months. Blood was collected from all animals before dosing, and at 3, 6, and 12 months of dosing, for measurement of haematological and clinical parameters. Urine analysis was performed on all animals before dosing, and at 3, 6, and 12 months. At the end of the study, a complete postmortem was carried out. The adrenals, brain, kidneys, liver, pituitary, thyroid and parathyroid, heart, salivary gland and testes/ovaries were weighed. The organs specified were examined microscopically. This study complied with GLP.

The test article was evenly distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. Measured test concentrations ranged between 97% and 101% of the target concentrations. No deaths occurred during the study. No treatment-related clinical signs, ophthalmoscopic findings, or abnormalities on the physical examination were observed. Body weights were comparable between treated and control animals throughout the study. Overall (weeks 0–52), body-weight gains were slightly decreased in the animals at 400 ppm (males, 14%; females, 19%) and 1000 ppm (males, 14%; females, 23%) animals. These differences were not statistically significant and reflected large, erratic changes in body-weight gains in all treated groups at various times during the study which were considered unrelated to treatment. Mean food consumption was decreased in males at 400 and 1000 ppm (17% and 12%, respectively). Decreased food consumption in males was not observed to occur in a dose-related manner and food consumption was not affected in females. Overall, food conversion efficiency values [100 × body-weight change (g) / food consumed (g)] were generally comparable, allowing for the observed erratic changes in body weight and food intake. Test article-related alterations in haematology parameters were observed at 400 and 1000 ppm at all analysis time-points (Table 16). The following increases in haematology parameters were observed at 1000 ppm at months 3, 6, and 12: (i) leukocytes in males; (ii) platelets in males and females; (iii) reticulocyte counts in females; (iv) segmented neutrophils in males; and (v) increased mean cell volume in females. The following decreases in haematology parameters were observed in the animals at the highest dose at months 3, 6, and 12: (i) erythrocytes in males and females; (ii) haemoglobin in males and females; and (iii) erythrocyte volume fraction in males and females. At 400 ppm, the following differences in haematological parameters were noted: (i) platelets were increased in males and females (months 3, 6, and 12); (ii) leukocytes were increased in males at months 3, 6 and 12; (iii) erythrocytes were decreased in males and females at months 3, 6, and 12; (iv) segmented neutrophils were increased in males (months 3, 6 and 12) and in females (months 3 and 6); (v) haemoglobin was decreased in females at month 3; (vi) mean cell volume was increased in males and females at months 3, 6, and 12; (vii) reticulocyte counts were increased in males and females at months 3, 6, and 12; and (viii) erythrocyte volume fraction was decreased in females at month 3.

Table 16. Haematological findings in beagle dogs fed diets containing bifenazate for 1 year

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	40	400	1000	0	40	400	1000
Erythrocyte count (10^6/mm^3):								
Before treatment								
3 months	5.36	5.55	5.40	5.52	5.37	5.63	5.64	5.95**
6 months	7.19	7.09	6.12*	5.33**	7.54	7.40	5.84**	5.56**
12 months	6.79	6.96	6.23	5.46*	6.47	6.89	5.76	5.43**
	7.35	7.43	6.38	5.50**	6.46	7.00	5.96	5.04**

Haemoglobin (g/dl):								
Before treatment	13.0	13.0	12.9	12.6	13.8	13.7	14.5	14.2
3 months	16.7	16.3	15.5	13.3**	16.7	17.2	15.2*	14.0**
6 months	16.0	16.5	15.5	13.4**	15.6	16.6	14.7	13.4*
12 months	17.5	18.1	16.4	13.7**	16.2	17.6	15.7	13.0**
Erythrocyte volume fraction:								
Before treatment	0.356	0.375	0.364	0.353	0.368	0.377	0.389	0.395
3 months	0.485	0.480	0.450	0.385**	0.488	0.493	0.432**	0.406**
6 months	0.473	0.491	0.460	0.399*	0.452	0.481	0.434	0.401
12 months	0.518	0.529	0.474	0.402**	0.463	0.495	0.449	0.378**
MCV (μm^3)								
Before treatment	66.6	67.7	67.5	64.3	68.6	67.0	69.1	66.4
3 months	67.5	67.8	73.4*	72.3	64.8	66.7	74.1**	73.0**
6 months	69.7	70.5	74.0*	73.5	69.9	69.8	75.3**	74.0*
12 months	70.5	71.3	74.4	73.3	71.7	70.6	75.5*	75.0*
Platelets ($10^3/mm^3$):								
Before treatment	316	312	303	345	293	337	296	290
3 months	192	231	341*	430**	243	258	394**	399**
6 months	232	226	340	422**	259	273	429**	414**
12 months	204	191	314*	409**	249	297	392*	418**
Reticulocytes (per 100 erythrocytes):								
Before treatment	0.9	0.8	0.9	0.5	0.5	1.1	0.6	1.0
3 months	1.5	1.9	4.2*	4.0*	1.0	1.7	2.9	3.7*
6 months	0.5	0.5	2.5	3.0	0.3	0.5	2.1*	3.6**
12 months	0.4	0.1	1.4	2.2**	0.3	0.4	1.7	3.1**
Leukocyte count ($10^3/mm^3$):								
Before treatment	9.5	10.6	9.5	9.0	8.6	10.2	8.2	8.8
3 months	9.2	11.9	14.1**	15.1**	9.3	11.0	11.4	13.7*
6 months	9.6	12.4	16.1*	15.9*	10.1	11.6	12.0	13.2
12 months	10.1	11.2	14.0	15.9*	10.6	10.2	9.6	14.7
Neutrophil count ($10^3/mm^3$):								
Before treatment	635	6.8	5.3	5.7	4.8	6.8	4.4	5.2
3 months	5.8	7.8	8.7*	8.9*	5.3	6.1	7.0	8.3
6 months	6.1	8.1	11.5*	10.6*	6.7	7.4	8.1	8.4
12 months	6.9	8.1	10.1	12.1*	7.7	7.3	6.4	9.5
Lymphocyte count ($10^3/mm^3$):								
Before treatment	2.8	3.6	4.1	3.2	3.7	3.2	3.7	3.5
3 months	3.2	3.7	4.8*	5.9*	3.6	4.4	4.3	4.9
6 months	3.0	4.0	3.9	4.8	3.2	3.7	3.6	4.2
12 months	2.6	2.5	3.0	3.1	2.5	2.3	2.9	4.3*

