

FENAMIDONE

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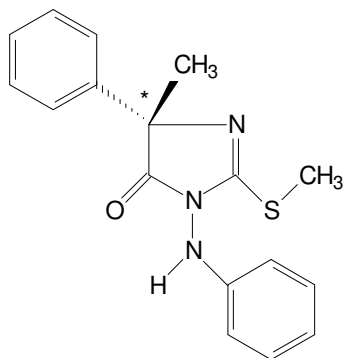
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Explanation

Fenamidone is the International Organization for Standardization–approved common name for (5*S*)-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-3,5-dihydro-4*H*-imidazol-4-one (International Union of Pure and Applied Chemistry [IUPAC]), with Chemical Abstracts Service No. 161326-34-7 (Fig. 1). There is no conversion to the *R*-enantiomer in biological systems. Fenamidone is a foliar fungicide used on vegetables and ornamentals.

Fig. 1. Structure of fenamidone (RPA 407213)



* Denotes the position of the chiral centre

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

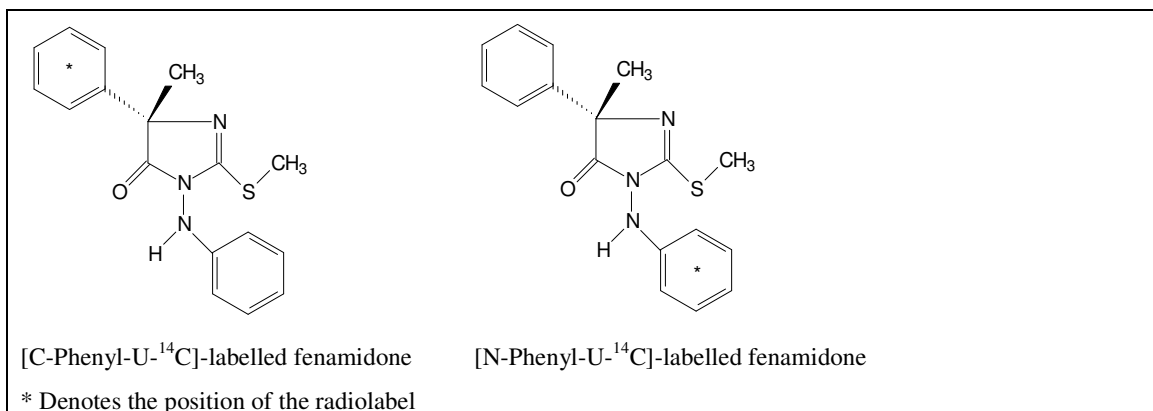
Unless otherwise stated, the contemporary unpublished studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and that complied, where appropriate, with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the European Union or United States Environmental Protection Agency. As these guidelines specify the clinical pathology tests normally performed and the tissues normally examined, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters.

Evaluation for acceptable daily intake

1. Biochemical aspects

Two different radiolabelled forms of [^{14}C]fenamidone were used in the pivotal rat metabolism study: [C-phenyl-U- ^{14}C]- and [N-phenyl-U- ^{14}C]-labelled preparations (Fig. 2).

Fig. 2. Position of the radiolabel in ^{14}C -labelled fenamidone used in rat metabolism studies



1.1 Absorption, distribution and excretion

Rats

Fenamidone was administered to Sprague-Dawley (SD) rats (five of each sex per dose) as a single gavage dose of [C-phenyl- ^{14}C -U]fenamidone (radiochemical purity 99.5%) in aqueous methyl cellulose (0.75% weight per volume [w/v]) at 3 mg/kg body weight (bw), a single gavage dose of radiolabelled fenamidone at 300 mg/kg bw or 14 daily gavage doses of unlabelled fenamidone (purity > 98.9%) at 3 mg/kg bw followed by a single gavage dose of radiolabelled fenamidone at 3 mg/kg bw. Separate groups of rats were dosed similarly with [N-phenyl-U- ^{14}C]fenamidone (98.7% purity) as either a single or repeated gavage dose of 3 mg/kg bw. Urine and faeces were collected at 24-hour intervals to 168 hours. Following termination at 168 hours, a range of tissues was sampled. Radioactivity was quantified in urine, faeces and tissues by liquid scintillation counting. Metabolites were analysed by thin-layer chromatography, high-performance liquid chromatography and liquid chromatography-mass spectrometry.

In a pharmacokinetics experiment, [C-phenyl-U- ^{14}C]fenamidone (radiochemical purity 99.5%) was administered to SD rats (five of each sex per dose) as a single gavage dose of 3 or 300 mg/kg bw. A separate group of five rats received a single gavage dose of [N-phenyl-U- ^{14}C]fenamidone at 3 mg/kg bw. Blood was sampled at 0.5, 1, 2, 3, 4, 5, 6, 8, 24, 48, 72, 96, 120, 144 and 168 hours for the analysis of standard pharmacokinetic parameters.

In a tissue kinetics experiment, [C-phenyl-U-¹⁴C]fenamidone (radiochemical purity 99.5%) was administered to SD rats (16 of each sex per dose) as a single gavage dose of 3 or 300 mg/kg bw. Four rats of each sex per dose were killed at 8, 56, 104 or 200 hours (males) or 24, 94, 168 or 292 hours (females). Radioactivity was analysed in various tissues.

The excretion of radioactivity was analysed in groups of bile duct-cannulated rats (five of each sex) that received a single gavage dose of [C-phenyl-U-¹⁴C]- or [N-phenyl-U-¹⁴C]fenamidone at 3 mg/kg bw. Bile and urine were collected at 0–6, 6–24 and 24–48 hours, whereas faeces were collected at 0–24 and 24–48 hours. Following termination at 48 hours, blood, the intestinal tract and its contents and the stomach and its contents were sampled for radiochemical analysis.

No signs of toxicity were reported.

The results of the excretion/mass balance phase of the study are summarized in Table 1. Mean recoveries of radioactivity across the five dose groups were greater than 95% of the administered dose. The majority of radioactivity was detected in the faeces (up to about 90% of the administered dose of [C-phenyl-U-¹⁴C]fenamidone and up to about 64% with [N-phenyl-U-¹⁴C]fenamidone, with the remainder mainly detected in urine (up to about 40% of the administered dose). Proportionally higher levels of radioactivity were detected in the urine following dosing with [N-phenyl-U-¹⁴C]fenamidone. The majority of radioactivity (> 80%) was eliminated in urine and faeces within 48 hours of dosing. The level of radioactivity remaining in tissues 168 hours after dosing was less than 0.7% of the administered dose. Following administration of [N-phenyl-U-¹⁴C]fenamidone as a single oral or repeated dose, the highest concentrations of radioactivity were detected in blood, liver, kidney, spleen and thyroid (all < 0.11 µg equivalents [eq]/g). In rats dosed with [C-phenyl-U-¹⁴C]fenamidone, the highest concentrations of radioactivity were detected in the thyroid, blood, skin and fur, and liver, with the thyroid having the highest tissue to plasma ratios (approximately 80–500, depending on the dosing regimen) (Table 2).

Plasma kinetic parameters are summarized in Table 3. At the low oral dose, the time ($T_{\max}$) to reach the maximum plasma concentration of radioactivity ($C_{\max}$) was approximately 2–4 hours, whereas it was approximately 14 hours in males and 26 hours in females at the high oral dose, suggesting saturation of binding at the higher dose. The plasma elimination half-life ($t_{1/2}$) was at least 60 hours. At the low oral dose, systemic exposure (as shown by the area under the plasma concentration–time curve [AUC]) was comparable between males and females. However, at the high dose, males were less exposed than females; non-linear kinetics were evident in males, as shown by the less than proportional increase in AUC from 3 to 300 mg/kg bw.

The results of the study on the excretion/mass balance of radioactivity in bile duct-cannulated rats are summarized in Table 4. Recovery of radioactivity was greater than 92%. The majority of radioactivity was detected in bile (approximately 70–80% of the administered dose), followed by urine (approximately 6–17% of the administered dose). Based on the level of radioactivity in urine, bile, cage wash and tissues, the level of gastrointestinal absorption is estimated to be greater than 80% of the administered dose (range of 81–96%). Comparison of the levels of radioactivity recovered in bile kinetics and absorption, distribution, metabolism and excretion (ADME) studies suggested that a part of the radioactivity excreted via the bile underwent enterohepatic circulation and was subsequently re-excreted via the urine.

In the tissue kinetics experiment, the $T_{\max}$ was approximately the same time as that for plasma. The highest levels of radioactivity were detected in the gastrointestinal tract, stomach, thyroid, liver, pancreas and blood (Totis, 1999).

1.2 Biotransformation

Rats

In the above-described study, the analysis of metabolites in pooled urine, faeces and bile detected up to 22, 24 and 22 radioactive species, respectively, indicating that fenamidone was extensively metabolized. The major metabolites (> 10% of the administered dose) of fenamidone were free RPA 409352, RPA 409361, RPA 412708 and RPA 412636 (Table 5). In rats receiving the

low oral dose, conjugated metabolites of RPA 409352 or RPA 409361 were detected in urine (2.58% and 14.40% of the administered dose in males and females, respectively) and bile (45.67% and 54.56% of the administered dose in males and females, respectively). A sulfo conjugate of RPA 408056 was detected in bile (1.99% and 3.10% of the administered dose in males and females, respectively). An examination of the chirality of selected metabolites by the author found no interconversion from the *S*- to *R*-enantiomer.

Table 1. Mass balance in rats following oral dosing with [C-phenyl-U-¹⁴C]- or [N-phenyl-U-¹⁴C]fenamidone

Sample	Mean % of administered radioactivity					
	3 mg/kg bw (single dose)		300 mg/kg bw (single dose)		3 mg/kg bw (repeated dose)	
	Males	Females	Males	Females	Males	Females
[C-phenyl-U-¹⁴C]Fenamidone						
<i>Urine</i>						
0–24 h	6.74	27.45	4.65	5.66	6.34	23.17
0–48 h	10.48	36.21	8.65	10.86	9.10	28.66
0–72 h	11.94	38.64	10.02	12.39	10.53	30.44
0–168 h	12.79	39.94	10.56	13.01	11.39	31.31
<i>Faeces</i>						
0–24 h	47.84	30.78	54.54	72.00	32.92	44.52
0–48 h	69.90	47.33	76.53	87.95	72.32	56.91
0–72 h	77.70	50.94	82.38	90.39	81.35	59.58
0–168 h	80.69	52.11	83.65	90.99	84.68	60.45
<i>Cage wash</i>	2.35	5.68	2.53	2.47	3.98	10.49
<i>Tissues</i>	0.65	0.45	0.29	0.27	0.54	0.52
<i>Recovery</i>	96.47	98.27	97.02	106.74	100.59	102.76
[N-phenyl-U-¹⁴C]Fenamidone						
<i>Urine</i>						
0–24 h	15.99	26.47	–	–	25.91	33.04
0–48 h	23.48	36.74	–	–	35.48	41.56
0–72 h	25.57	39.28	–	–	38.72	44.73
0–168 h	26.59	40.45	–	–	40.58	46.49
<i>Faeces</i>						
0–24 h	41.56	32.53	–	–	13.82	28.94
0–48 h	57.57	44.80	–	–	31.94	39.45
0–72 h	63.08	47.85	–	–	38.87	41.68
0–168 h	64.28	49.55	–	–	52.03	44.71
<i>Cage wash</i>	4.09	5.44	–	–	4.68	7.82
<i>Tissues</i>	0.53	0.62	–	–	0.66	0.65
<i>Recovery</i>	95.49	96.05	–	–	97.95	99.66

Source: Totis (1999)

Table 2. Tissue to plasma ratios of radioactivity following oral dosing with [C-phenyl-U-¹⁴C]- or [N-phenyl-U-¹⁴C]fenamidone

Sample	Tissue : plasma ratio					
	3 mg/kg bw (single dose)		300 mg/kg bw (single dose)		3 mg/kg bw (repeated dose)	
	Males	Females	Males	Females	Males	Females
[C-phenyl-U-¹⁴C]Fenamidone						
Liver	8.34	8.03	7.20	4.67	6.92	10.94
Kidneys	3.97	2.50	2.96	2.32	3.90	3.20
Lungs	2.57	2.89	3.64	4.66	2.75	4.04
Blood	7.61	10.62	11.74	13.95	7.94	9.73
Skin and fur	6.65	3.99	4.02	3.42	3.17	4.86
Thyroid	494.01	394.79	105.29	83.51	523.51	467.88
[N-phenyl-U-¹⁴C]Fenamidone						
Liver	29.30	27.38	–	–	14.14	15.02
Kidneys	15.90	9.95	–	–	11.09	6.37
Heart	6.44	5.73	–	–	3.33	3.19
Lungs	6.78	10.02	–	–	3.94	5.21
Spleen	10.44	13.07	–	–	6.49	11.34
Blood	34.94	38.78	–	–	14.61	18.18
Skin and fur	7.17	11.73	–	–	3.39	6.00
Thyroid	NA	16.47	–	–	10.71	15.24

NA: not available

Source: Totis (1999)

Table 3. Kinetic parameters (expressed as means) in rats following a single oral dose of [C-phenyl-U-¹⁴C]- or [N-phenyl-U-¹⁴C]fenamidone

Parameter	3 mg/kg bw		300 mg/kg bw	
	Males	Females	Males	Females
[C-phenyl-U-¹⁴C]Fenamidone				
$C_{\max}$ (µg eq/g)	0.29	0.31	12.16	17.70
$T_{\max}$ (min)	4.29	3.71	14.64	25.68
$t_{1/2}$ (h)	61.5	72.8	72	84
$AUC_{(0-n)}$ (µg eq·h/g)	12.79	16.61	775.66	1 674.82
[N-phenyl-U-¹⁴C]Fenamidone				
$C_{\max}$ (µg eq/g)	0.34	0.31	–	–
$T_{\max}$ (min)	3.02	2.63	–	–
$t_{1/2}$ (h)	109.24	129.60	–	–
$AUC_{(0-n)}$ (µg eq·h/g)	15.37	17.59	–	–

AUC: area under the plasma concentration–time curve; $C_{\max}$: maximum concentration in plasma; $t_{1/2}$: half-life;

$T_{\max}$: time to reach $C_{\max}$

Source: Totis (1999)

Table 4. Mass balance of radioactivity in bile duct-cannulated rats following a single oral dose (3 mg/kg bw) of [C-phenyl-U-¹⁴C]- or [N-phenyl-U-¹⁴C]fenamidone