From Goldenthal (1999)
MCV, mean corpuscular volume.
* $p < 0.05$; ** $p < 0.01$

Relative percentages of protein electrophoresis peak 4 were decreased at months 3, 6, and 12 in males at 400 ppm and 1000 ppm. It was stated that electrophoresis peak 4 corresponded to beta-globulins, "many of which function in the transport of haem or Fe and a decrease in these values may reflect increased utilisation of haem or iron, secondary to a haemolytic process." Total bilirubin

was increased in females at months 3, 6, and 12 at 400 and 1000 ppm and at some intervals in males at 400 and 1000 ppm. Other changes in clinical parameters were minor, not dose-dependent, and/or not sustained over time and considered not to be treatment-related. Increased urinary bilirubin and brown coloration of the urine were noted at 400 and 1000 ppm in males only. No treatment-related gross pathological findings were observed. Statistically significant increases in relative liver weights were observed in females at 1000 ppm. Absolute and relative weights of the adrenals and pituitary were slightly decreased in males at 1000 ppm and slightly increased in females at 1000 ppm. These were considered to be incidental findings. Kidney weights (both absolute and relative to body weight) were increased (statistically non-significant) in males and females at 400 and 1000 ppm. Test article-related microscopic pathology changes were noted in the bone marrow, kidney, and liver at 400 and 1000 ppm. Mild to moderate hyperplasia of the bone marrow of the rib, femur and sternum of the males and females was noted at 400 and 1000 ppm. This hyperplasia was characterized by a reduction in the amount of adipose tissue present in the marrow cavity, with an apparent increase in the numbers of cells of both the erythroid and myeloid series. Brown pigment was present within the tubular epithelial cells of the kidney in trace to mild amounts in males and females at 400 and 1000 ppm. The pigment occurred as discrete granules within the cytoplasm and was morphologically consistent in appearance with haemosiderin. Brown pigment was present in the liver, primarily within the Kupffer cells lining the hepatic sinusoids, in trace amounts in males and females in groups at 400 and 1000 ppm. As with the pigment in the kidney, it generally occurred as discrete granules within the cytoplasm and was morphologically consistent in appearance with haemosiderin. The changes in the haematology parameters indicate blood loss with regeneration and correspond to the increased haemosiderin pigment in the liver and kidney and hyperplasia in the bone marrow observed in the microscopic evaluation of these organs. The presence of haemosiderin, the regenerative response, and the presence of bilirubinaemia and bilirubinuria are consistent with a haemolytic mechanism.

The NOAEL was 40 ppm, equal to 1.01 mg/kg bw per day in males and 1.05 mg/kg bw per day in females. The LOAEL was 400 ppm, equal to 8.9 mg/kg bw per day in males and 10.4 mg/kg bw per day in females for changes in haematology (decreases in erythrocyte counts, haemoglobin and erythrocyte volume fraction values; increases in reticulocyte counts and MCV), clinical chemistry and urine analysis parameters (increases in serum total bilirubin, urinary bilirubin and brown coloration of the urine), and histopathological changes (mild to moderate hyperplasia of the bone marrow of the rib, femur and sternum and brown pigment in liver and kidney) (Goldenthal, 1999).

(b) Dermal administration

Rats

In a 21-day study of dermal toxicity with repeated doses, groups of 10 male and 10 female Sprague-Dawley rats (Crl:CD(BR)VAX/Plus) received dermal applications of bifenazate (lot No. PP159981B; purity, 92.5%) at a dose of 0, 80, 400 or 1000 mg/kg bw per day, 6 h per day for a total of 21 to 22 days. The test substance was formulated in distilled water. The hair was clipped from the back of each rat before the first application, then periodically as required. This clipped area comprised not less than 15% of the total body surface area. The test article was applied uniformly to the dose site by gentle inunction using a glass stirring rod. The application site was then wrapped in a gauze bandage and secured with tape for 6 h. At the end of exposure, the gauze and tape were removed and the application site rinsed with tap water and dried. Animals were observed twice daily for signs of mortality, morbidity, toxicity, and the presence of dermal irritation. A detailed clinical examination was done weekly, including observations of general condition, skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs, feet, respiration and palpation for tissue masses. Dermal reactions at the application site were scored daily (before dosing) using the Draize method. Body weight was measured before dosing, then weekly. Food consumption was measured

weekly. Blood and urine samples were taken at the end of the study, and the standard test parameters were examined. An ophthalmoscopic examination was done before dosing and at termination. At the end of the study, a postmortem examination was done on all animals. The adrenals, kidneys, liver, spleen and testes were weighed, and a microscopic examination conducted on the adrenals, kidneys, liver, lung, skin (treated and untreated) and spleen. Where no significant abnormalities were seen at 1000 mg/kg bw per day, tissues from rats at 80 and 400 mg/kg bw per day were not examined. The spleen was determined to be a target organ and was microscopically examined for the groups at 80 and 400 mg/kg bw per day. This study complied with GLP.

There were no treatment-related effects in clinical signs of toxicity, mortality, ophthalmoscopic evaluations and gross histopathological findings at necropsy. Dermal irritation (erythema/oedema) occurred more frequently in some of the treated rats of both sexes in comparison to controls, but without any dose–response relationship. The irritation was minimal and only lasted for short durations. Body weight was statistically significantly decreased in males at the highest dose (11%) and females (10%) and females at the intermediate dose (9%). Food consumption was decreased at 400 and 1000 mg/kg bw per day. In males at the highest dose, haemoglobin was decreased (5%) and platelets were increased significantly (13%). In females at the highest dose, significant decreases were seen in erythrocytes (12%), haemoglobin (10%) and erythrocyte volume fraction (9%). Slight decreases in haematological findings showed a trend toward anaemia, which was compensated by extramedullary haematopoiesis in the spleen. Mild erythrocyte hypochromasia and anisocytosis were noted in two out of ten females in the groups at 400 and 1000 mg/kg bw per day. Mild hypochromasia was also noted in three out of ten females at 1000 mg/kg bw per day. Total bilirubin was statistically significantly increased in femalea at 1000 mg/kg bw per day. Creatine phosphokinase activities were decreased in males at 400 and 1000 mg/kg bw per day, by 35% and 46% respectively, which was considered to be treatment-related. A mild increase in urinary ketones, protein, and specific gravity and a decrease in urinary volumes were seen at 400 and 1000 mg/kg bw per day. A mild increase in ketonuria was likely due to the decreased body weight of the animals. Proteinuria was not associated with any renal tubular dysfunction on the basis of histopathological examination. Absolute testes weights were unaffected by treatment; the increased relative testes weights were a result of decreased body-weight gain. Absolute and relative adrenal and spleen weights were increased in males at 1000 mg/kg bw per day, while absolute and relative spleen weights were increased in females at 1000 mg/kg bw per day. Extramedullary haematopoiesis of the spleen was seen at 400 and 1000 mg/kg bw per day. No other treatment-related histopathological changes were observed. No treatment-related effects were seen at 80 mg/kg bw per day.