Sample	Mean % of administered radioactivity			
	[C-phenyl-U- ¹⁴ C]Fenamidone		[N-phenyl-U- ¹⁴ C]Fenamidone	
	Males	Females	Males	Females
Urine				
0–6 h	4.39	4.46	3.18	7.04
0–24 h	9.91	10.75	6.07	15.76
0–48 h	10.67	11.69	6.33	17.62
Faeces				
0–24 h	2.73	0.21	2.46	0.11
0–48 h	4.11	1.08	4.46	1.36
Bile				
0–6 h	70.71	49.64	76.76	35.95
0–24 h	79.44	68.05	83.15	61.01
0–48 h	79.72	72.62	83.41	71.30
Cage wash	1.13	1.90	0.92	1.50
Tissues	0.68	5.37	1.01	5.43
Total	96.31	92.66	96.13	97.21

Source: Totis (1999)

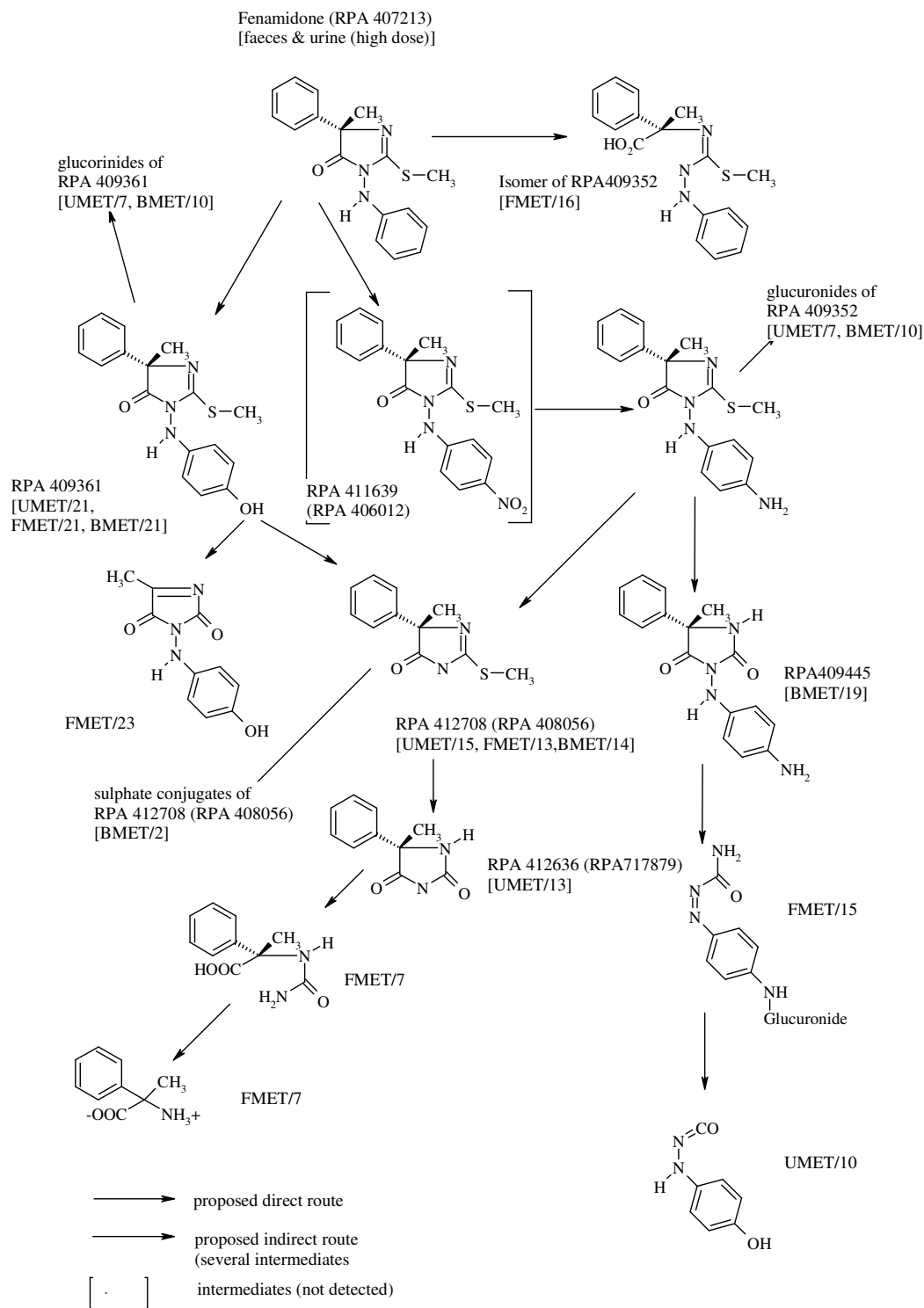
Table 5. Main fenamidone metabolites detected in rat excreta

Metabolite	% of administered dose					
	3 mg/kg bw (single dose)		300 mg/kg bw (single dose)		3 mg/kg bw (repeated dose)	
	Males	Females	Males	Females	Males	Females
RPA 407213: Fenamidone						
Urine	–	–	0.13	0.25	–	–
Faeces	–	–	49.92	67.78	–	–
Bile	–	–	–	–	–	–
RPA 409352: 3-(4-Aminophenylamino)-5-methyl-2-methylthio-5-phenyl-3,5-dihydroimidazol-4-one						
Urine	0.24	1.91	0	0	0.21	1.32
Faeces	7.20	4.19	5.55	7.53	3.44	5.69
Bile	0.18	0	–	–	–	–
RPA 409361: 1-(4-Hydroxyphenylamino)-4-methyl-2-methylthio-4-phenylimidazolin-5-one						
Urine	0.64	4.76	0.52	3.06	0.47	2.17
Faeces	7.67	12.88	6.18	6.90	5.41	14.87
Bile	0.47	0.24	–	–	–	–
RPA 412708: (5S)-5-Methyl-2-(methylthio)-5-phenyl-3,5-dihydro-4H-imidazol-4-one						
Urine	0.05	0.25	0.10	0.09	0.27	0.34
Faeces	10.47	8.40	1.99	0.84	10.33	13.01
Bile	15.34	2.10	–	–	–	–
RPA 412636: (S)-5-Methyl-5-phenyl-2,4-imidazolidine-dione						
Urine	0.53	2.94	0.27	0.84	0.70	0.75
Faeces	0	0	0	0	0	0
Bile	0	0	–	–	–	–

Source: Totis (1999)

The proposed metabolic pathway for fenamidone in rats is given in Fig. 3 (Totis, 1999).

Fig. 3. Proposed metabolic pathway of [^{14} C]fenamidone in rats



In a subsequent discussion on the formation of the metabolite designated RPA 409352, Totis (2000) proposed that fenamidone was metabolized by gut bacteria to the 4-nitrophenyl metabolite RPA 406012, which was rapidly reduced to the amide derivative RPA 409352, which is then reabsorbed and re-excreted via the bile. This is based on the observation that free RPA 409352 is predominantly detected in faeces, with the conjugated form detected in bile.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity tests on fenamidone in rats are summarized in Table 6. Clinical signs observed for up to 2 days after an acute oral dose greater than or equal to 1000 mg/kg bw included reduced motor activity, hunched posture, staggering gait, prostration, bradypnoea and piloerection (Dange & Foulon, 1997). No clinical signs of toxicity were observed following an acute dermal exposure to 2000 mg/kg bw lasting 24 hours (Dange, 1997). Clinical signs observed for up to 2 days after an acute inhalational exposure (4 hours, nose only) included lethargy, uncoordinated limb movements, wet fur and brown staining around the snout, jaw, body and eyes (Coombs, Horrell & Bannerman, 1998). Additional clinical signs observed in females included gasping and noisy respiration. All treated rats lost body weight over the 1st day after exposure.

Table 6. Results of studies of acute toxicity on fenamidone

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw) or LC ₅₀ (mg/L)	Reference
Rat	SD Ico:OFA-SD (IOPS Caw)	Male and female	Oral	99.8	0.5% (w/v) aqueous CMC	> 5 000 (males) 2 028 (females)	Dange & Foulon (1997)
Rat	SD Ico:OFA-SD (IOPS Caw)	Male and female	Dermal	99.8	Powder moistened in 0.9% (w/v) saline	> 2 000 (24 h exposure)	Dange (1997)
Rat	SD	Male and female	Inhalation MMAD = 2.5 µm	99.8	Acetone	> 2.1 mg/L (4 h, nose-only exposure)	Coombs, Horrell & Bannerman (1998)

CMC: carboxymethyl cellulose; LC₅₀: median lethal concentration; LD₅₀: median lethal dose; MMAD: mass median aerodynamic diameter; SD: Sprague-Dawley; w/v: weight per volume

(b) Dermal and ocular irritation

The results of skin and eye irritation tests on fenamidone conducted in rabbits are summarized in Table 7.

Table 7. Results of studies of dermal and ocular irritation on fenamidone in rabbits

Strain	Sex	Purity (%)	Application site	Exposure period	Result	Reference
NZW	Male	99.8	6 cm ² , non-abraded skin	4 h, semi-occluded	Not a skin irritant	Chuzel (1997a)
NZW	Male	99.8	Left eye, right eye control	Eye unwashed for up to 72 h	Not an eye irritant	Chuzel (1997b)

NZW: New Zealand White

(c) *Dermal sensitization*

Fenamidone (purity 99.8%) was analysed for its skin sensitization potential in the guinea-pig maximization test. Fifteen male and 15 female guinea-pigs (Dunkin Hartley strain) were included in the test group, and five of each sex were included in the control group. In the induction phase (day 1), animals were injected intradermally on either side of the dorsal midline with 1% (w/v) fenamidone in paraffin oil and Freund's complete adjuvant (1 : 1). On day 7, the same region received a topical application of sodium lauryl sulfate in petrolatum (10% weight per weight [w/w]) in order to induce local irritation. On day 8, this same test site was treated by topical application of undiluted fenamidone or the vehicle (control group) and was covered by an occlusive dressing for 48 h. On day 22, all animals were challenged by a topical application of undiluted fenamidone under an occlusive dressing, with the left flank serving as the vehicle control. No skin reactions were observed at 24 and 48 hours. On the basis of these findings, fenamidone is not a skin sensitizer (de Jouffrey, 1997).

2.2 *Short-term studies of toxicity*

Mice

A 14-day range-finding study was conducted in groups of CD-1 mice (five of each sex) that were gavaged with fenamidone (purity 99.9%) in 0.5% (w/v) carboxymethyl cellulose at a dose of 0, 10, 100, 300 or 1000 mg/kg bw per day. There were no deaths or treatment-related clinical signs. There was no treatment-related effect on body weight, feed consumption or clinical chemistry parameters. The absolute liver weight of males at 300 and 1000 mg/kg bw per day was 23% ($P < 0.05$) and 29% ($P < 0.01$) higher than the control values, respectively. The absolute liver weight of high-dose females was 45% higher ($P < 0.01$) than the control value. Relative liver weight was approximately 18% and 30% higher than the control values ($P < 0.05$) in high-dose males and females, respectively. At necropsy, mild liver enlargement was observed in one high-dose female, with a pale-yellowish discoloration (graded as moderate) observed in 0/5, 0/5, 1/5, 0/5 and 2/5 males and 0/5, 0/5, 0/5, 2/5 and 1/5 females at 0, 30, 100, 300 and 1000 mg/kg bw per day, respectively. Histopathological examination revealed centrilobular hypertrophy (graded as slight to mild) in three males each at 300 and 1000 mg/kg bw per day and in three females at 1000 mg/kg bw per day (Dange, 1994).

In a non-guideline study, fenamidone (purity 98.4%) was admixed in the diet at a concentration of 0, 70, 700 or 7000 ppm and fed ad libitum to groups of 10 CD-1 mice of each sex for 90 days. The achieved doses were 0, 11.4, 110 and 1102 mg/kg bw per day for males and 0, 14.5, 146 and 1468 mg/kg bw per day for females at 0, 70, 700 and 7000 ppm, respectively. Observations for mortalities and clinical signs were made daily. Body weight and feed consumption were recorded weekly. Blood was sampled on the last day of dosing for the analysis of limited haematology and clinical chemistry parameters. Following termination, all mice were necropsied, organs weighed and selected tissues examined histopathologically.

Two high-dose males were killed in a moribund condition on days 12 and 41, with a further two found dead on days 51 and 53. There were no other deaths. Clinical signs were observed in five high-dose males (including the four that died or were killed) and included pallor, reduced motor activity, staggering gait, prostration and dyspnoea. No treatment-related clinical signs were observed in females. There was no treatment-related effect on body weight gain or feed consumption. Haematology parameters were unremarkable. Mean total bilirubin was significantly higher ($P < 0.05$) than the control values in females at 700 and 7000 ppm (Table 8). In males, total bilirubin was elevated across all treated groups, but the differences were not statistically significant and did not follow a dose-response relationship. Given the inconsistent findings between males and females and that similar increases in total bilirubin did not occur in the preceding 14-day study by Dange (1994) at comparable doses, the significant increase in total bilirubin in females is not clearly treatment related.

At the highest dose (both sexes), absolute and relative liver weights were significantly higher ($P < 0.01$ or 0.05) than the control values (21% and 15%, respectively, in males and 14% and 18%,

respectively, in females) (Table 8). In the four high-dose male decedents, pale livers (four) and hepatomegaly (two) were observed macroscopically, with these findings also observed across the majority of groups following scheduled termination (Table 8). However, in the absence of a clear dose–response relationship and the occurrence of a relatively high background incidence in the control groups, these macroscopic findings (in survivors) cannot definitively be attributed to treatment. Histopathology revealed centrilobular hypertrophy of hepatocytes (graded as mild to moderate) in all treated groups of males and in females at 700 and 7000 ppm (Table 8). The occurrence of liver hypertrophy without accompanying evidence of hepatotoxicity is considered to be an adaptive response to treatment.

Table 8. Liver findings in mice following 13 weeks of dietary exposure to fenamidone

Parameter	0 ppm		70 ppm		700 ppm		7 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Clinical chemistry^a								
Total bilirubin (µmol/L)	1.4	1.2	2.1	1.1	2.0	2.1*	2.1	2.2*
Liver weights^a								
Absolute (g)	1.4	1.4	1.5	1.3	1.5	1.3	1.7*	1.6*
Relative ^b (%)	3.9	4.4	4.1	4.2	4.1	4.5	4.5**	5.2**
Macroscopic liver findings^b								
<i>N</i>	10	10	10	10	10	10	6	10
Enlargement	0	4	1	6	1	7	2	4
Pale colour	4	2	0	8	1	5	4	7
Histopathological liver findings^c								
Centrilobular hypertrophy	0	0	2	0	3	4	5	6

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

^a Results expressed as the mean.

^b Relative to body weight.

^c Results expressed as the absolute number of mice with the finding.