The NOAEL was 80 mg/kg bw per day on the basis of decrease in body-weight gains, decrease in food consumption, clinical haematology (decreased haemoglobin, erythrocyte volume fraction and erythrocyte counts), urine analysis (increase in ketones, protein and specific gravity and decrease in urinary volume) and histopathology (extramedullary haematopoiesis) observed at 400 mg/kg bw per day, the LOAEL, and above (Goldenthal, 1998).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of oncogenicity, groups of 50 male and 50 female Crl:CD-1(ICR)BR mice were given diets containing bifenazate (lot/batch No. PP159945; purity, 90.2%) at a concentration of 0, 10, 100, or 225 ppm (males) and 175 ppm (females), equal to 0, 1.5, 15.4 and 35.7 mg/kg bw per day in males and 0, 1.9, 19.7 and 35.7 mg/kg bw per day in females, for up to 79 weeks. Diets were prepared weekly. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for mortality and morbidity. Changes in clinical condition or

behaviour were recorded daily. Detailed clinical observations were recorded weekly. Body weight and food consumption were measured weekly for the first 13 weeks, then monthly for the rest of the study. Water consumption was not measured. An ophthalmoscopic examination was not done. Blood was collected from all surviving animals in weeks 52 and 78, and the cell morphology, erythrocyte count and total and differential leukocyte counts were determined. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed. Tissues were collected for histological examination from the control group and from animals at the highest dose, animals that died prematurely, and animals sacrificed in extremis. All gross lesions and masses from all animals and the lungs, liver, and kidneys from mice at 10 and 100 ppm were examined microscopically. This study complied with GLP.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The measured test concentrations ranged between 89.6% and 110% of the target concentrations.

Survival was not significantly affected by treatment, with 80%, 84%, 94% and 90% of males and 82%, 74%, 90% and 78% of females surviving at 0, 10, 100 and 225/175 ppm, respectively. The only clinical sign which was associated with treatment was a swollen abdomen, seen in 5, 1, 6 and 12 males and 5, 3, 9 and 9 females at 0, 10, 100 and 225/175 ppm respectively. Body weights were consistently decreased in females at 175 ppm. Small but statistically significant decreases in body weight were also observed in males at 225 ppm. Body-weight gains (overall) were decreased in females (16%) at 175 ppm. Overall body-weight gains were comparable to those of controls in all males and females at 10 and 100 ppm. No treatment-related differences in food consumption or use were observed in any treated group. Erythrocyte count was decreased in males at 225 ppm, and slightly decreased in females at 175 ppm. Leukocyte and lymphocyte counts were decreased in males at 100 and 225 ppm. While all treated females had a decreased leukocyte count at 78 weeks, this would appear to be an artefact related to the increased value in controls. Absolute and relative liver weights were increased in males at the highest dose and relative weights in females at the highest dose. A decrease in kidney weights (absolute and relative) was seen in males at the intermediate and highest doses and decreased absolute kidney weights in females at the highest dose. In the absence of corroborating histopathology, the decreases in kidney weights were considered to be of equivocal toxicological importance. An increased incidence (%) of liver masses was detected in males at the highest dose (16% treated vs 6% controls). It was stated that these masses generally correlated to hepatocellular adenomas. No other treatment-related gross pathological changes were noted in any treated group. An increased incidence of primary, benign hepatocellular adenomas was observed in males at the highest dose (21% in treated groups vs 10% in controls). This incidence was greater than historical control incidences (4.3–14.9%); however, it was not statistically significant and hepatocellular carcinomas were not identified in these animals. In addition, benign hepatocellular adenoma and hepatocellular carcinoma had low occurrences in the females at the highest dose (2% each vs 0% in controls) and were within historical control ranges (benign adenoma, 0–2%; carcinoma, 0–2%).

The NOAEL was 10 ppm (equal to 1.5 mg/kg bw per day) in males and 100 ppm (equal to 19.7 mg/kg bw per day) in females. The LOAEL was 100 ppm (equal to 15.4 mg/kg bw per day) in the males (for changes in haematology parameters and possibly kidney weights) and 175 ppm (equivalent to 35.7 mg/kg bw per day) in the females (for decreased body weights and body-weight gains) (Ivett, 1999a).

Rats

In a long-term combined study of toxicity/oncogenicity, groups of 50 male and 50 female Sprague-Dawley (Crl:CD BR) rats were given diets containing bifenazate (lot/batch No. PP159945; purity, 90.2–92.5%) at a nominal concentration of 0, 20, 80, or 200 ppm (males) and 160 ppm

(females) (equal to 0, 1.0, 3.9, or 9.7 mg/kg bw per day in males and 0, 1.2, 4.8, and 9.7 mg/kg bw per day in females) for up to 104 weeks. An additional 10 rats of each sex per dose were sacrificed at 53 weeks. Diets were prepared weekly. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for mortality and morbidity. Changes in clinical condition and behaviour were recorded daily. Detailed clinical observations were recorded weekly. Body weight and food consumption were measured weekly for the first 13 weeks, then monthly for the rest of the study. Water consumption was not measured. An ophthalmoscopic examination was performed before treatment and during week 104. Blood and urine were collected from 10 rats of each sex per dose in weeks 13, 26, 52, and week 104. Haematological parameters examined were erythrocyte count, leukocyte count, erythrocyte volume fraction, haemoglobin and platelet count. Differential leukocyte count and cell morphology were assessed on the control rats and on those at the highest dose, although slides were prepared for all groups. Standard clinical chemistry and urine analysis parameters were examined. All rats dying during the study were examined grossly, and all tissues were prepared and examined histopathologically. After 52 weeks, 10 rats of each sex per group were killed and examined, and all rats were killed at the end of the study. After scheduled sacrifice, a postmortem examination was done. The adrenals, brain, kidneys, liver, ovaries, spleen and testes with epididymides were removed and examined. All tissues were examined from control rats and rats at the highest dose. At 20 and 80 ppm, the lungs, liver, spleen and kidneys, as well as any gross lesions from all rats were examined. This study complied with GLP.

The test article was uniformly distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. The measured test concentrations ranged between 90.8 to 105% of the target concentrations.

Survival was not significantly affected by treatment in either males or females. At termination (104 weeks), survival rates ranged from 40% to 78% in males and from 28% to 44% in females. No treatment-related clinical signs were observed. Slight decreases in body weights and body-weight gains were observed in females at 80 and 160 ppm and in males at 200 ppm, particularly early in the study. The body weights for females at the intermediate dose (80 ppm) and males at the highest dose (200 ppm) subsequently recovered to similar mean concurrent values. The mean body weights of females at the highest dose generally remained slightly lower than the concurrent controls for the remainder of the study. The mean overall food consumption in the animals at the highest dose was decreased but this was not statistically significant. Overall food conversion efficiency was reduced in females at the highest dose (statistically non-significant).

At terminal sacrifice, lens opacity (unilateral/bilateral/focal/multifocal) was observed in males at the intermediate and highest doses (11 and 21% respectively vs 0% in controls) and in females at the intermediate dose (14% treated vs 0% controls); the majority of the lesions occurred unilaterally. Bilateral diffuse lens opacity was not observed in any of the animals. The submitted ophthalmology report stated that the ophthalmoscopic findings may have been confounded by trauma associated with blood collection from the orbital venous plexus (of the first 10–20 rats of each sex per dose) during the study and by the higher survival rate in males at the intermediate and highest doses compared to controls (n = 35 and 39 rats, respectively, vs 27 controls). The sponsor concluded that the relationship of the observed ophthalmoscopic findings to treatment with bifenazate was uncertain.

In females at the highest dose at weeks 13, 26, and 52, decreased erythrocyte counts (6–10%), haemoglobin (5–8%), and erythrocyte volume fraction (5–7%) were detected. These parameters were also decreased in females at the highest dose at weeks 78 and/or 105 (6–9%; not statistically significant). In addition, a decrease in erythrocyte count (7%) was detected in females at the intermediate dose at week 26. The values for these haematological parameters were within historical control values. No treatment-related differences from concurrent controls

were observed in the blood smears obtained from the animals at the highest dose at weeks 13, 26, 52, 78, and 104 or in any of the moribund animals at time of sacrifice. No treatment-related differences from controls were observed in any clinical chemistry parameter. Decreases in total cholesterol were observed at weeks 26, 52, and 78 in males at the highest dose. However, no corroborating gross or histological data were observed.

There were no treatment-related abnormalities on urine analysis. There were no treatment-related macroscopic findings. Spleen weight was slightly increased in both sexes receiving the highest dose at the interim sacrifice, and in females at 160 ppm at the terminal sacrifice. Liver weight was slightly increased in males at 200 ppm at interim and terminal sacrifice and in females at 160 ppm at the terminal sacrifice. None of the organ-weight changes achieved statistical significance. At the interim sacrifice, pigment that was morphologically comparable with haemosiderin was seen as a treatment-related histomorphological finding in the spleen sections. Although the pigmentation was similar between treated and untreated animals, the severity of the lesion increased slightly in the groups of animals at 80 ppm and at the highest dose, with females exhibiting slightly more severe lesions than males. The incidence and/or severity of the spleen pigment, as seen in the interim-sacrifice animals, were not increased in the animals sacrificed at week 105 or animals dying during the study. No treatment-related neoplastic changes were observed.

The NOAEL was 80 ppm, equal to 3.9 mg/kg bw per day in males and 4.8 mg/kg bw per day in females. The LOAEL was 200 ppm for males and 160 ppm for females (equal to 9.7 mg/kg bw per day in males and females) for decreases in body weight, body-weight gain, food consumption and haematological parameters. The study author concluded that the NOEL was 20 ppm (equal to 3.9 mg/kg bw per day in males and 4.8 mg/kg bw per day in females). The difference in the NOAEL value between the study author and this reviewer is due to the difference between NOEL andNOAEL. The Meeting considered that the effects seen at 80 ppm (i.e. transient slight decreases in body weight and body-weight gain early in the study and a transient slight decrease in erythrocyte counts) were not adverse (Ivett, 1999b).

2.4 Genotoxicity

Results of studies of genotoxicity with bifenazate are shown in Table 17.

Table 17. Results of studies of genotoxicity with bifenazate

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation (Ames test)	*S. typhimurium* TA98, TA100, TA1535 and TA1537 *E. coli* strain WP2 *uvrA*	10–5000 µg/plate ± S9 in DMSO	90.2	Negative[a,b]	Wagner & Coffman (1996)
Forward mutation	L5178Y/*Tk*$^{+/-}$ mouse lymphoma cells	Initial assay: 100–5000 µg/ml − S9 25–2500 µg/ml + S9 in DMSO Independent repeat assay: 5–50 µg/ml − S9 25–500 µg/ml + S9 in DMSO	90.2	Negative[c,d,e]	San & Clarke (1996)

Cytogenetic test	Chinese hamster ovary cells (CHO-K1 cells)	Initial assay: 12–375 µg/ml −S9 20–1250 µg/ml + S9 in DMSO Independent repeat assay:[f] 12–94 µg/ml −S9 20–236 µg/ml + S9 in DMSO	90.2	Negative	Gudi & Schadly (1996)
In vivo					
Micronucleus formation[g]	Mouse (ICR)	Males: 96, 192, 384 mg/kg bw Females: 50, 100, 200 mg/kg bw	90.2	Negative[h]	Gudi (1996)

DMSO, dimethyl sulfoxide; S9, exogenous metabolic activation system from 9000 × *g* fraction of rat liver induced with Aroclor 1254.

[a] Tested in triplicate, positive controls included, statements of compliance with good laboratory practice and quality assurance included.

[b] Precipitate was observed at concentrations greater than 1000 µg per plate, but no appreciable toxicity was observed.

[c] Tested in duplicate, positive controls included, statements of compliance with good laboratory practice and quality assurance included.

[d] Initial assay failed owing to excess toxicity and high mutant frequencies among S9-activated solvent controls. The assay was repeated at 10–100 µg/ml activation −S9 and 25–1000 µg/ml +S9. The non-activated portion failed owing to excess toxicity and was repeated at 5–50 µg/ml.

[e] In the independent repeat assay, visible precipitate present at concentrations ≥ 100 µg/ml with S9 in treatment medium. Toxicity in cultures was observed at doses ≥ 20 µg/ml −S9 and ≥ 100 µg/ml +S9.

[f] In the repeat assay, cells were exposed for 20 h −S9 and 6 h +S9.

[g] Five of each sex per dose in the range-finding study; 15 of each sex per dose in the definitive study, with 20 of each sex in the group at the highest dose. Bone marrow collected at 24, 48 and 72 h. Administered via intraperitoneal injection in corn oil. Positive controls included (cyclophosphamide, 60 mg/kg bw; five males, five females). Statements of compliance with good laboratory practice and quality assurance were included.

[h] Mortality observed in 3 out of 20 males at 384 mg/kg bw. Clinical signs after dosing included lethargy at the intermediate and highest doses.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproduction toxicity, groups of 30 male and 30 female Crl:CD(SD) BR rats were given diets containing bifenazate (lot/batch No. PP15 9981-B; purity, 92.5%) at a dose of 0, 20, 80, or 200 ppm, equal to 0/0, 1.5/1.7, 6.1/6.9 and 15.3/17.2 mg/kg bw per day in the parental animals and 0/0, 1.7/1.9, 6.9/7.8, and 17.4/19.4 mg/kg bw per day in F_1 animals (males/emales, respectively)]. Exposure of parental animals began at age approximately 6 weeks and lasted for 10 weeks before mating. F_1 pups (30 of each sex per dose) selected to produce the F_2 generation were exposed to the same doses as their parents, beginning on postnatal day 22. F_1 animals were dosed with the test article for 10 weeks before mating to produce the F_2 litters. Mating to produce a second F_{2b} generation was not performed.

Diets were prepared weekly and stored at room temperature. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected twice daily for mortality and morbidity and detailed examinations were performed weekly. Females were weighed weekly before mating and on days 0, 4, 7, 11, 14 and 20 of gestation, and days 1, 4, 7, 14 and 21 of lactation. Food consumption was measured weekly for both sexes during the period before mating and daily during

gestation and lactation for the females; food consumption for males was recorded on a weekly basis after mating and until scheduled necropsy.