Source: Berthe (1995)

The no-observed-adverse-effect level (NOAEL) was 700 ppm (equal to 110 mg/kg bw per day), based on clinical signs and deaths in males at 7000 ppm (equal to 1102 mg/kg bw per day) (Berthe, 1995).

In a subsequent 90-day study, fenamidone (purity 98.9%) was admixed in the diet at a concentration of 0, 50, 200, 1000 or 5000 ppm and fed ad libitum to groups of 10 C57 Black 10J mice of each sex. The mean achieved doses were 0, 11.3, 44.5, 220 and 1064 mg/kg bw per day for males and 0, 13.7, 54, 274 and 1375 mg/kg bw per day for females at 0, 50, 200, 1000 and 5000 ppm, respectively. Observations for mortalities and clinical signs were made daily. Body weight and feed consumption were recorded weekly. Blood was sampled 1 day prior to study termination for the analysis of limited clinical chemistry parameters. Following termination, all mice were necropsied, organs weighed and selected tissues examined histopathologically.

There were no treatment-related deaths, clinical signs or effects on body weight gain or feed consumption. In females, plasma cholesterol was significantly lower than the control value (0.88 mmol/L) at 1000 ppm (0.63 mmol/L; $P < 0.05$) and 5000 ppm (0.47 mmol/L; $P < 0.01$). Selected

organ weight, macroscopic and histopathological findings are summarized in Table 9. At the highest dose, absolute and relative liver weights of males were approximately 15% higher than the control values ($P < 0.01$ or 0.05). In high-dose females, only relative liver weight was significantly ($P < 0.05$) higher than the control value (by about 12%). At necropsy, there was a slight increase in the incidence of pale liver in high-dose males; however, the background incidence in the control was relatively high, and the dose–response relationship was limited. Histopathological examination indicated a slight increase in hepatocellular microvacuolation in males, but again there was a high background occurrence in the control group.

Table 9. Liver findings in mice following 90 days of dietary exposure to fenamidone

Parameter	0 ppm		50 ppm		200 ppm		1 000 ppm		5 000 ppm	
	M	F	M	F	M	F	M	F	M	F
Liver weight^a										
Absolute (g)	1.02	0.87	1.04	0.80	1.04	0.87	1.09	0.91	1.16**	0.95
Relative ^b (%)	4.22	4.22	4.24	4.03	4.36	4.29	4.43	4.41	4.85*	4.72*
Macroscopic liver findings^c										
<i>N</i>	8	10	10	10	9	10	10	10	10	9
Pale appearance	3	0	3	2	5	3	5	2	7	2
Histopathological liver findings^c										
Hepatocellular microvacuolation	4	1	5	1	6	2	8	5	8	2

F: female; M: male; *: $P < 0.05$; **: $P < 0.01$

^a Results expressed as the mean.

^b Relative to body weight.

^c Results expressed as the absolute number of mice with the finding.

Source: Bigot & Tassel (1997)

The NOAEL was 1000 ppm (equal to 220 mg/kg bw per day) for equivocal histopathological findings in the liver at 5000 ppm (equal to 1064 mg/kg bw per day) (Bigot & Tassel, 1997).

Rats

In a 14-day range-finding study, groups of SD rats (five of each sex) were administered fenamidone (purity 99.9%) in 0.5% (w/v) carboxymethyl cellulose by gavage at a dose of 0, 100, 300 or 1000 mg/kg bw per day (males) or 0, 30, 100 or 300 mg/kg bw per day (females). The dose selection was based on differences in acute toxicity between males and females. Satellite groups of three rats of each sex were added to the high-dose and control groups for the analysis of hepatic cellular proliferation and liver histology after 3 days of treatment. Rats were observed daily for mortality and clinical signs. Body weight was recorded on days 1, 7 and 14, with feed consumption recorded weekly. Blood was sampled prior to terminal sacrifice for the analysis of haematology and clinical chemistry parameters. All rats were necropsied, and various organs were weighed and examined histopathologically.

There were no deaths or treatment-related clinical signs. Body weight, feed consumption and haematology parameters were unaffected by treatment. In high-dose males, significantly elevated total bilirubin (approximately 78%, $P < 0.01$), total protein (approximately 6%, $P < 0.05$) and cholesterol (approximately 31%, $P < 0.05$) were determined. In high-dose male rats killed after 3 days, absolute and relative liver weights were approximately 22% higher ($P < 0.01$) than the control values. At terminal sacrifice, a 31% increase ($P < 0.01$) in relative liver weight was noted in high-dose males. In high-dose females killed after 3 days, relative liver weight was about 15% higher ($P < 0.05$) than the

control value. No macroscopic abnormalities were observed at the interim sacrifice, whereas liver enlargement was noted in 0/5, 1/5, 1/5 and 3/5 males at 0, 100, 300 and 1000 mg/kg bw per day, respectively, in rats killed after 14 days. Centrilobular hepatocytic hypertrophy was observed histopathologically in 2/3 high-dose males killed after 3 days and in 3/5 high-dose males killed after 14 days. Thyroid follicular cell hyperplasia occurred in 2/5 and 3/5 female rats at 100 and 300 mg/kg bw per day, respectively.

Analysis of the cytochrome P450 isozyme profile by western blotting revealed a dose-related increase in isoform IIB, which was not detected in controls or low-dose females. Benzoxyresorufin *O*-debenzylation (BROD) was increased by 2.7-, 8.5- and 17.1-fold in males and 1.3-, 4.9- and 25.4-fold in females at the low, middle and high doses, respectively. In comparison, treatment with 75 mg/kg bw phenobarbital resulted in increases of 127.4- and 31.2-fold in males and females, respectively. Pentoxyresorufin *O*-depropylation (PROD) was increased by 1.4-, 2.8- and 4.5-fold in males and 1.1-, 4.3- and 15.7-fold in females at the low, middle and high-doses, respectively. Treatment with 75 mg/kg bw phenobarbital resulted in increases of 89.7- and 37.9-fold in males and females, respectively. There was no treatment-related effect on ethoxyresorufin *O*-deethylation (EROD).

In rats killed after 3 days, the numbers of proliferating cell nuclear antigen (PCNA) positive cells in the liver were increased by about 2.9-fold in males and 22.5-fold in females at 100 and 300 mg/kg bw per day, respectively. In rats killed at the end of the study, the number of PCNA positive cells was increased by 2.2- and 4.1-fold in high-dose males and females, respectively (Dange, 1995a).

Fenamidone (purity > 99%) was admixed in the diet at a concentration of 0, 500, 5000 or 15 000 ppm and fed ad libitum to groups of 10 SD rats of each sex for 28 days. The achieved doses were 0, 39, 389 and 1203 mg/kg bw per day for males and 0, 42, 405 and 1194 mg/kg bw per day for females at 0, 500, 5000 and 15 000 ppm, respectively. Rats were observed daily for deaths and clinical signs. Body weight and feed consumption were recorded weekly. Ophthalmoscopy was performed pretreatment and during weeks 2 and 4 of treatment. Blood and urine were collected at the end of the study for the analysis of standard haematology, clinical chemistry or urine analysis parameters. Following sacrifice, rats were necropsied, and organs were weighed and examined histopathologically.

There were no treatment-related deaths or clinical signs. Ophthalmoscopy was unremarkable. In males, mean body weight gain was significantly lower ($P < 0.01$ or 0.05) than the control values at 5000 ppm (days 0–8 and 15–22) and 15 000 ppm (days 0–8), with no significant intergroup differences in females (Table 10). Feed consumption was significantly lower ($P < 0.01$ or 0.05) than the control values in males at every dose and in females at 5000 and 15 000 ppm (Table 10).

Selected haematology, clinical chemistry, organ weight, and macroscopic and histopathological findings are also summarized in Table 10. There were no treatment-related urine analysis findings. At 5000 ppm (males) and 15 000 ppm (both sexes), red cell parameters were significantly lower ($P < 0.01$) than the control values. At 5000 and 15 000 ppm, significantly reduced plasma glucose (both sexes; $P < 0.01$ or 0.05) and increased cholesterol (males; $P < 0.01$) occurred. Additional clinical chemistry findings in high-dose males included reduced urea and increased total bilirubin ($P < 0.01$ or 0.05).

There were a number of organ weights that were significantly different from the control values at and above 5000 ppm (Table 10), but only the increases in liver and spleen weights correlated with increases in their respective relative weights. In addition, both of these organs had treatment-related macroscopic and/or histopathological abnormalities. At necropsy, enlargement and a dark abnormal colour of the spleen were noted at 5000 ppm (males) and 15 000 ppm (both sexes), with mild to marked hyperplasia of the germinative follicle of the white pulp observed microscopically in both sexes at these same dietary concentrations. There were no macroscopic liver abnormalities, whereas histopathology revealed mild to moderate diffuse hepatocellular hypertrophy at 5000 and 15 000 ppm in both sexes. The only other treatment-related histopathological finding was an increase

Table 10. Treatment-related findings in rats following 28 days of dietary exposure to fenamidone^a

Parameter	0 ppm		500 ppm		5 000 ppm		15 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight gain (g)								
Days 0–8	7.62	2.88	7.30	2.57	5.11**	2.41	–0.07**	1.90
Days 8–15	6.42	2.47	4.67	2.92	5.20	2.20	5.62	2.30
Days 15–22	5.27	1.30	5.17	2.45	3.92*	2.55	4.35	1.91
Days 22–28	2.94	1.30	2.26	0.33	2.03	0.55	1.33	0.86
Feed consumption (g/day)								
Days 0–8	28.5	20.9	27.2	20.4	25.7**	18.8*	19.3**	17.1**
Days 8–15	28.1	21.9	26.9	21.1	26.5	20.1	26.3	20.2
Days 15–22	28.6	21.0	26.3*	20.8	24.8**	20.1	25.9*	19.1
Days 22–28	26.5	18.4	24.2*	18.1	23.3**	17.7	21.8**	17.3
Haematology								
RBC ($\times 10^{12}$)	8.53	8.32	8.61	8.46	8.53	8.34	7.76**	7.62**
Hb (g/100 mL)	15.9	15.3	15.7	15.6	14.8**	15.4	14.6**	14.2**
Hct (%)	47.8	45.9	47.1	46.7	45.2**	46.5	43.9**	43.1**
Clinical chemistry								
Glucose (mmol/L)	8.34	7.79	8.48	7.33	7.02*	6.56**	5.93**	6.33**
Urea (mmol/L)	4.29	4.56	4.36	4.56	4.08	4.91	3.42*	4.52
Cholesterol (mmol/L)	0.69	0.74	0.75	0.81	1.06**	0.86	1.53**	0.86
Bilirubin (μ mol/L)	1.7	2.1	1.8	1.8	2.2	2.0	2.6**	1.7
Organ weights								
<i>Brain</i>								
Absolute (g)	1.96	1.83	1.94	1.89	1.89	1.84	1.85*	1.81
Relative ^b (%)	0.52	0.77	0.54	0.77	0.57**	0.79	0.62**	0.79
<i>Heart</i>								
Absolute (g)	1.27	0.88	1.18	0.87	1.13*	0.84	1.05**	0.85
Relative (%)	0.33	0.37	0.33	0.35	0.33	0.36	0.35	0.37
<i>Liver</i>								
Absolute (g)	10	6.6	10	6.8	11.1*	7.4**	11.4**	7.9**
Relative (%)	2.6	2.7	2.8	2.8	3.3**	3.2**	3.8**	3.4**
<i>Pituitary</i>								
Absolute (g)	0.011	0.011	0.011	0.012	0.009	0.010	0.009*	0.010
Relative (%)	0.002	0.004	0.003	0.005	0.002	0.004	0.003	0.004
<i>Spleen</i>								
Absolute (g)	0.80	0.57	0.79	0.62	0.84	0.61	1.09**	0.67
Relative (%)	0.21	0.24	0.22	0.25	0.25	0.26	0.36**	0.29**
<i>Kidney</i>								
Absolute (g)	2.92	1.87	2.87	2.10	2.62	2.00	2.43**	1.96
Relative (%)	0.78	0.78	0.80	0.86	0.78	0.85	0.81	0.86

Table 10 (continued)

Parameter	0 ppm		500 ppm		5 000 ppm		15 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
<i>Epididymis</i>								
Absolute (g)	1.20	–	1.11	–	1.10*	–	1.06**	–
Relative (%)	0.32	–	0.31	–	0.32	–	0.35	–
<i>Prostate</i>								
Absolute (g)	0.58	–	0.51	–	0.44**	–	0.41**	–
Relative (%)	0.15	–	0.14	–	0.13	–	0.13	–
Macroscopic findings^c								
<i>Spleen</i>								
Enlarged	0/9	0/10	0/10	0/10	2/10	0/10	6/10	3/10
Dark abnormal colour	0/9	0/10	0/10	1/10	3/10	1/10	9/10	5/10
Microscopic findings^c								
Liver: Diffuse hypertrophy	0/9	0/10	0/10	0/10	6/10	5/9	10/10	10/10
Spleen: Follicular hyperplasia	0/9	0/10	0/10	0/10	10/10	2/9	9/10	5/10
Pituitary gland: Increased chromophobe cells	0/9	0/10	5/10	0/10	7/10	0/9	7/10	0/10

Hb: haemoglobin; Hct: haematocrit; RBC: red blood cells; *: $P < 0.05$; **: $P < 0.01$

^a Results expressed as the mean unless otherwise specified.

^b Relative to body weight.

^c Absolute number of rats with the finding.

Source: Dange (1995b)

in the number of chromophobe cells in males only at every dietary concentration; the toxicological significance of this finding is unclear.

The NOAEL was 500 ppm (equal to 39 mg/kg bw per day), based on decreased red cell parameters and increased spleen weights coincident with hyperplasia of the germinative follicle of the white pulp at and above 5000 ppm (equal to 389 mg/kg bw per day) (Dange, 1995b).

In a second 28-day study, fenamidone (purity > 99%) was admixed in the diet at a concentration of 0, 60, 150, 1000 or 5000 ppm and fed ad libitum to groups of 10 SD rats of each sex. The achieved doses were 0, 4.9, 12.3, 82.7 and 418.5 mg/kg bw per day for males and 0, 5.3, 13.9, 90.6 and 450.1 mg/kg bw per day for females at 0, 60, 150, 1000 and 5000 ppm, respectively. Observations for mortalities and clinical signs were made daily. Body weight and feed consumption were recorded weekly. Blood was sampled during weeks 1, 2 and 4 for the analysis of thyroid stimulating hormone (TSH), triiodothyronine (T_3) and thyroxine (T_4). No haematology, clinical chemistry or urine analysis parameters were analysed. At the end of the study, all rats were necropsied; the brain, liver and thyroid were weighed and examined histopathologically.