Estrous cycles were monitored with vaginal smears for 3 weeks before mating and until mating was confirmed or the mating period was completed. Semen from each adult male was evaluated for sperm concentration and motility; sperm morphology was examined in control and adult males at 200 ppm. Duration of gestation was calculated using the date delivery began. Females were allowed to deliver normally and rear young to weaning on day 21. Litters were examined after delivery and pups were sexed, examined for gross abnormalities and the number of stillborn and live pups recorded. Litters were then examined twice per day for survival. Pups dying on days 0 to 4 were examined, and pups with external abnormalities suggestive of skeletal changes were cleared and stained for skeletal examination. A detailed postmortem was done on all pups dying between day 4 and weaning, with all gross abnormalities preserved for histopathological examination. On postnatal day 4, all litters were reduced to eight pups per litter, with four pups of each sex where possible. A detailed physical examination of all pups was done on days 1, 4, 7, 14 and 21, with pups sexed and weighed at this time.

At weaning of the F_1 pups, 30 pups of each sex per dose were selected to become F_1 parents. These pups were offered treated diet from day 22. F_1 pups were examined to determine the time of balano-preputial separation (males from day 40) or vaginal perforation (females from day 30). The F_0 and F_1 adults were killed after either selection of F_1 generation or weaning of F_2 pups respectively. A complete postmortem was done. The weights of the adrenals, brain, epididymides, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thymus and uterus were measured. A histopathological examination was done on rats at 0 and 200 ppm, and on all rats dying during the study. The adrenals, brain, epididymis, cervix, coagulating gland, kidney, liver, ovaries, pituitary, prostate, seminal vesicle, spleen, testes, thymus, uterus, vagina, vas deferens and any gross lesions present were examined. The non-selected F_1 weanlings and all F_2 weanlings were killed on postnatal day 21, with terminal investigations focusing on developmental landmarks (including balano-preputial separation, vaginal perforation, and spermatogenic end-points). The brain, spleen and thymus were collected and weighed.

The test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks at room temperature. Measured test concentrations in the diet ranged between 96.2% and 101% of the target concentrations.

There were no treatment-related clinical signs or mortalities observed in the parental or F_1 animals. One F_0 male at 20 ppm died during study week 8, but no deaths were observed at the higher doses, and therefore, the death at the lowest dose was not considered to be treatment-related. One F_1 female at 200 ppm was found dead during study week 37; necropsy findings suggested that this female had kidney disease as evidenced by white areas on the kidneys, dilated renal pelvis, and distended ureter, and this death was considered not to be attributed to treatment. Reduced mean body-weight gains occurred at 200 ppm in males during weeks 2–5 and in females during weeks 0–3, but body-weight gains were not significantly affected in either sex for the remainder of the 10-week period before breeding.

Mean body weights of animals at 200 ppm were lower than controls from weeks 3–19 (males) and week 1 through gestation and lactation (females). Mean body weights and body-weight gains of F_0 animals at 20 and 80 ppm were largely unaffected by test article administration through the study. In the F_1 animals at 20 and 80 ppm, decreased body-weight gains were observed throughout the interval before mating in both sexes and in females during gestation and lactation. Sporadic reductions in body-weight gains were observed in the F_1 females at 20 ppm during the period before mating. In order to assess the equivocal body-weight gain effect at this dose, a supplementary two-generation reproduction study (WIL-155040), included with the current submission, was conducted to investigate the parental body-weight reductions observed at 20 ppm. In this supplemental study,

groups of 30 males and 30 female rats were fed bifenazate at a concentration of 7.5, 15, or 20 ppm, equal to 0, 0.6, 1.1, or 1.5 mg/kg bw per day for males and 0, 0.6, 1.2, or 1.7 mg/kg bw per day for females. Mean body weights and body-weight gains were unaffected by treatment, and therefore, the decreased body-weight gains observed in the current study at 20 ppm were considered not to be treatment-related. Food consumption was not affected in F_0 rats, but was decreased intermittently in F_1 males at 80 and 200 ppm and F_1 females at 200 ppm in the period before mating.

There were no treatment-related effects on fertility or gestation length in either generation, nor were there any treatment-related effects on sperm numbers, production, motility or abnormalities. There were no treatment-related effects on the mean litter size in either generation. In both generations, there was a slight decrease in the percentage of males at 200 ppm, but this was within the range for historical control values and was not considered to be treatment-related. There were no treatment-related effects on survival during lactation, and no developmental effects detected in pups dying during the first 4 days. No treatment-related effects on pup body weight were seen during lactation, and no effects were seen on postmortem examination of surplus pups in the F_1 generation or of weanling F_2 pups. There were no effects on balano-preputial separation; however, vaginal perforation was delayed in female pups at 80 and 200 ppm, occurring at 36, 34, 40 and 47 days at 0, 20, 80 and 200 ppm. There were no treatment-related gross pathological findings in the F_0 or F_1 adults. No treatment-related changes in organ weights were observed in the F_0 or F_1 adults. A decrease in absolute liver weight was seen in F_1 females at 200 ppm, which paralleled a slightly lower body weight and was not considered to be toxicologically significant. The spleen weight (absolute and relative) was increased in F_0 females at 200 ppm, with the relative spleen weight increased in F_1 females at 200 ppm. Increases were also seen in relative adrenal and kidney weights in females at 200 ppm; however, these were not considered to be treatment-related, but to be related to body-weight changes. No treatment-related microscopic changes were observed in the F_0 or F_1 animals.

The NOAEL for parental toxicity was 20 ppm, equal to 1.5 and 1.7 mg/kg bw per day in males and females, respectively. The LOAEL for parental toxicity was 80 ppm, equal to 6.1 and 6.9 mg/kg bw per day in males and females, respectively, on the basis of decreased body weights and body-weight gains. The NOAEL for reproductive and neonatal toxicity was 200 ppm, equal to 15.3 and 17.2 mg/kg bw per day in males and females, respectively, the highest dose tested (Schardein, 1996).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 25 pregnant Sprague-Dawley (Crl:CD BR) rats were given bifenazate (lot No. PP159981-B; purity, 92.5%) at a dose of 0, 10, 100 or 500 mg/kg bw per day (not adjusted for purity) in 0.5% carboxymethyl cellulose by gavage on days 6–15 of gestation, inclusive. Stability, homogeneity and dose concentrations were confirmed analytically. All animals were observed twice daily for mortality and moribundity and once per day for clinical signs of toxicity. Animals were also observed for signs of toxicity approximately 1 h after dosing. Maternal body weights and food consumption were recorded on day 0 of gestation, daily on days 6–16 of gestation, and on day 20. On day 20 of gestation, all surviving dams were sacrificed and subjected to gross necropsy. The uterus and ovaries were exposed and the number of corpora lutea on each ovary was recorded. Gravid uteri were weighed, opened, and the location and number of viable and non-viable fetuses, early and late resorptions, and the total number of implantations were recorded. Uteri from females that appeared non-gravid were opened and placed in 10% ammonium sulfide to detect any early implantation loss. Maternal tissues were preserved for histological examination only as indicated by gross findings. All fetuses were weighed, sexed, and examined for external malformations/variations. Each fetus was examined viscerally by fresh dissection and the sex verified. Kidneys were graded for renal papillae development by the method of Woo & Hoar (1972). Heads from approximately one-half

of the fetuses in each litter were placed in Bouin's fixative for subsequent section. The heads from the remaining one-half of the fetuses were examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

All animals survived to terminal sacrifice. At the highest dose, treatment-related clinical signs of toxicity included pale extremities, dried red material on the forelimbs or around the nose, decreased defecation, and dried brown vaginal discharge. Dams at 100 mg/kg bw per day also had an increase in the incidence of dried red material around the nose. Mean maternal body weights were significantly lower than those of the controls for the groups at the intermediate and highest doses beginning on day 8 and 7 of gestation, respectively, and continuing until termination. Final body weights of the groups at the intermediate and highest doses were 93% and 90%, respectively, of the level for the control group. The most pronounced effect on body weights of these treated groups was an overall weight loss during day 6–9 of gestation. Maternal food consumption by the groups at the intermediate and highest doses was significantly less than that by the control group throughout the dosing interval. No treatment-related effects were seen on reproductive indices of controls and treated groups.

No treatment-related effects were seen in the mean number of corpora lutea, implantation sites, pre- and postimplantation loss, or early and late resorptions. There were no statistically significant differences in litter size, number of fetuses, number of implantations, mean fetal weight, or fetal sex ratio. The number of early resorptions was increased at 100 and 500 mg/kg bw per day, with 10, 15, 25 and 19 fetuses (or 2.7, 3.9, 9.7 and 5.4%) resorbed. This was not dose-related, and was within the historical control range of 2.2-13.5%. Additionally, the control value at 2.7% was low, as only 5 out of 143 data sets in the historical control group had a resorption rate of less than 3%. Given this information, the increase in resorptions was not considered to be treatment-related. Postimplantation loss was significantly higher at 100 and 500 mg/kg bw per day compared with controls. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetuses. The incidence rate of litters containing fetuses with malformations in the groups at 0, 10, 100, and 500 mg/kg bw per day was 0/25, 0/22, 1/24, and 2/24, respectively. There was one fetus at 100 mg/kg bw per day in one litter with umbilical herniation and one fetus from one litter at 500 mg/kg bw per day with umbilical herniation. These were within historical control range, and considered incidental. There were also two fetuses from one litter at 500 mg/kg bw per day with retroesophageal aortic arch, which was not considered to be treatment-related since it was only slightly above the historical control incidence and was seen in only one litter. Variations common to fetuses from treated and control litters included ossification of the cervical centrum, 14th rudimentary ribs, and unossified sternebrae.

The NOAEL for maternal toxicity was 10 mg/kg bw per day. The LOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of abnormal clinical signs, reduced body weights and body-weight gains and reduced food consumption. The NOAEL for developmental toxicity was 500 mg/kg bw per day, the highest dose tested (Schardein, 1997b).

Rabbits

In a range-finding study of developmental toxicity, five pregnant New Zealand White SPF rabbits per treatment group were given bifenazate (lot No. CPL00376RC; purity, 90.1%) at a dose of 0, 125, 250, 500, 750 or 1000 mg/kg bw per day (not adjusted for purity) by gavage in 0.5% carboxymethylcellulose on days 7–20 of gestation, inclusive. All animals were observed twice per day for mortality and moribundity and once per day for clinical signs of toxicity. Maternal body weights were recorded on days 0, 7, 10, 13, 16, 19, 21, 25, and 29 of gestation. On day 29 of gestation, all surviving does were killed and subjected to gross necropsy. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. Gravid uteri were weighed, opened, and the location and number of viable and non-viable fetuses, early and late resorptions, and the total number of implantations were recorded. Uteri from females that appeared non-gravid were opened

and placed in 10% ammonium sulfide to detect any early implantation loss. Maternal tissues were preserved for histological examination only as indicated by gross findings. All fetuses were weighed and examined for external malformations/variations. Crown-rump measurements were recorded for late resorptions and the tissues were discarded. Each fetus was examined viscerally by fresh dissection and the sex determined. The brain from each fetus was examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

One control animal died due to gavage error. One animal at 750 mg/kg bw per day died on day 17 of gestation. Two animals were found dead, one on day 11 and one on day 21 of gestation at 1000 mg/kg bw per day. One animal at 500 mg/kg bw per day was euthanized in extremis on day 21 of gestation after the observation of prostration, rigid body, and loss of righting reflex. On postmortem examination of this rabbit, foci in the lungs and liver were found. Discoloured urine was observed in five out of five animals in all treatment groups. Decreased defaecation was observed at 250 mg/kg bw per day and above. Abortions were seen in 0, 0, 3, 2, 4 and 1 rabbit at 0, 125, 250, 500, 750 and 1000 mg/kg bw per day, respectively. Decreased body weights and body-weight gains were seen during the treatment at 500 mg/kg bw per day and above, with marginal effects at 250 mg/kg bw per day. The mean number of live fetuses were 3.6, 5.0, 3.0, 5.5, 0 and 3.3 at 0, 125, 250, 500, 750 and 1000 mg/kg bw per day respectively, with 5, 5, 1, 2, 1 and 3 pregnant rabbits examined in each of these groups, respectively. There were insufficient pups to allow an evaluation of developmental toxicity in this study.

On the basis of abortions at 250 mg/kg bw per day and above, the highest dose for the definitive study was suggested to be not more than 250 mg/kg bw per day (Denny, 1996).

In a study of developmental toxicity, groups of 20 pregnant New Zealand White rabbits were given bifenazate (lot No. PP159981-B; purity, 92.5%) at a dose of 0, 10, 50, or 200 mg/kg bw per day by gavage in 0.5% carboxymethylcellulose on days 7–19 of gestation, inclusive. Stability, homogeneity and dose concentrations were confirmed analytically. All animals were observed twice daily for mortality and moribundity and once daily for clinical signs of toxicity. Animals were also observed for signs of toxicity at approximately 1 h after dosing. Maternal body weights were recorded on day 0 of gestation, daily on days 7–20 of gestation, and on days 24 and 29. Food consumption was measured daily. A postmortem examination was done on all females aborting during the study. On day 29 of gestation, all surviving does were killed and subjected to gross necropsy. The uterus and ovaries were excised and the number of corpora lutea on each ovary was recorded. Gravid uteri were weighed, opened, and the location and number of viable and non-viable fetuses, early and late resorptions, and the total number of implantations were recorded. Uteri from females that appeared non-gravid were opened and placed in 10% ammonium sulfide to detect any early implantation loss. Maternal tissues were preserved for histological examination only as indicated by gross findings. All fetuses were weighed and examined for external malformations/variations. Crown-rump measurements were recorded for late resorptions and the tissues were discarded. Each fetus was examined viscerally by fresh dissection and the sex determined. The brain from each fetus was examined by mid-coronal slice. All carcasses were eviscerated and processed for skeletal examination.