There were no treatment-related mortalities or clinical signs. There were no significant intergroup differences in body weight, body weight gain or feed consumption. There was no treatment-related effect on TSH, T_3 or T_4 . Absolute and relative liver weights were significantly higher ($P < 0.01$) than the control values in high-dose males (29% and 39%, respectively) and females

(21% and 28%, respectively) (Table 11). There were no significant intergroup differences in thyroid weights. At necropsy, mild to marked liver enlargement was noted predominantly at the highest dose, whereas histopathology revealed slight to moderate diffuse hypertrophy of hepatocytes at 1000 ppm (males) and 5000 ppm (both sexes) (Table 11). The increase in liver weight and hypertrophy were considered to be an adaptive response to treatment. In high-dose males, slight to mild thyroid follicular cell hypertrophy was also observed (Table 11).

Table 11. Liver and thyroid findings in rats exposed to fenamidone in the diet for 28 days^a

Parameter	0 ppm		60 ppm		150 ppm		1 000 ppm		5 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Organ weights										
<i>Liver</i>										
Absolute (g)	8.13	5.48	8.20	5.37	8.77	5.70	8.67	5.66	10.53**	6.61**
Relative ^b (%)	2.71	2.84	2.72	2.82	2.85	3.02	2.92	2.93	3.77**	3.63**
Macroscopic findings^c										
Enlarged liver	1	0	1	0	2	1	3	0	7	3
Microscopic findings^c										
Liver: Diffuse hypertrophy	0	0	0	0	0	0	4	0	10	4
Thyroid: Follicular cell hypertrophy	0	0	0	0	0	0	0	0	3	0

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

^a Results expressed as the mean unless otherwise specified.

^b Relative to body weight.

^c Absolute number of rats with the finding ($n = 10$).

Source: Dange (1999a)

The NOAEL was 1000 ppm (equal to 82.7 mg/kg bw per day) for follicular cell hypertrophy in males at 5000 ppm (equal to 418.5 mg/kg bw per day) (Dange, 1999a).

Fenamidone (purity 98.4%) was admixed in the diet at a concentration of 0, 50, 150, 500 or 5000 ppm and fed ad libitum to groups of 10 SD rats of each sex for up to 90 days. The achieved doses were 0, 3, 9, 30 and 305 mg/kg bw per day for males and 0, 3, 11, 35 and 337 mg/kg bw per day for females at 0, 50, 150, 500 and 5000 ppm, respectively. Clinical signs were recorded daily. Body weight and feed consumption were recorded weekly. An ophthalmological examination was performed on control and high-dose rats prior to the commencement of dosing and then during week 13. Blood was collected 1 week prior to terminal sacrifice for the analysis of standard haematology and clinical chemistry parameters. Urine was collected overnight prior to necropsy for urine analysis. Selected organs were weighed and tissues examined histopathologically.

There were no deaths or treatment-related clinical signs. At the highest dose, mean absolute body weight was approximately 10% lower ($P < 0.01$ or 0.05) than the control values throughout the exposure period in males and from approximately week 9 in females. Body weight gain was also reduced at the highest dose throughout the study (by up to about 10% in both sexes), reaching

statistical significance in males over days 1–8 ($P < 0.01$), 78–84 ($P < 0.01$) and 84–90 ($P < 0.05$) and in females over days 1–8 ($P < 0.05$) and 78–84 ($P < 0.05$). Feed consumption (week 1 in males, approximately 20% lower; weeks 1, 3, 8, 9, 10 and 12 in females, approximately 10–20% lower) was also significantly lower ($P < 0.01$ or 0.05) than the control values. There were no treatment-related ophthalmological abnormalities. At the highest dose, red blood cells and haemoglobin were significantly lower than the control values (red blood cells: 6% [$P < 0.05$] and 8% [$P < 0.01$] lower in males and females, respectively; haemoglobin: 6% and 7% lower [$P < 0.01$] in males and females, respectively). Also at the highest dose, blood glucose was approximately 20% lower ($P < 0.05$) than the control values in both sexes.

At the highest dose, a range of absolute and/or relative organ weights were significantly ($P < 0.01$ or 0.05) different from the control values. In males, absolute and relative thymus weights were approximately 34% and 26% lower than the control values, respectively; similar differences did not occur in females, and there was no corroborating histopathology. Absolute (males) and relative (both sexes) thyroid weights were approximately 37% and 50% higher than the control values, but there were also no corroborating microscopic findings. The author stated that all organ weights were within the normal range for age- and sex-matched SD rats.

There were no treatment-related macroscopic findings. Histopathology revealed a slight increase in the incidence of bile duct hyperplasia in high-dose males (5/10 versus 1/10 in the control; graded as slight) and prominent germinal centres of the white pulp of the spleen, also in high-dose males (8/10 versus 2/10 in the control). However, these findings were considered by the author to represent normal background findings.

The NOAEL was 500 ppm (equal to 30 mg/kg bw per day) for reduced body weight gain, reduced feed consumption and changes in red cell parameters at 5000 ppm (equal to 305 mg/kg bw per day) (Dange, 1995c).

In a subsequent 90-day dietary study in rats, fenamidone (purity 99.8%) was admixed in the diet at a concentration of 0, 60, 150, 1000 or 5000 ppm and fed ad libitum to groups of 10 SD rats of each sex. The achieved doses were 0, 4.1, 10.4, 68.3 and 344 mg/kg bw per day for males and 0, 4.8, 12.0, 83.3 and 381 mg/kg bw per day for females at 0, 60, 150, 1000 and 5000 ppm, respectively. Observations for deaths and clinical signs were made daily. Body weight and feed consumption were recorded weekly. Ophthalmological examinations were made on all rats prior to the commencement of dosing and then in the control and high-dose groups during week 12. Blood was collected during week 12 for the analysis of haematology and clinical chemistry parameters. One day prior to sacrifice, overnight urine was collected from all rats for the analysis of standard urinary parameters. Following termination, all rats were necropsied, organs weighed and tissues examined histopathologically.

There were no deaths or treatment-related clinical signs. Ophthalmological examinations were unremarkable. In high-dose males, mean absolute body weight was significantly lower ($P < 0.01$ or 0.05) than the control values throughout most of the exposure period (approximately 10% lower than the control values). Body weight gain of high-dose males was also significantly lower ($P < 0.01$ or 0.05) than the control values mainly during the first 5 weeks of exposure (20–30% lower). There were no significant intergroup differences in body weight or body weight gain in females. Feed consumption was inconsistently significantly lower ($P < 0.01$ or 0.05) than the control values in high-dose males (up to about 10% lower) and females (approximately 11–17% lower).

Treatment-related haematological findings were confined to high-dose males and included reduced haemoglobin (approximately 6% lower than the control, $P < 0.05$) and mean corpuscular haemoglobin concentration (MCHC) (approximately 4% lower than the control value, $P < 0.01$). Clinical chemistry findings included increased cholesterol in high-dose males ($P < 0.01$) and females ($P < 0.05$) (about 30% above the control), reduced glucose (approximately 26% lower than the control in high-dose males, $P < 0.01$) and increased phosphorus (approximately 20% higher than the control in females, $P < 0.05$). There were no treatment-related urine analysis findings.

Necropsy revealed a dark liver in four high-dose males and two high-dose females. Selected organ weights and histopathological findings are presented in Table 12. In high-dose females, absolute and relative liver weights were significantly higher ($P < 0.01$ or 0.05) than the control values (13% and 22%, respectively), concomitant with ground glass cytoplasm in hepatocytes (graded as mild) attributable to the proliferation of smooth endoplasmic reticulum. In males, relative but not absolute liver weights were significantly higher ($P < 0.01$ or 0.05) than the control values at 1000 and 5000 ppm (12% and 23%, respectively), with the difference at the highest dose most likely reflecting the lower terminal body weight of this group. Histopathology revealed ground glass cytoplasm in hepatocytes at these same dietary concentrations, a finding consistent with liver enzyme induction. Absolute and relative thyroid weights were significantly higher ($P < 0.01$) than the control values in high-dose males, but there was no supporting histopathology.

Table 12. Organ weights and microscopic findings in rats following 13 weeks of dietary exposure to fenamidone

Parameter	0 ppm		60 ppm		150 ppm		1 000 ppm		5 000 ppm	
	M	F	M	F	M	F	M	F	M	F
Terminal body weight (g)	430	270	477*	277	468	267	435	254	383*	250
Organ weights^a										
<i>Liver</i>										
Absolute (g)	10.36	6.55	11.82	6.69	1.77	6.52	11.70	6.43	11.49	7.39*
Relative ^b (%)	2.40	2.43	2.51	2.40	2.53	2.43	2.69**	2.54	3.00**	2.96**
<i>Thyroid</i>										
Absolute (g)	0.020	0.022	0.023	0.016	0.023	0.016	0.024	0.019	0.029**	0.019
Relative (g)	0.004 7	0.007 8	0.004 9	0.005 6*	0.004 9	0.005 6*	0.005 5	0.007 3	0.007 5**	0.007 5
Histopathology^c										
<i>N</i>	10	10	10	10	10	10	10	10	10	10
Hepatocytes – ground glass cytoplasm	0	0	0	0	0	0	2	0	10	7

*, $P < 0.05$; **, $P < 0.01$

^a Results expressed as means.

^b Relative to body weight.

^c Results expressed as the absolute number of rats with the finding.

Source: Bigot (1997)

The NOAEL was 1000 ppm (equal to 68.3 mg/kg bw per day) for reduced body weight gain at 5000 ppm (equal to 344 mg/kg bw per day) (Bigot, 1997).

Dogs

In a non-guideline study, groups of two Beagle dogs of each sex were offered 300 g diet containing fenamidone (purity > 99%) at a concentration of 0, 280, 2800 or 28 000 ppm for 28 days. The achieved doses were 0, 9.1, 89.2 and 595.3 mg/kg bw per day for males and 0, 9.7, 92.7 and 428.8 mg/kg bw per day for females at 0, 280, 2800 and 28 000 ppm, respectively. Observations for mortalities and clinical signs were made daily. Feed consumption was recorded daily, and body weight twice weekly. At the end of the treatment period, an ophthalmological examination was performed on all dogs. Blood and urine were sampled for the analysis of haematology, clinical chemistry or urine analysis parameters. Following terminal sacrifice, dogs were necropsied, selected organs weighed and tissues examined histopathologically.

There were no deaths. Clinical signs were observed at the highest dose and included emaciation (two females, one male), diarrhoea and/or a reduction in faeces (all dogs), vomiting (one male) and ocular discharge (two males). All high-dose dogs lost up to 15–25% of their pretreatment body weight, coincident with reduced feed intake (approximately 50–70% lower than the respective pretreatment value). Ophthalmoscopy confirmed the discharge in the two high-dose males. Treatment-related haematological findings were confined to the highest dose and included (relative to the respective pretreatment value) reduced red blood cells (approximately 15% in males and 28% in one female), haemoglobin (approximately 18% in two males and 30% in one female), haematocrit (approximately 11% in two males and 26% in one female), platelet counts (approximately 48% in one female), neutrophil counts (approximately 52% in one female) and increased prothrombin time and activated partial thromboplastin time (approximately 25% in one female). Effects on clinical chemistry parameters also occurred at the highest dose and included reduced total protein (up to approximately 10% in two males and one female), albumin (up to approximately 20% in two males and one female), cholesterol (approximately 40% in two males and one female), inorganic phosphate (approximately 36% in one female) and calcium (approximately 9% in two females) and increased bilirubin (twice the pretreatment value in one male, 4 times higher in one female) and alanine aminotransferase (ALAT) (twice the pretreatment value in one male). There was no treatment-related effect on any urine analysis parameters.

As a result of high intragroup variation, there were no clear treatment-related effects on organ weights. Necropsy revealed atrophy of the thymus in all high-dose dogs and one female at each of 280 and 2800 ppm. Histopathology revealed abnormalities in the liver (slight to moderate centrilobular sinusoidal dilatation; slight to mild bile duct hyperplasia) and thymus (mild to marked cortical atrophy) (Table 13).

Table 13. Histopathological findings in dogs exposed to fenamidone in the diet for 28 days

Parameter	Incidence of finding							
	0 ppm		280 ppm		2 800 ppm		28 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
<i>N</i>	2	2	2	2	2	2	2	2
Liver								
Centrilobular sinusoidal dilatation	1	0	1	2	1	1	2	2
Bile duct hyperplasia	0	0	2	1	2	1	1	2
Thymus								
Cortical atrophy	1	0	1	1	0	1	2	2

Source: Dange & Katchadourian (1995)

No NOAEL was identified. The lowest-observed-adverse-effect level (LOAEL) was 280 ppm (equal to 9.1 mg/kg bw per day) for bile duct hyperplasia at all dietary concentrations tested (Dange & Katchadourian, 1995).

In a second 28-day range-finding study, groups of three Beagle dogs of each sex received fenamidone (purity 98.3%) via gelatine capsules at a dose of 0, 3, 10 or 100 mg/kg bw per day. The NOAEL was 100 mg/kg bw per day, the highest tested dose (Fisch, 1999a).

Fenamidone (purity > 98%) was administered to groups of four Beagle dogs of each sex in gelatine capsules at a dose of 0, 10, 100 or 500 mg/kg bw per day for 13 weeks. Observations for mortalities and clinical signs were made daily. Feed consumption was recorded daily, and body

weight weekly. An ophthalmological examination was performed on all dogs prior to the commencement of dosing and during week 13. Blood was collected pretreatment and during weeks 5, 8 and 13 for the analysis of standard haematology and clinical chemistry parameters. Urine was collected pretreatment and in week 13 for the analysis of standard urinary parameters. At the end of the study, dogs were killed and necropsied, their organs were weighed and their tissues were examined histopathologically.

All dogs survived to scheduled termination. Treatment-related clinical signs included reddish coloration of the tongue (all high-dose dogs) and ptialism (three high-dose males and four high-dose females); neither of these findings was considered toxicologically significant. There was no treatment-related effect on body weight gain, feed consumption or ophthalmological findings. Haematology and urine analysis were unremarkable. Plasma cholesterol of high-dose females (4.5 mmol/L) was higher than the control value (3.4 mmol/L) at week 13, but at no other sampling times or in males; on this basis, it was considered an incidental finding. There were no treatment-related organ weight, macroscopic or histopathological findings.

The NOAEL was 500 mg/kg bw per day, the highest dose tested (Fisch, 1999b).

In a 52-week study, fenamidone (purity > 99%) was administered to groups of four Beagle dogs of each sex in gelatine capsules at a dose of 0, 10, 100 or 1000 mg/kg bw. Recorded end-points were consistent with the preceding study, with the exception that ophthalmological examination, blood sampling and urine sampling occurred pretreatment and at weeks 13, 26 and 52.

Treatment-related findings are summarized in Table 14. There were no deaths. Hypersalivation and vomiting were observed at 100 mg/kg bw per day (females) and 1000 mg/kg bw per day (both sexes), with a dose-related increase in their frequency and severity. The hypersalivation that occurred at 100 mg/kg bw per day was slight and occurred prior to dosing, with the vomiting occurring only on two or three occasions. In contrast, these clinical signs occurred frequently at the highest dose. With the exception of one high-dose male that began vomiting from 3 days after the commencement of dosing, vomiting began approximately 3–15 weeks after the commencement of dosing in all other dogs. There was no treatment-related effect on body weight gain, feed consumption, ophthalmological findings, urine analysis parameters or macroscopic findings. At the highest dose, mean haemoglobin and MCHC were significantly lower ($P < 0.01$ or 0.05) than the control values on occasion, but the levels showed no consistency over time or between sexes. Further, the author stated that the results were within the historical control range (data not provided). Significantly elevated ($P < 0.01$ or 0.05) alkaline phosphatase (AP) activity occurred at the highest dose (2.3-fold higher than the control value). Absolute liver weights were significantly higher ($P < 0.05$) than the control values at 100 and 1000 mg/kg bw per day in females (approximately 40% and 32%, respectively) and at 1000 mg/kg bw per day in males (approximately 33%). Corresponding relative liver weights were approximately 23% and 29% higher than the control values in males and females, respectively, but the increases were not statistically significant. In high-dose males, the absolute weight of spleen was approximately 43% higher ($P < 0.05$) than the control value, with the relative spleen weight approximately 38% higher, but not significantly so; in the absence of any supporting microscopic changes, this finding is not considered treatment related. Histopathologically, the incidence of biliary proliferation was increased in high-dose males.

The NOAEL was 100 mg/kg bw per day for clinical signs (hypersalivation and vomiting in both sexes), increased AP activity (both sexes), increased liver weight (males) and increased bile duct hyperplasia (males) at 1000 mg/kg bw per day (Fisch, 1999c).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an 80-week oncogenicity study, C57BL/10J_rCD-1 Alpk mice (55 of each sex per group) were exposed to fenamidone (purity 99.8%) in the diet at a concentration of 0, 70, 350, 3500 or 7000

Table 14. Findings in dogs over 52 weeks of oral exposure (capsules) to fenamidone

Parameter	0 mg/kg bw per day		10 mg/kg bw per day		100 mg/kg bw per day		1 000 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
<i>N</i>	4	4	4	4	4	4	4	4
Clinical signs^a								
Vomiting	0	1	0	0	0	2	4	4
Hypersalivation	0	0	0	0	0	1	4	4
Haematology^b								
Hb (g/dL)								
- Pretreatment	14.0	14.8	13.6	13.7	14.3	14.5	14.8	14.6
- Week 13	16.2	16.9	15.3	15.8	15.0	16.4	14.4*	15.5
- Week 26	16.4	17.5	16.1	16.4	15.3	15.8	14.9	13.9**
- Week 52	16.3	18.1	16.5	17.7	15.7	17.8	16.5	16.0*
MCHC (g/dL)								
- Pretreatment	32.6	32.8	32.3	32.6	32.7	32.8	33.0	32.8
- Week 13	33.0	32.7	32.6	32.7	32.8	33.2	31.8*	32.3
- Week 26	32.8	32.8	32.8	32.7	32.3	32.2	30.7*	31.6
- Week 52	32.3	32.3	32.1	32.6	32.1	31.9	31.5	31.9
Clinical chemistry^b								
AP (IU/L)								
- Pretreatment	267	298	315	325	400	318	368	315
- Week 13	159	149	170	194	257	214	261	316*
- Week 26	105	116	121	151	203	188	266**	262*
- Week 52	96	102	96	131	176	158	262**	284*
Organ weights^b								
<i>Liver</i>								
Absolute (g)	235.4	174	243.5	225.6	263.3	244.3*	313.6*	228.9*
Relative ^c (%)	2.25	1.95	2.22	2.13	2.46	2.39	2.76	2.51
<i>Spleen</i>								
Absolute (g)	28.03	27.89	29.46	27.05	29.89	28.79	40.05*	31.27
Relative (%)	0.26	0.32	0.27	0.26	0.28	0.28	0.36	0.35
Histopathology^a								
Bile duct hyperplasia	1	0	0	1	0	0	4	1

AP: alkaline phosphatase; Hb: haemoglobin; IU: International Units; MCHC: mean corpuscular haemoglobin concentration; *: $P < 0.05$; **: $P < 0.01$

^a Results expressed as the absolute number of dogs with the finding.

^b Results expressed as means.

^c Relative to body weight.

Source: Fisch (1999c)

ppm. The mean achieved doses were 0, 9.5, 47.5, 525.5 and 1100 mg/kg bw per day for males and 0, 12.6, 63.8, 690.5 and 1393 mg/kg bw per day for females at 0, 70, 350, 3500 and 7000 ppm, respectively. Additional groups of 10 mice of each sex were similarly exposed for 52 weeks. Observations for mortalities and clinical signs were made daily. Body weight and feed consumption were recorded weekly for the first 14 weeks and every 4 weeks thereafter. Blood was sampled during

week 53 and at the end of the study for the analysis of haematology parameters. Decedents and mice killed at the end of the designated exposure period were necropsied, organs weighed and tissues examined histopathologically.

There were no treatment-related mortalities or clinical signs. Mean body weight was significantly lower ($P < 0.01$) than the control values at 3500 and 7000 ppm in both sexes throughout the dosing period (up to about 8–12% lower in both sexes). Body weight gain was not determined. Mean feed consumption was significantly higher ($P < 0.01$ or 0.05) than the control values at 3500 and 7000 ppm in both sexes (up to about 15%), mainly over the first 40–50 weeks. Overall feed conversion efficiency (weeks 1–12) was significantly lower ($P < 0.01$) than the control values at 3500 and 7000 ppm in both sexes (approximately 15% and 48% lower in males and 14% and 23% lower in females, respectively).

There were a number of haematology parameters that were slightly, albeit significantly, different from the control values, but these showed little consistency over time or between sexes. In females, increased platelet counts occurred at 3500 and 7000 ppm (+23% and +29% during week 53 and +27% and +40% during week 81, respectively).

There were no treatment-related gross lesions. Selected liver findings are presented in Table 15. Absolute liver weights were significantly ($P < 0.01$) higher than the control values at 3500 and 7000 ppm (approximately 10–30% higher than the controls) in mice killed after 53 weeks and at and above 350 ppm in mice killed after 80 weeks (approximately 10–20% higher than the controls). Although relative liver weights were increased at these same dietary concentrations, they were not significantly different from the control values. The increase in liver weight was corroborated histopathologically at 3500 and 7000 ppm by an increase in nuclear pleomorphism with or without increased cytoplasmic eosinophilia plus occasional giant cells and eosinophilic globules in the cytoplasm. At these same dietary concentrations, there was an increase in clear cell foci and/or basophilic foci in the liver. There were no treatment-related neoplastic findings. There was an increase in the incidence of hepatocellular adenocarcinoma in 3500 ppm males (4/65 versus 0/65 in the controls), which the author noted was above the historical control incidence. However, as this increase was not statistically significant and did not occur at 7000 ppm, it was not considered treatment related.

The NOAEL for chronic toxicity was 350 ppm (equal to 47.5 mg/kg bw per day) for lower body weight, increased liver weight and histopathological findings in the liver (nuclear pleomorphism, eosinophilia and giant cells) at 3500 ppm (equal to 525.5 mg/kg bw per day). The NOAEL for carcinogenicity was 7000 ppm (equal to 1100 mg/kg bw per day), the highest dietary concentration tested (Milburn, 1999).

Rats

Fenamidone (purity 98.9% or 99.8%) was admixed in the diet at a concentration of 0, 60, 150, 1000 or 8000 ppm and fed ad libitum to groups of SD rats for 52 weeks (10 rats of each sex per dose) or 104 weeks (60 rats of each sex per dose). Separate groups of 15 rats of each sex in the control and high-dose groups were treated similarly for 52 weeks followed by a 3-month recovery phase. The concentration of fenamidone in the high-dose diet was decreased to 4000 ppm from day 17 because of marked body weight loss. However, the study author (Bigot, 1999a) removed all rats from the reversibility phase on day 20. An additional high-dose group (5000 ppm) and control group formed part of a second study (Bigot, 1999b). Observations for mortality and clinical signs were made daily. Body weight and feed consumption were recorded weekly for the first 14 weeks and every 4 weeks thereafter. Ophthalmological examinations were performed prior to the commencement of dosing and at 1 and 2 years. Blood and urine were collected at approximately 6-month intervals for the analysis of standard haematology, clinical chemistry or urine analysis parameters. Decedents and rats killed at the end of the designated exposure period were necropsied, organs weighed and tissues examined histopathologically.

Table 15. Liver findings in mice following 80 weeks of dietary exposure to fenamidone

Parameter	0 ppm		70 ppm		350 ppm		3 500 ppm		7 000 ppm	
	M	F	M	F	M	F	M	F	M	F
Liver weight^a										
<i>Week 53</i>										
Absolute (g)	1.54	1.26	1.58	1.34	1.55	1.32	1.70**	1.45**	1.71**	1.60**
Relative ^b (%)	4.35	4.65	4.39	4.76	4.78	4.71	5.22	5.68	5.50	6.11
<i>Week 81</i>										
Absolute (g)	1.57	1.37	1.59	1.32	1.70**	1.52**	1.81**	1.52**	1.83**	1.57**
Relative (%)	4.47	4.69	4.65	4.72	4.90	5.34	5.61	5.76	5.91	6.09
Liver histopathology^c										
<i>N</i>	65	55	65	65	65	65	65	65	65	65
Nuclear pleomorphism ± cytoplasmic eosinophilia ± giant cells ± eosinophilic droplets	0	0	0	0	0	0	43	50	57	59
Altered hepatocytes: Clear cell foci	1	0	1	0	2	0	1	3	3	5
Altered hepatocytes: Basophilic foci	0	0	1	0	0	0	3	1	0	3

F: female; M: male; *: $P < 0.05$; **: $P < 0.01$ ^a Results expressed as means.^b Relative to body weight.^c Results expressed as the absolute number of mice with the findings.

Source: Milburn (1999)

The achieved doses from weeks 1 to 49 were 0, 3.4, 8.6, 54.4 and 309 mg/kg bw per day for males and 0, 4.3, 10.9, 70.5 and 380 mg/kg bw per day for females at 0, 60, 150, 1000 and 5000 ppm, respectively. The achieved doses from weeks 1 to 101 were 0, 2.8, 7.1, 47.7 and 260 mg/kg bw per day for males and 0, 3.6, 9.2, 60.9 and 335 mg/kg bw per day for females at 0, 60, 150, 1000 and 5000 ppm, respectively.

There were no treatment-related mortalities or clinical signs. There were no treatment-related effects on body weight, body weight gain or feed consumption at and below 1000 ppm. During weeks 1 and 2 of exposure to the diet containing 8000 ppm fenamidone, mean body weight was, respectively, about 14% and 26% lower than the control values in males and about 5% and 6% lower than the control values in females; these differences were statistically significant ($P < 0.01$). This lower body weight gain was concomitant with a decrease in feed consumption (approximately 16% lower than the controls; $P < 0.01$). In the additional study, reduced body weight gain occurred at 5000 ppm (up to 10% lower than the controls in the 1st year and about 7% lower in the 2nd year in males; up to about 14% lower than the controls in the 1st year and up to about 18% lower in the 2nd year in females). These effects on body weight gain were reversible after the 1st year of treatment. Feed consumption was also reduced at 5000 ppm (up to about 10% lower than the controls in males and 13% lower in females).

There were no treatment-related ophthalmological abnormalities or effects on haematology, clinical chemistry or urine analysis parameters.

In rats killed after 52 or 104 weeks of treatment, absolute and relative kidney weights were significantly higher ($P < 0.01$ or 0.05) than the control values at and above 150 ppm, whereas liver weights were significantly increased at and above 1000 ppm (Table 16). Absolute and relative thyroid weights were significantly higher than the control values ($P < 0.01$) at 5000 ppm (Table 16). In rats killed after 52 weeks, no treatment-related macroscopic abnormalities occurred at or below 1000 ppm. In rats dying or killed in a moribund condition, the incidence of enlarged livers and enlarged thyroid glands in 1000 ppm males was higher than the control values (20/36 versus 14/38 and 15/36 versus 7/38, respectively). In rats killed after 104 weeks, the incidence of liver enlargement was increased in 1000 ppm males (16/24 versus 8/22 in the controls). In 150 and 1000 ppm males, the incidence of kidneys with a granular appearance was increased relative to the controls (10/32 and 9/24, respectively, versus 2/22 in the controls). Also at 1000 ppm, there was an increase in males with enlarged kidneys (5/24 versus 1/22 in the controls), consistent with chronic progressive nephropathy. In the second study, large thyroid glands were observed in 5000 ppm males (3/10), but not in the controls.

In rats killed after 52 weeks, there was a dose-related increase in the incidence of slight diffuse thyroid follicular cell hypertrophy and/or hyperplasia at and above 150 ppm (Table 16). At 5000 ppm in the second study, the incidence of slight centrilobular hepatocyte hypertrophy was increased in females (5/10 versus 0/10 in the controls); in males, the incidences of foamy cytoplasm (4/10 mild, 2/10 moderate) and eosinophilic inclusions (2/10 slight, 1/10 mild and 2/10 moderate) in periportal hepatocytes were higher than the control incidences (0/10); these differences were not statistically significant. Similar histopathological changes in the liver were not evident after 104 weeks of treatment.

In rats killed after 104 weeks, hypertrophy of centrilobular hepatocytes (graded as slight or moderate) was significantly higher ($P < 0.05$) than the control incidence in 5000 ppm females (14/60 versus 0/60, respectively). Vacuolation of centrilobular hepatocytes (graded as slight to moderate) was also significantly higher ($P < 0.05$) than the control incidence in 5000 ppm females (12/60 versus 0/60, respectively). In the thyroid, the incidence of focal follicular cell hyperplasia was significantly higher ($P < 0.01$) than the control incidence in males at 1000 and 5000 ppm; no such differences were noted in females (Table 16). The thyroid follicular cell hyperplasia observed in males at 60 and 150 ppm was not statistically different from the control and on this basis was not considered treatment related. Colloid basophilia was significantly increased ($P < 0.001$) in both sexes at 1000 and 5000 ppm. While there was a slight increase in the severity of C-cell hyperplasia at 1000 and 5000 ppm in males and at and above 150 ppm in females, this increase was not statistically significant, nor was there any difference in the total number of rats with C-cell hyperplasia.