There were no deaths and no treatment-related clinical signs during the study. One doe in each of the groups at 0, 50, and 200 mg/kg bw per day aborted and was necropsied on day 21, 20, and 26 of gestation, respectively. These abortions were not considered to be treatment-related. No statistically or biologically significant differences in absolute body-weight changes occurred between the treated and control groups during the study. No statistically significant differences in food consumption were seen between the treated groups and the control group at any time during the study. No treatment-related findings were observed in any animals at necropsy. No treatment-related differences were observed between the treated and control groups for number of corpora lutea per doe, implantations per doe, pre- or postimplantation loss, resorptions per doe, fetal body weights, or fetal sex ratios. The slight (not significant) increase in early resorptions per doe in the group at the highest dose was due to

two animals with whole litter resorption which consisted of early resorptions of 1 and 7 implantation sites, respectively. This resulted in a corresponding increase (not significant) in postimplantation loss for the group at the highest dose. No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetuses.

The NOAEL was 200 mg/kg bw per day, the highest dose tested (Schardein, 1997a).

2.6 Special studies: studies on metabolites

No studies on toxicity of metabolites were available.

3. Observations in humans

A toxicological working paper on bifenazate prepared for the Meeting by Crompton Corporation stated that "monitoring of production plant personnel following normal safety precautions have not indicated any significant adverse effects". It also stated that "a review of incidental reports regarding bifenazate showed that the incidents were minor in nature and dissipated within a reasonable period of time. No oral studies have been performed with bifenazate in volunteers".

Comments

Biochemical aspects

In studies of toxicokinetics, rats were given a single dose of radiolabelled bifenazate (10 or 1000 mg/kg bw) or were pre-treated with unlabelled bifenazate (10 mg/kg bw per day for 14 days) then given a dose of radiolabelled bifenazate administered by gavage. On the basis of urinary and biliary excretion, approximately 79–85% and 22–29% of the orally administered dose was absorbed within 72 h at 10 and 1000 mg/kg bw. A peak in plasma concentrations of radioactivity was observed 5–6 h and 18–24 h after dosing at 10 and 1000 mg/kg bw, respectively. The elimination half-life at 10 and 1000 mg/kg bw was between 11.5 and 15.6 h. Approximately 90% of the administered dose was eliminated in excreta within 48 h and 96 h at 10 and 1000 mg/kg bw, respectively. Faeces were the major route of excretion (66–82% of the administered dose), with 8–24% of the administered dose being recovered in the urine. Approximately 68–73% of the administered dose was excreted in the bile within 72 h at 10 mg/kg bw and 21–26% of the administered dose at 1000 mg/kg bw. Approximately 0.5% of the administered dose was detected in the tissues and residual carcass at 168 h, with highest concentrations of radioactivity in the liver, kidney and blood. Systemically absorbed bifenazate was extensively metabolized. The major metabolites of bifenazate in the faeces and urine resulted from hydrazine oxidation, demethylation, ring hydroxylation, removal of the hydrazine-carboxylic acid side-chain, and conjugation of the biphenyl ring moiety with glucuronic acid and sulfate. A total of eight metabolites and bifenazate were identified in the faeces and three metabolites were identified in the urine. The primary metabolite(s) in the urine were sulfate and glucuronide conjugates. Unchanged bifenazate was isolated in the faeces of males and females (5–7% and 48–61% of the faecal radioactivity at 10 and 1000 mg/kg bw, respectively). In faeces, metabolite D3598 (a product of oxidation of the hydrazine moiety) was detected (4–5% and 1–2% of the faecal radioactivity at 10 and 1000 mg/kg bw, respectively). Excretion, distribution and metabolite profiles were essentially independent of pre-treatment and sex.

Toxicological data

Bifenazate has low toxicity when administered orally, dermally or by inhalation. The LD_{50} after oral administration was > 5000 mg/kg bw in mice and rats. The LD_{50} in rats treated dermally

was > 5000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was > 4.4 mg/l (dust). Bifenazate was slightly irritating to the eyes and skin of rabbits. Bifenazate was not a skin sensitizer in guinea-pigs (Buehler test), but gave a positive response (mild sensitizer) in a Magnusson & Kligman (maximization) test in guinea-pigs.

In the absence of any specific studies addressing effects after single doses, attention was paid to effects after one or several doses in short-term studies with repeated doses. In a 28-day feeding study in mice, there were no deaths at 200 ppm (equivalent to 34 mg/kg bw per day). Deaths were observed starting on day 3 at 1000 ppm and above. At a dose of 2500 or 5000 ppm, all treated animals died. Antemortem findings (hunched posture, hypoactive behaviour, pale appearance, urine staining, tremors, dyspnoea, thinness and/or partial eye closure) were not reported before days 6–8 in 5 out of 20 animals at approximately 275 mg/kg bw per day, or before days 7–8 in 2 out of 10 males at approximately 550 mg/kg bw per day. In a 28-day feeding study in rats, there were no deaths before dosing day 14 in animals given bifenazate at a dose of up to 10 000 ppm (equal to 410 mg/kg bw per day) and there were no clinical signs before week 2 of dosing at up to 10 000 ppm. Toxicologically significant reductions in erythrocyte counts, haemoglobin and erythrocyte volume fraction were observed at 10 000 ppm at termination.

In short-term and long-term studies in mice, rats and dogs, the primary effects of bifenazate were on the haematopoietic system and the liver.

In a 90-day dietary study of toxicity in mice, an increase in the incidence of haemosiderin pigment and/or severity of this effect was seen in the spleen at 100 ppm (equal to 16.2 mg/kg bw per day) and above, although no significant changes in blood parameters were seen. The NOAEL was 50 ppm (equal to 8.0 mg/kg bw per day).

In a 90-day dietary study of toxicity in rats, decreased body-weight gains (females), decreases in erythrocytes and haemoglobin (females), increases in relative spleen and kidney weights (females), hepatocellular necrosis (males), increased pigment in the spleen (both sexes) extramedullary haematopoiesis in the spleen (females), vacuolation of the zona fasciculata of the adrenal cortex (males) and hepatocellular hypertrophy were seen at 200 ppm (equal to 13.3 mg/kg bw per day). The NOAEL was 40 ppm (equal to 8.0 mg/kg bw per day).

In a 90-day study of toxicity in dogs, alterations in haematological and related parameters (decreases in erythrocytes, haemoglobin and erythrocyte volume fraction, increases in reticulocytes, MCV, MCH, anisocytosis and platelets, a decrease in protein peak 4 (females only), brown-coloured urine (males only), brown pigment in Kupffer cells), increases in absolute and relative liver weights and hepatocellular hypertrophy (females only) were seen at 400 ppm (equal to 10.4 mg/kg bw per day). The NOAEL was 40 ppm (equal to 0.9 mg/kg bw per day). In a 52-week study of toxicity in dogs, haematological changes (decreases in erythrocyte counts, haemoglobin and erythrocyte volume fraction; increases in reticulocyte counts and MCV), changes in clinical chemistry and urine analysis parameters (increased concentration of serum total bilirubin, urinary bilirubin, and brown coloration of the urine), and histopathological changes (mild to moderate hyperplasia of the bone marrow of the rib, femur and sternum and brown pigment in liver and kidney) were seen at the LOAEL of 400 ppm (equal to 8.9 mg/kg bw per day). The NOAEL was 40 ppm (equal to 1.01 mg/kg bw per day).