The NOAEL was 60 ppm (equal to 2.8 mg/kg bw per day), based on a dose-related increase in follicular cell hypertrophy and/or hyperplasia in both sexes after 52 weeks at 150 ppm (equal to 7.1 mg/kg bw per day). The NOAEL for carcinogenicity was 5000 ppm (equal to 260 mg/kg bw per day in), the highest dietary concentration tested (Bigot, 1999a, 1999b).

2.4 Genotoxicity

The results of genotoxicity assays on fenamidone are summarized in Table 17. In the majority of experimental systems, fenamidone tested negative for genotoxicity. The two in vitro studies in which positive responses were noted are described more fully below. In the mouse lymphoma forward mutation assay, fenamidone tested positive in the presence of an exogenous source of metabolic activation. In a chromosomal aberration assay conducted on human peripheral lymphocytes, fenamidone tested positive in both the presence and the absence of metabolic activation.

Table 16. Organ weight, macroscopic and histopathological findings in rats exposed to fenamidone for up to 2 years in the diet

Parameter	0 ppm		60 ppm		150 ppm		1 000 ppm		0 ppm		5 000 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
Organ weights^a (chronic phase – 52 weeks on treatment)												
<i>Kidney</i>												
Absolute (g)	3.28 (0)	2.13 (0)	3.69 (+13)	2.12 (0)	3.62 (+10)	2.38 (+12)	3.78* (+14)	2.48* (+16)	3.70 (0)	2.20 (0)	4.40* (+19)	2.54** (+15)
Relative ^b (%)	0.53 (0)	0.58 (0)	0.55 (+4)	0.59 (0)	0.55 (+4)	0.66* (+14)	0.60* (+13)	0.68** (+17)	0.56 (0)	0.61 (0)	0.69** (+23)	0.77** (+26)
<i>Liver</i>												
Absolute (g)	13.42 (0)	7.94 (0)	15.30 (+14)	7.78 (–2)	15.47 (+15)	8.13 (+2)	16.07* (+20)	8.40 (+6)	14.98 (0)	7.87 (0)	18.99* (+27)	9.83** (+25)
Relative (%)	2.14 (0)	2.14 (0)	2.28 (+7)	2.13 (0)	2.36 (+10)	2.23 (+4)	2.53* (+18)	2.29 (+7)	2.29 (0)	2.17 (0)	2.98** (+30)	2.98* (+37)
<i>Thyroid</i>												
Absolute (g)	0.030 (0)	0.021 (0)	0.031 (+3)	0.020 (+5)	0.031 (+3)	0.022 (+5)	0.034 (+13)	0.024 (+14)	0.033 (0)	0.024 (0)	0.046** (+39)	0.023 (–4)
Relative (%)	0.005 (0)	0.006 (0)	0.005 (0)	0.006 (0)	0.005 (0)	0.006 (0)	0.005 (0)	0.007 (+17)	0.005 (0)	0.006 (0)	0.007** (+40)	0.007 (+17)
Organ weights^a (carcinogenicity phase – 104 weeks on treatment)												
<i>Kidney</i>												
Absolute (g)	4.07 (0)	2.77 (0)	4.18 (+3)	3.01 (+9)	4.51* (+11)	3.17** (+14)	4.58* (+13)	3.26** (+18)	4.48 (0)	2.83 (0)	4.86 (+8)	2.97 (+13)
Relative (%)	0.63 (0)	0.57 (0)	0.62 (0)	0.63 (+10)	0.72* (+14)	0.66** (+16)	0.73 (+16)	0.71** (+25)	0.74 (0)	0.58 (0)	0.82 (+11)	0.74** (+28)
<i>Liver</i>												
Absolute (g)	14.03 (0)	12.95 (0)	14.52 (+3)	12.39 (–4)	14.34 (+2)	12.42 (–4)	15.81* (+13)	12.95 (0)	14.76 (0)	13.66 (0)	17.86** (+21)	13.47 (0)

Parameter	0 ppm		60 ppm		150 ppm		1 000 ppm		0 ppm		5 000 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
Relative (%)	2.16 (0)	2.59 (0)	2.14 (-1)	2.56 (-1)	2.27 (+5)	2.57 (-1)	2.46* (+14)	2.75 (+6)	2.38 (0)	2.75 (0)	2.97** (+25)	3.28** (+19)
<i>Thyroid</i>												
Absolute (g)	0.049 (0)	0.038 (0)	0.043 (-12)	0.031 (-18)	0.047 (-4)	0.036 (-5)	0.057 (+16)	0.036 (-5)	0.051 (0)	0.056 (0)	0.067* (+31)	0.039 (-30)
Relative (%)	0.008 (0)	0.008 (0)	0.006 (-25)	0.007 (-12)	0.008 (0)	0.008 (0)	0.009 (+13)	0.008 (0)	0.009 (0)	0.011 (0)	0.011 (+22)	0.01 (+10)
Histopathological findings^c – thyroid (chronic phase – 52 weeks on treatment)												
Follicular cell hypertrophy and/or hyperplasia												
- Slight	0/9	0/10	0/10	0/10	2/9	2/10	3/10	5/10	0/10	0/10	9/10	9/10
Histopathological findings^c – thyroid (carcinogenicity phase – 104 weeks on treatment)												
Focal follicular cell hyperplasia												
- Slight	0/59	0/59	3/58	0/59	2/59	0/60	6/60	1/60	0/60	1/60	8/60	3/60
- Mild	0/59	0/59	0/58	0/59	0/59	0/60	0/60	0/60	0/60	0/60	5/60	0/60
- Moderate	0/59	0/59	0/58	0/59	0/59	0/60	2/60	0/60	0/60	0/60	0/60	0/60
- Marked	0/59	0/59	0/58	0/59	0/59	0/60	1/60	0/60	0/60	0/60	0/60	0/60
- Total	0/59	0/59	3/58	0/59	2/59	0/60	9/60**	1/60	0/60	1/60	13/60**	3/60
Basophilia, colloid												
- Slight	0/59	0/59	0/58	0/59	0/59	0/60	19/60	2/60	0/60	0/60	24/60	16/60
- Mild	0/59	0/59	0/58	0/59	0/59	0/60	6/60	0/60	0/60	0/60	20/60	6/60
- Total	0/59	0/59	0/58	0/59	0/59	0/60	25/60***	2/60***	0/60	0/60	44/60***	22/60***
Diffuse follicular cell hypertrophy												
- Slight	0/59	0/59	0/58	0/59	0/59	0/60	6/60	0/60	0/60	0/60	16/60	16/60
- Mild	0/59	0/59	0/58	0/59	0/59	0/60	0/60	0/60	0/60	0/60	1/60	1/60
- Total	0/59	0/59	0/58	0/59	0/59	0/60	6/60	1/60	0/60	0/60	17/60	17/60

Table 16 (continued)

Parameter	0 ppm		60 ppm		150 ppm		1 000 ppm		0 ppm		5 000 ppm	
	M	F	M	F	M	F	M		F	M	F	M
Increased follicular diameter												
- Slight	0/59	0/59	0/58	0/59	0/59	0/60	3/60	0/60	0/60	0/60	12/60	2/60
C-cell hyperplasia												
- Slight	20/59	23/59	17/58	19/59	22/59	22/60	15/60	18/60	16/60	23/60	26/60	19/60
- Mild	0/59	1/59	0/58	3/59	1/59	5/60	8/60	7/60	2/60	0/60	12/60	4/60
- Moderate	0/59	0/59	0/58	0/59	0/59	1/60	0/60	2/60	0/60	1/60	0/60	0/60
- Total	20/59	24/59	17/58	22/59	23/59	28/60	23/60	27/60	18/60	24/60	38/60	23/60

F: females; M: males; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

^a Results expressed as means.

^b Relative to body weight.

^c Results expressed as the number of rats with the finding/total number of rats sampled.

Source: Bigot (1999a, 1999b)

Table 17. Results of genotoxicity assays on fenamidone

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro studies					
Gene mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535 and TA1537	<i>Cytotoxicity test</i> : 1–5 000 µg/plate (±S9) <i>Mutagenicity test 1</i> : 50–2 500 µg/plate (±S9) <i>Mutagenicity test 2</i> : 10–1 000 µg/plate (–S9); 50–2 500 µg/plate (+S9) DMSO vehicle	98.9	Negative ^a	Katchadourian, Berniaud & Fourmer (1996)
Gene mutation	Mouse lymphoma cells (L5178Y)	<i>Cytotoxicity test</i> : 18.75–600 µg/mL (±S9) <i>Experiment 1</i> : 12.5–150 µg/mL (–S9); 3.125–50 µg/L (+S9) <i>Experiment 2</i> : 50–200 µg/mL (–S9); 6.25–43.75 µg/mL (+S9) DMSO vehicle	99.8	Positive ^b	Fellows (1999a)
Chromosomal aberrations	Human lymphocytes	<i>Experiment 1</i> : 2.91–5.93 µg/mL (–S9); 147–300 µg/mL; 3 or 20 h incubation <i>Experiment 2</i> : 22.53–40.05 µg/mL (–S9); 168.8–300 µg/mL (+S9); 3 or 20 h incubation DMSO vehicle	99.8	Positive ^b	Marshall (1999a)
Unscheduled DNA synthesis	Rat hepatocytes	<i>Experiment 1</i> : 0.064–40 µg/mL <i>Experiment 2</i> : 1.25–30 µg/mL DMSO vehicle	99.8	Negative ^c	Fellows (1999b)
In vivo studies					
Micronucleus	Swiss CD-1 mice, bone marrow (5/sex/group)	0, 500, 1 000 or 2 000 mg/kg bw, ip; killed 24 or 48 h after dosing 0.5% (w/v) CMC vehicle	99.8	Negative ^d	Marshall (1999b)
Unscheduled DNA synthesis	Male Wistar rats, hepatocytes (5/group)	0, 80 or 2 000 mg/kg bw, po; killed after 12–14 h (Experiment 1) or 2–4 h (Experiment 2) after dosing 0.5% (w/v) CMC vehicle	99.8	Negative	Fellows (1999c)

CMC: carboxymethyl cellulose; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; ip: intraperitoneal; po: per os (by mouth); S9: 9000 × g supernatant fraction of rat liver homogenate; w/v: weight per volume

^a Cytotoxicity at and above 1000 µg/plate (standard plate incorporation) or 250 µg/plate (liquid preincubation); precipitation at and above 1000 µg/plate.

^b See evaluation in text.

^c Cytotoxicity at ≥ 30 µg/mL.

^d The majority of mice showed signs of toxicity at the highest dose (lethargy, hunched appearance, eye closure and distended abdomen).

Table 18. Effect of fenamidone on survival of mouse lymphoma L5178Y cells (cytotoxicity test)

Concentration (µg/mL)	Mean relative survival (%)	
	–S9	+S9
0	100	100
18.75	120.8	58.4
37.5	106.3	5.5
75	91.8	0
150	0	1
300	0	0
600	0	0

S9: 9000 × g supernatant fraction of rat liver homogenate

Source: Fellows (1999a)

Fellows (1999a) analysed the potential of fenamidone (purity 99.8%) to cause mutations at the *tk* locus in mouse lymphoma L5178Y cells. 4-Nitroquinoline 1-oxide (NQO) and benzo(*a*)pyrene (BP) in dimethyl sulfoxide (DMSO) were used as positive controls. Experiments were conducted in the presence and absence of an exogenous source of metabolic activation (S9 post-mitochondrial liver fraction from Aroclor-induced rats). In a cytotoxicity/range-finding experiment, fenamidone in DMSO was tested at concentrations from 18.75 to 600 µg/mL. The highest concentration was the limit of solubility for the test material. Toxicity occurred at and above 150 µg/mL in the absence of S9 and at and above 37.5 µg/mL in the presence of S9 (Table 18). Based on the results of the range-finding experiment, two mutagenicity experiments were conducted, which tested concentrations of 3.125–200 µg/mL (±S9). In both experiments, fenamidone increased the mutation frequency only in the presence of S9 (Table 19). Although there were increases in both small and large colonies, the increase in the frequency of small colony mutants was more pronounced.

Fenamidone (99.8% purity) in DMSO was tested for its ability to cause chromosomal aberrations in peripheral human lymphocytes in the presence and absence of S9. In Experiment 1, concentrations ranging from 2.9 to 300 µg/mL were tested. Cells were exposed for 20 hours in the absence of S9 and 3 hours (with a 17-hour recovery phase) in the presence of S9. Concentrations tested in Experiment 2 ranged from 0.95 to 300 µg/mL. NQO and cyclophosphamide (CPA) were used as positive controls. There was a significant ($P < 0.05$ or 0.01) treatment-related increase in cells with chromosomal aberrations in both experiments following 3 or 20 hours of exposure (Table 20). The effect was more pronounced in the presence of S9 (Marshall, 1999a).

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

Fenamidone (purity 99.8%) was admixed in the diet at a concentration of 0, 60, 1000 or 5000 ppm and fed ad libitum to two parental generations of Crl:CD[®](SD)BR rats (28 of each sex per group) and their offspring. Achieved doses during the premating period in F₀ rats were 0, 3.9, 63.8 and 328.3 mg/kg bw per day for males and 0, 5.15, 84.4 and 459.6 mg/kg bw per day for females at 0, 60, 1000 and 5000 ppm, respectively. Achieved doses during the premating period in F₁ rats were 0, 4.0, 68.6 and 353.2 mg/kg bw per day for males and 0, 5.45, 89.22 and 438.34 mg/kg bw per day for females at 0, 60, 1000 and 5000 ppm, respectively.

Rats were observed for mortalities and clinical signs 3 times daily. Parental rats were weighed weekly throughout the premating phase. Maternal rats were weighed on gestation days 0, 7, 14, 17 and 21 and on lactation days 0 (day of parturition), 7, 14 and 21. Feed consumption was recorded weekly throughout the premating phase, on days 7, 14 and 21 during gestation and on days 7 and 14 of lactation. Standard reproduction and litter parameters were recorded. Following death or scheduled

Table 19. Mutation frequency in L5178Y cells 2 days after exposure to fenamidone^a

-S9			+S9		
Concentration (µg/mL)	Relative survival (%)	Mutation frequency	Concentration (µg/mL)	Relative survival (%)	Mutation frequency ^b
Experiment 1					
0	100	78.46	0	100	68.58
12.5	108	–	3.125	90.87	77.61
25	109.4	–	6.25	79	85.68
50	112.9	70.44	12.5	62	118.78*
75	93.6	67.42	25	32.8	240.87*
100	77.6	73.21	37.5	6.2	377.91
125	76	72.93	50	5.1	–
150	45	77.80	–	–	–
NQO = 0.05	116.6	357.48	BP = 2	64.6	596.5
NQO = 0.1	76.3	481.93	BP = 3	41.5	1074
Experiment 2					
0	100	106.54	0	100	75
50	88.8	111.08	6.25	88.8	72
75	75	123.21	12.5	63.8	234.9*
100	60.5	105.66	18.75	37.1	391.8*
125	40.7	97.03	25	4.8	529.6
150	10.6	120.16	31.25	7.2	410.9
175	1.7	–	37.5	6.0	–
200	0.93	–	43.75	7.7	–
NQO = 0.05	84.5	499.20	2	75.1	729.60
NQO = 0.1	77.8	442.46	3	37.1	752.75

BP: benzo(a)pyrene; NQO: 4-nitroquinoline 1-oxide; S9: 9000 × g supernatant fraction of rat liver homogenate;

*: $P < 0.05$

^a Results expressed as the mean.

^b Significant ($P < 0.001$) linear trend.

Source: Fellows (1999a)

termination, necropsies were performed on all parental rats and offspring. The following organs from parental rats were weighed: testes, epididymides, seminal vesicles, prostate, uterus, ovaries, brain, pituitary, liver, kidneys, adrenal glands and spleen. The following tissues from control and high-dose parental rats were examined histopathologically: right testis, right epididymis, seminal vesicles, coagulating gland, prostate, pituitary, adrenal glands, ovaries, uterus, oviduct, cervix and vagina.

There were no treatment-related mortalities or clinical signs. At 5000 ppm, mean absolute body weight of F₀ parental rats was significantly lower ($P < 0.01$ or 0.05) than the control values during the premating phase (up to about 7% lower than the controls in males and 9% lower in females). Body weight gain was also significantly lower ($P < 0.01$ or 0.05) than the control values during this same period at 1000 and 5000 ppm in males (up to about 20% and 36% lower, respectively) and at 5000 ppm in females (up to about 36% lower). Mean feed consumption was significantly lower ($P < 0.01$ or 0.05) than the control values at these same doses. On occasion in both sexes, feed conversion efficiency was significantly lower ($P < 0.01$ or 0.05) than the control values during premating (up to about 50% lower than the controls). Throughout gestation and in the first week or two of lactation, the mean absolute body weight of 5000 ppm females was also significantly

Table 20. Proportion of human lymphocytes with chromosomal aberrations following exposure to fenamidone^a

-S9			+S9		
Treatment (µg/mL)	Proportion of cells with aberrations excluding gaps	Mitotic index	Treatment (µg/mL)	Proportion of cells with aberrations excluding gaps	Mitotic index
Experiment 1					
0	0.030	3.6	0	0.020	5.9
2.907	0.025	3.0	147	0.18**	3.2
4.152	0.050	1.8	210	0.22**	3.1
5.932	0.060*	1.2	300	0.21**	1.6
NQO = 2.5	0.22**	Not reported	CPA = 25	0.56**	Not reported
Experiment 2					
0	0.020	4.1	0	0.025	5.6
22.53	0.040	2.3	168.8	0.13**	3.6
30.03	0.045	2.3	225	0.12**	3.0
40.05	0.065*	1.5	300	0.12**	3.3
NQO = 2.5	0.34**	Not reported	CPA = 25	0.40**	Not reported
			0 ^b	0.005	5.7
			300 ^c	0.080**	2.7

CPA: cyclophosphamide; NQO: 4-nitroquinoline 1-oxide; S9: 9000 × *g* supernatant fraction of rat liver homogenate; *: $P < 0.05$; **: $P < 0.01$

^a Results expressed as means.

^b Additional solvent control group.

^c Additional fenamidone treatment group.

Source: Marshall (1999a)

lower ($P < 0.01$) than in the controls (up to approximately 10% lower), but there was no difference in body weight gain. No effect on feed consumption occurred during lactation or gestation.

There was no treatment-related effect on reproduction, sperm or litter parameters in any generation.

There were no significant intergroup differences in F₁ pup weights during lactation. Following weaning, the mean absolute body weights of F₁ rats were significantly lower ($P < 0.001$ or 0.01) than the control values at 5000 ppm (weeks 10 and 12 in males, approximately 7% and 10% lower, respectively; weeks 7 and 9 in females, approximately 9% and 8% lower, respectively). During gestation, the mean absolute body weight of F₁ dams was significantly lower ($P < 0.05$; approximately 7% lower) than in the controls at 5000 ppm only during week 3; there was no difference in body weight gain. There were no intergroup differences in maternal body weight or body weight gain during lactation. Feed consumption was generally unremarkable in F₁ parental rats, with a reduction of about 13% ($P < 0.01$ or 0.05) occurring in dams during lactation at 1000 and 5000 ppm. Feed conversion efficiency was significantly higher ($P < 0.01$) at 1000 and 5000 ppm in F₁ males (up to about 2.5 times higher than in the controls).

F₂ pup weights were significantly lower ($P < 0.01$ or 0.05) than in the controls in males at 5000 ppm (days 8 and 21; 17% and 21% lower, respectively) and in females at 1000 and 5000 ppm (day 21; 21% lower, $P < 0.01$ or 0.05).

At 5000 ppm, absolute liver and spleen weights of F₀ maternal rats were significantly higher ($P < 0.01$ or 0.05) than those of the controls (7% and 11% higher, respectively). In F₀ males and females at the same dose, relative liver and spleen weights were significantly higher ($P < 0.01$ or 0.05) than those of the controls (17% and 6% higher, respectively, in males; 15% and 16% higher,

respectively, in females). In F₁ females, absolute liver and spleen weights were significantly higher ($P < 0.01$ or 0.05) than those of the controls at 5000 ppm (16% higher for both), but there were no differences in males. Relative liver and spleen weights were significantly higher ($P < 0.01$) than those of the controls in 5000 ppm F₁ males (10% and 19%, respectively) and females (21% and 26%, respectively). In F₂ male pups, the absolute weights of the spleen and thymus were significantly lower than those of the controls (26% and 22% lower, respectively), but there was no similar effect in females or any difference in relative organ weights. None of the differences in organ weights in this study was accompanied by any histopathological findings.

The NOAEL for reproductive toxicity was 5000 ppm (equal to 328.3 mg/kg bw per day), the highest dietary concentration tested. The NOAEL for parental toxicity was 60 ppm (equal to 3.9 mg/kg bw per day), for lower body weight, reduced body weight gain and reduced feed consumption at 1000 ppm (equal to 63.8 mg/kg bw per day). The NOAEL for offspring toxicity was 1000 ppm (equal to 63.8 mg/kg bw per day) for lower F₁ and F₂ pup weights at 5000 ppm (equal to 328.3 mg/kg bw per day) (Bussi, 1999).

(b) *Developmental toxicity*

Rats

Fenamidone (99.8% purity) in 0.5% (w/v) carboxymethyl cellulose was administered by gavage to groups of 25 sperm-positive CrI: CD (SD) BR rats at a dose of 0, 25, 150 or 1000 mg/kg bw per day from day 6 to day 15 of gestation. Dams were observed daily throughout gestation for clinical signs of toxicity, with body weight and feed consumption recorded throughout this period. On day 20 of gestation, surviving dams were killed and fetuses examined for external, visceral and skeletal abnormalities.

There were no maternal deaths or treatment-related clinical signs. At 1000 mg/kg bw per day, dams lost a small amount (~8 g) of body weight over the first 2 days of dosing, with mean body weight gain significantly lower ($P < 0.01$) than in the controls from days 6 to 16 of gestation (35% lower). Feed consumption was significantly lower than in the controls from days 6 to 9 and from days 9 to 12 (40% and 15% lower, respectively). There were no treatment-related macroscopic findings in dams. Other than significantly lower ($P < 0.01$) fetal weight at 1000 mg/kg bw per day (5% lower than in the controls in both sexes), there were no treatment-related effects on litter parameters.

There were no treatment-related external abnormalities. Internal examination revealed a slight increase in the incidence of enlarged thymus at 150 and 1000 mg/kg bw per day (4.1% and 4.4%, respectively, compared with the control incidence of 1.1%); however, these values were well below the historical control mean (8.5%) and maximum (14.7%). The slight increase in incomplete ossification at 1000 mg/kg bw per day (Table 21) was not statistically significant and was considered a secondary effect of the reduction in fetal weight.

Table 21. Summary of skeletal fetal findings in rats

Observation	Incidence of finding (%)			
	0 mg/kg bw per day	25 mg/kg bw per day	150 mg/kg bw per day	1 000 mg/kg bw per day
Interparietal bone incompletely ossified				
Fetal incidence	1.5	3.2	3.3	5.6
Litter incidence	12	21.7	18.2	25
Hyoid body incompletely ossified				
Fetal incidence	7	7	8	15
Litter incidence	5	6	5	11

Source: Foulon & Wason (1997)

The NOAEL for maternal toxicity was 150 mg/kg bw per day for reduced body weight gain and feed consumption at 1000 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 150 mg/kg bw per day for lower fetal weight at 1000 mg/kg bw per day (Foulon & Wason, 1997).

Rabbits

Groups of 30 pregnant New Zealand White rabbits were exposed to fenamidone (purity 99.8%) in 0.5% (w/v) aqueous carboxymethyl cellulose by gavage from days 6 to 28 of gestation at a dose of 0, 10, 30 or 100 mg/kg bw per day. Dams were observed daily throughout gestation for clinical signs of toxicity, with body weight and feed consumption recorded regularly. Surviving dams were killed on day 29 of gestation and necropsied, with standard litter parameters recorded. Fetuses were examined for external, visceral and skeletal abnormalities.

There were no treatment-related deaths or clinical signs. Significantly reduced ($P < 0.01$) body weight gain occurred in dams at 100 mg/kg bw per day from days 6 to 10 of gestation (10 g versus 50 g in the control), but not from days 6 to 8 of gestation, when feed consumption was approximately 15% lower ($P < 0.01$) than in the controls. There were no significant intergroup differences in feed consumption at other times or in absolute body weight at any time. Mean absolute liver weight was significantly higher ($P < 0.01$) than in the controls at 30 and 100 mg/kg bw per day (19% and 37% higher, respectively). Litter parameters were unremarkable. There were no treatment-related external, visceral or skeletal abnormalities in fetuses.

The NOAEL for maternal toxicity was 30 mg/kg bw per day for decreased body weight gain at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested (Foulon, 1999).

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study, fenamidone (purity 99.8%) in 0.5% (w/v) aqueous carboxymethyl cellulose was administered by gavage to groups of 10 Crl:CD Br rats of each sex at a dose of 0, 125, 500 or 2000 mg/kg bw. Observations for mortalities and clinical signs were made daily. Body weight was recorded prior to dosing, on the day of dosing and weekly thereafter. Feed consumption was recorded weekly. A functional observational battery was performed on all rats pretreatment and at 4 hours, 7 days and 14 days after dosing. Survivors were killed after 14 days, and nervous tissue was examined histopathologically.

There were no deaths. Clinical signs observed 24 hours after dosing included soiling of the anogenital region (2/10 males at 2000 mg/kg bw) or stained/wet anogenital region (1/10 females at 500 mg/kg bw and 2/10 females at 2000 mg/kg bw). Abnormalities observed during the functional observational battery were confined to the 4-hour time point and included increased urination in 2000 mg/kg bw females (6/10 compared with 1/10 in the controls; $P < 0.01$); soiling or staining of the fur around the anogenital region (2/10 males at 2000 mg/kg bw; 4/10 and 5/10 females at 500 and 2000 mg/kg bw, respectively; $P < 0.05$); mucoid faeces at 500 and 2000 mg/kg bw (2/10 males and 3/10 females at both doses); hunched posture (3/10 females at 2000 mg/kg bw; $P < 0.05$); unsteady gait (2/10 and 2/10 females at 500 and 2000 mg/kg bw, respectively); significantly lower ($P < 0.01$) rectal temperature in 2000 mg/kg bw females (0.3 °C lower than the control); and significantly reduced locomotor activity at 2000 mg/kg bw in both sexes ($P < 0.01$ in females and $P < 0.05$ in males) at 4 hours after dosing. There were no treatment-related histopathological abnormalities of the brain or nervous tissues.

The NOAEL was 125 mg/kg bw for nonspecific findings in the functional observational battery at 4 hours after dosing at 500 mg/kg bw (Hughes & Crome, 1999).

In a 13-week neurotoxicity study, groups of 10 Crl:CD BR rats of each sex per group were exposed to fenamidone (purity 98.9%) at a dietary concentration of 0, 150, 1000 or 5000 ppm. The mean achieved doses over 13 weeks were 0, 11.2, 73.5 and 392.3 mg/kg bw per day for males and 0,

12.7, 83.4 and 414.2 mg/kg bw per day for females at 0, 150, 1000 and 5000 ppm, respectively. Mortalities, clinical signs, body weight and feed consumption were recorded regularly throughout the study. A functional observational battery and motor activity assessment were performed prior to the commencement of dosing and then after 4, 8 and 13 weeks. Survivors were killed at the end of the exposure period and their brain weights recorded. Nervous tissue from five rats of each sex per dose was examined histopathologically.

There were no treatment-related deaths or clinical signs. Mean overall body weight gain (weeks 0–13) was significantly lower ($P < 0.01$) than in the controls at 5000 ppm (26% and 21% lower in males and females, respectively). Feed consumption was also significantly lower ($P < 0.05$) than in the controls at 5000 ppm (6% and 9% lower in males and females, respectively). There were no treatment-related functional observational battery findings or effects on locomotor activity. There were no intergroup differences in brain weight and no treatment-related histopathological abnormalities of the brain or nervous tissues.

The NOAEL was 1000 ppm (equal to 73.5 mg/kg bw per day) for reduced body weight gain at 5000 ppm (equal to 392.3 mg/kg bw per day) (Hooks, 2001).