When administered dermally, bifenazate displayed toxic effects that were qualitatively similar to those seen after oral administration. In a 21-day study of dermal toxicity in rats, the NOAEL was 80 mg/kg bw per day based on haematological effects seen at 400 mg/kg bw per day and above.

Bifenazate gave negative results in an adequate range of studies of genotoxicity in vitro and in vivo.

The Meeting concluded that bifenazate is unlikely to be genotoxic.

In long-term studies of toxicity and carcinogenicity in mice and rats, there was no treatment-related neoplasticity. Survival was not affected by treatment with bifenazate in mice and rats. At

100 ppm (equal to 19.7 mg/kg bw per day), decreases in counts for leukocytes and lymphocytes were observed in male mice. In female mice, decreases in body-weight gains were observed at 175 ppm (equal to 35.7 mg/kg bw per day). The NOAEL was 10 ppm (1.5 mg/kg bw per day) in males and 100 ppm (equal to 19.7 mg/kg bw per day) in females. In rats, decreases in body-weight gain, food consumption and haematological parameters were seen at the LOAEL of 200 ppm (equal to 9.7 mg/kg bw per day). The NOAEL for systemic toxicity was 80 ppm (equal to 3.9 mg/kg bw per day). Bifenazate was not carcinogenic in mice or rats.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that bifenazate is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproduction in rats, offspring toxicity or reproductive parameters were not affected at the highest dose tested (200 ppm, equal to 15.3 mg/kg bw per day). The NOAEL for parental systemic toxicity was 20 ppm (equal to 1.5 mg/kg bw per day) on the basis of decreases in body-weight gains. Bifenazate was not embryotoxic, fetotoxic or teratogenic at doses of 500 (the highest dose tested) or 200 mg/kg bw per day (the highest dose tested) in rats and rabbits, respectively.

The Meeting concluded that bifenazate is neither teratogenic nor a reproductive toxicant.

No treatment-related clinical signs of neurotoxicity were observed in the studies that were provided. Therefore, no specific studies of neurotoxicity were necessary.

No studies of toxicity with metabolites of bifenazate were submitted. Parent bifenazate and bifenazate-diazene (D 3593) readily undergo chemical interconversion, so the Meeting considered that these compounds were assessed in the studies with bifenazate. Since other major metabolites of bifenazate are polar glucuronide or sulfate conjugates that are rapidly excreted, the Meeting concluded that these metabolites are likely to be less toxic than bifenazate.

No significant adverse effects were reported in personnel of production plants.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw based on a NOAEL of 40 ppm (equal to 1.0 mg/kg bw per day) for compensatory haematopoiesis, alteration in urine analysis parameters and liver toxicity seen at 400 ppm (equal to 9.0 mg/kg bw per day) and above in a 52-week study in dogs fed bifenazate, and using a 100-fold safety factor.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for bifenazate on the basis of its low acute toxicity, lack of haemolytic effects in a 28-day study of toxicity in mice, the absence of developmental toxicity in rats and rabbits, the lack of clinical signs of neurotoxicity in the database, and the absence of any other toxicological end-point that would be likely to be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	79-week study of toxicity and carcinogenicity[a]	Toxicity	10 ppm, equal to 1.5 mg/kg bw per day	100 ppm, equal to 15.4 mg/kg bw per day
		Carcinogenicity	175 ppm, equal to 35.7 mg/kg bw per day[c]	
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	80 ppm, equal to 3.9 mg/kg bw per day	160 ppm, equal to 9.7 mg/kg bw per day

		Carcinogenicity	160 ppm, equal to 9.7 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	20 ppm, equal to 1.5 mg/kg bw per day	80 ppm, equal to 6.1 mg/kg bw per day
		Offspring toxicity	200 ppm equal to 15.3 mg/kg bw per day	
	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	100 mg/kg bw per day
		Embryo/fetotoxicity	500 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	200 mg/kg bw per day[c]	—
		Embryo-fetotoxicity	200 mg/kg bw per day	—
Dog	90-day study of toxicity[a]	Toxicity	40 ppm, equal to 0.9 mg/kg bw per day	400 ppm, equal to 10.4 mg/kg bw per day
	One-year study of toxicity[a]	Toxicity	40 ppm, equal to 1.0 mg/kg bw per day	400 ppm, equal to 8.9 mg/kg bw per day

[a] Dietary administration
[b] Gavage administration
[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for continued evaluation of the compound

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

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

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Moderate and incomplete; maximum blood concentration reached by 5–6 h; later at higher doses. Approximately 79–85% and 22–29% absorbed within 72 h at 10 and 1000 mg/kg bw, respectively
Distribution	Widely distributed in tissues
Potential for accumulation	No evidence of significant accumulation
Rate and extent of excretion	Approximately 90% (27% in urine and 63% in faeces) within 48 h at 10 mg/kg bw per day
Metabolism in animals	Extensive; metabolic pathways include hydrazine oxidation, demethylation, ring hydroxylation, cleavage of the hydrazine-carboxylic acid portion of the molecule and conjugation with glucuronic acid and sulfate
Toxicologically significant compounds	Bifenazate and bifenazate-diazene (compounds readily interconvert)

Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.4 mg/l dust (4-h exposure, nose only)
Rabbit, skin irritation	Minimal irritation
Rabbit, eye irritation	Minimal irritation
Guinea-pig, skin sensitization	Not a sensitizer (Buehler test) Mild sensitizer (maximization)
Short-term studies of toxicity	
Target/critical effect	Haematopoietic system
Lowest relevant oral NOAEL	1.0 mg/kg bw per day (90-day and one-year study in dogs)
Lowest relevant dermal NOAEL	80 mg/kg bw per day (21-day study in rats)
Lowest relevant inhalation NOAEL	No data
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Haematopoietic system
Lowest relevant NOAEL	1.5 mg/kg bw per day (78-week study in mice)
Carcinogenicity	Not carcinogenic in mice and rats
Reproductive toxicity	
Reproduction target/critical effect	No toxicologically relevant effects
Lowest relevant reproductive NOAEL	15.3 mg/kg bw per day (rats, highest dose tested)
Developmental target/critical effect	No developmental toxicity in rats and rabbits at highest dose tested
Lowest relevant developmental NOAEL	200 mg/kg bw per day (rabbits; highest dose tested)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	No clinical signs observed in available toxicological studies

Medical data	
	No significant adverse health effects reported

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Dog, one-year study of toxicity	100
ARfD	Unnecessary	—	—

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