In a developmental neurotoxicity study, fenamidone (purity 99.2%) was admixed in the diet at concentrations of 0, 60, 250, 1000 and 4700 ppm and fed ad libitum to groups of 30 pregnant female Wistar rats from day 6 of gestation to day 21 of lactation. The mean achieved doses in maternal rats over gestation and lactation were 0, 5.5, 23, 92.3 and 429 mg/kg bw per day at 0, 60, 250, 1000 and 4700 ppm, respectively. Observations for deaths and clinical signs were made regularly. Body weight and feed consumption were recorded regularly. Maternal rats were subjected to a functional observational battery during days 13 and 30 of gestation and days 11 and 21 of lactation. Following delivery, pups were examined and weighed. On postnatal days 4, 11, 21, 35, 45 and 60, at least 10 pups of each sex per dose were subjected to a functional observational battery. Pup weights were recorded on postnatal days 0, 4, 11, 17 and 21 and weekly thereafter. Female pups were examined daily for vaginal patency from postnatal day 29, whereas males were examined for preputial separation from postnatal day 38. A motor activity assessment was performed on one male and/or one female from each litter on postnatal days 13, 17, 21 and 60. The acoustic startle response was evaluated on postnatal days 22 and 60 on one male and/or one female from each litter. Ophthalmoscopy was performed on at least 10 rats of each sex per dose at about 50–60 days of age. Following sacrifice, all rats were necropsied, brain morphometry was recorded and brain tissue was examined histopathologically in the control and high-dose groups.

There were no treatment-related maternal deaths or clinical signs. Maternal body weight gain was significantly lower ($P < 0.05$) than in the controls from day 0 to day 20 of gestation at 4700 ppm (8% lower). There were no significant intergroup differences in maternal body weight gain during gestation. Feed consumption was unaffected by treatment. The functional observational battery was unremarkable.

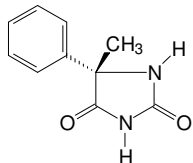
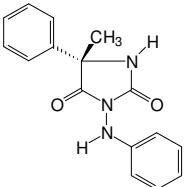
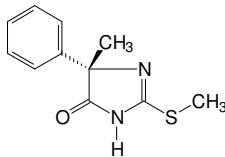
In offspring, there were no treatment-related deaths or clinical signs. At the highest dose, mean absolute body weight was significantly lower ($P < 0.01$) than in the controls on postnatal days 4, 11, 17 and 21 (up to 10% lower than in the controls in both sexes). Mean body weight gain was also significantly lower ($P < 0.01$ or 0.05) than in the controls during lactation (up to 20% lower than in the controls in both sexes). There were no treatment-related functional observational battery findings and no effects on motor activity, startle response, the incidence of gross lesions, brain weight or brain morphometry.

The NOAEL for maternal toxicity was 1000 ppm (equal to 92.3 mg/kg bw per day) for reduced body weight gain at 4700 ppm (equal to 429 mg/kg bw per day). The NOAEL for offspring toxicity was also 1000 ppm (equal to 92.3 mg/kg bw per day), for lower absolute body weight and reduced body weight gain at 4700 ppm (equal to 429 mg/kg bw per day) (Sheets, Gilmore & Hoss, 2005).

(b) *Studies on metabolites*

Additional toxicity studies were conducted on three fenamidone metabolites: RPA 412636 (*S*-enantiomer of RPA 717879), RPA 410193 (*S*-enantiomer of RPA 405862) and RPA 412708 (*S*-enantiomer of RPA 408056). Details of these metabolites are provided in Table 22.

Table 22. Fenamidone metabolites on which additional toxicity tests were conducted

Code	Chemical name (IUPAC)	Structure	Description
RPA 412636	(<i>S</i>)-5-Methyl-5-phenyl-2,4-imidazolidine-dione		Present in rat urine at < 1% of the administered dose
RPA 410193	(5 <i>S</i>)-5-Methyl-5-phenyl-3-(phenylamino)-2,4-imidazolidine-dione		Not detected in rat metabolism studies; identified as a metabolite in grapes, grape juice and wine
RPA 412708	(5 <i>S</i>)-5-Methyl-2-(methylthio)-5-phenyl-3,5-dihydro-4 <i>H</i> -imidazol-4-one		Biliary or faecal metabolite in rats (> 10% of the administered dose)

IUPAC: International Union of Pure and Applied Chemistry

Acute toxicity

The results of acute oral (gavage) toxicity tests of fenamidone metabolites in SD rats are summarized in Table 23. Sedation, lateral recumbency, piloerection, dyspnoea, coma, tremors and hypoactivity were observed from 1 to 7 days after dosing with RPA 412636 at 500, 1000 or 2000 mg/kg bw, with recovery in survivors evident from 4 to 6 days after dosing (Manciaux, 1999a). No clinical signs were observed in the acute toxicity study on RPA 410193 (Manciaux, 1999b). A range of clinical signs was observed at and above a single dose of RPA 412708 at 100 mg/kg bw, including hypoactivity or sedation, piloerection, dyspnoea, lateral recumbency and ptosis; these signs resolved within 2 days (Manciaux, 1999c). Reduced body weight gain was also noted at 150 or 200 mg/kg bw.

Table 23. Results of studies of acute oral toxicity of fenamidone metabolites in SD rats

Metabolite/strain	Sex	Purity (%)	Vehicle	LD ₅₀ (mg/kg bw)	Reference
RPA 412636					
ICO: OFA-SD (IOPS Caw)	Male and female	99.8	0.5% CMC	1 520	Manciaux (1999a)
RPA 410193					
ICO: OFA-SD (IOPS Caw)	Male and female	99.8	0.5% CMC	> 2 000	Manciaux (1999b)
RPA 412708					
ICO: OFA-SD (IOPS Caw)	Male and female	99.8	0.5% CMC	100–200	Manciaux (1999c)

CMC: carboxymethyl cellulose; LD₅₀: median lethal dose

Short-term studies of toxicity

In a non-guideline study, RPA 412636 (purity 99.8%) was admixed in the diet at a concentration of 0, 300, 1200 or 3000 ppm and fed ad libitum to groups of five ICO: OFA-SD (IOPS Caw) rats of each sex per dose for 14 days. The mean achieved doses were 0, 23, 90 and 215 mg/kg bw per day for males and 0, 24.5, 96.7 and 233 mg/kg bw per day for females at 0, 300, 1200 and 3000 ppm, respectively. A satellite group of three rats of each sex was added to the control and high-dose groups and killed after 3 days for the analysis of liver weight, hepatic cellular proliferation and liver histopathology. Rats were observed daily for mortalities and clinical signs. Body weight was recorded on days 1, 7 and 14. Feed consumption was recorded weekly. Blood was sampled prior to terminal sacrifice for the analysis of standard haematology and clinical chemistry parameters. Following necropsy, the following organs were weighed: brain, kidneys, liver, ovaries, spleen, testes and thyroid. The following organs were examined histopathologically: duodenum, kidneys, liver, ovaries, spleen, testes and thyroid gland. Hepatic cellular proliferation was assessed by immunostaining. Microsomes were prepared for the analysis of cytochrome P450 isoenzyme profile.

There were no deaths or clinical signs. During the 1st week of treatment, mean absolute body weight was significantly lower ($P < 0.05$) than that of the controls in 3000 ppm males (4% lower). At 3000 ppm, body weight gain was significantly lower ($P < 0.05$) than that of the controls in both sexes (males: 40% lower than in the controls; females: body weight loss of 0.5 g). Significantly reduced ($P < 0.05$) feed consumption also occurred at this same dose during the 1st week (14% lower in males and 17% lower in females). Haematology parameters were unremarkable. In 3000 ppm females, urea and cholesterol were increased by 26% ($P < 0.05$) and 41% ($P < 0.01$), respectively.

The relative liver weight of high-dose males killed after 3 days of treatment was approximately 18% higher than that of the controls ($P < 0.05$). Mild liver enlargement was noted macroscopically in two males and one female at 3000 ppm. Hepatocytic mitosis was observed histopathologically in all males and two females at 3000 ppm. Mild hepatic (mainly centrilobular) hypertrophy was observed in all 3000 ppm males.

In males killed after 14 days of treatment, mean absolute and relative liver weights were significantly higher ($P < 0.01$) than those of the controls at 1200 and 3000 ppm (absolute: 30% and 34%; and relative: 28% and 40%, respectively). In 3000 ppm females, relative liver weight was significantly higher ($P < 0.05$) than that of the controls (16%). Enlargement of the liver was observed macroscopically in all males at 1200 and 3000 ppm in association with prominent lobulation in two or three males. One 500 ppm male and one female at each of 1200 and 3000 ppm had liver enlargement without prominent lobulation. Histopathology revealed a dose-related increase in the incidence and grade of hepatic hypertrophy in both sexes (Table 24). In the kidneys of all treated groups of males, an increase of eosinophilic droplets occurred in cortical tubular cells (Table 24). Additionally, multifocal cortical tubular hyaline degeneration occurred in all 1200 and 3000 ppm males and in one 500 ppm male. Staining of α_{2u} -globulin was evident in all 3000 ppm males (one graded as moderate, four graded as marked) compared with only one control male (graded as moderate). These kidney findings in males are not considered relevant to humans.

The results of the liver enzyme analysis are summarized in Table 25. There was a slight increase in total cytochrome P450, whereas BROD and PROD were increased, markedly consistent with the effect of phenobarbital. There was no treatment-related effect on EROD or lauric acid hydroxylation. In rats killed after 3 days, the numbers of PCNA positive cells in the liver were increased by 253% in males and by 276% in females at 3000 ppm, relative to the control group. In rats killed at the end of the study, the numbers of PCNA positive cells were increased at and above 300 ppm (6%, 44% and 133% in males and 14%, 0% and 66% in females at 300, 1200 and 3000 ppm, respectively).

The NOAEL was 1200 ppm (equal to 90 mg/kg bw per day) for reduced body weight gain and feed consumption at 3000 ppm (equal to 215 mg/kg bw per day) (Bigot, 1999c).

Table 24. Histopathological observations in rats following 14 days of dietary exposure to the fenamidone metabolite RPA 412636

Parameter	Incidence of finding (n = 5)							
	0 ppm		300 ppm		1 200 ppm		3 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Hepatocytic hypertrophy								
- Slight	0	0	4	0	0	1	0	1
- Mild	0	0	0	0	2	2	0	1
- Moderate	0	0	0	0	2	0	1	3
- Marked	0	0	0	0	1	0	4	0
- Total	0	0	4	0	5	3	5	5
Eosinophilic droplets (kidney)								
- Slight	1	0	2	0	0	0	0	0
- Mild	0	0	1	0	3	0	0	0
- Moderate	0	0	1	0	2	0	5	0
- Total	1	0	4	0	5	0	5	0
Tubular degeneration								
- Slight	1	0	1	0	1	0	2	0
- Mild	0	0	0	0	2	0	2	0
- Moderate	0	0	0	0	2	0	1	0
- Total	1	0	1	0	5	0	5	0

Source: Bigot (1999c)

Table 25. Induction of rat liver enzymes by the fenamidone metabolite RPA 412636

Mean fold increase relative to the solvent control											
RPA 412636						Positive controls					
300 ppm		1 200 ppm		3 000 ppm		PB		β-NF		CLA	
Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Total cytochrome P450											
1.8	1.25	2.2	1.3	2.3	1.5	2.2	1.8	1.3	1.5	1.2	0
BROD											
35	14	44	18	56	24	43	31	2.1	2.3	—	—
PROD											
20	17	27	22	39	39	28.8	20	3.4	5.1	—	—

β-NF: beta-naphthoflavone at 75 mg/kg bw per day; BROD: benzoxyresorufin *O*-debenzylation; CLA = clofibric acid at 300 mg/kg bw per day; PB: phenobarbital at 75 mg/kg bw per day; PROD: pentoxyresorufin *O*-debenzylation

Source: Bigot (1999c)

In a similarly designed 14-day study, RPA 410193 (purity 99.8%) was admixed in the diet at concentrations of 0, 450, 4500 and 15 000 ppm and fed ad libitum to groups of five ICO: OFA-SD (IOPS Caw) rats of each sex per dose. The achieved doses were 0, 30, 299 and 1098 mg/kg bw per

day for males and 0, 37, 374 and 1133 mg/kg bw per day for females at 0, 450, 4500 and 15 000 ppm, respectively.

There were no deaths or treatment-related clinical signs or effects on body weight or feed consumption. At the highest dose, small reductions in red blood cells (males), haemoglobin (females) and haematocrit (females) were considered to be incidental findings. Cholesterol was significantly higher ($P < 0.01$) than in the controls at 4500 and 15 000 ppm (1.4- and 2-fold higher in males, respectively; 1.5- and 1.7-fold higher in females, respectively). In high-dose rats killed on day 4, absolute and relative liver weights were higher than those of the controls (absolute: 13% and 42% in males and females, respectively; relative: 14% and 44% in males and females, respectively), with only the relative weight of males significantly different from that of the controls ($P < 0.05$). No gross pathological changes were observed in these interim-killed rats, whereas slight centrilobular hypertrophy was observed histopathologically (two males and one female).

In rats killed on day 15, both relative and absolute liver weights were significantly higher ($P < 0.01$ or 0.05) than those of the controls at 4500 and 15 000 ppm. The magnitude of this increase was approximately 35% and 50% for both absolute and relative liver weights in males at 4500 and 15 000 ppm, respectively. In females, the increases were approximately 17% and 30% for both absolute and relative weights at 4500 and 15 000 ppm, respectively. Macroscopically, liver enlargement was noted in all high-dose rats and all but one female at 4500 ppm; prominent lobulation and/or a dark coloration were associated with this enlargement. Histopathological examination revealed centrilobular hypertrophy at 4500 ppm (all males and 3/5 females) and 15 000 ppm (all rats), with the severity of this finding more pronounced in males. Histopathological findings in the thyroid gland comprised follicular cell hypertrophy characterized by a high columnar epithelial lining of the thyroid follicles and the presence of smaller follicles and loss of colloid (1/5, 2/5, 5/5 and 4/5 males and 0/5, 0/5, 4/5 and 4/5 females at 0, 450, 4500 and 15 000 ppm, respectively).

Results of liver enzyme analysis in rats killed on day 4 are given in Table 26. There was a dose-related increase in total cytochrome P450 in males, with a relatively modest increase in high-dose females. There was no treatment-related effect on EROD, whereas BROD and PROD were increased in both sexes. There was no effect on lauric acid hydroxylation. Cell cycling analysis of rats killed on day 4 revealed an approximate 5-fold increase in PCNA positive cells in high-dose rats.

Table 26. Induction of rat liver enzymes by fenamidone metabolite RPA 410193

Mean fold increase relative to the solvent control											
RPA 410193						Positive controls					
450 ppm		4 500 ppm		15 000 ppm		PB		β -NF		CLA	
Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Total cytochrome P450											
1.4	0	1.9	0	2.2	1.4	2.2	1.8	1.3	1.5	1.2	0
BROD											
6	0	66	24	78	102	43	31	2.1	2.3	–	–
PROD											
1.2	0	16	9.7	15.5	23	28.8	20	3.4	5.1	–	–

β -NF: beta-naphthoflavone at 75 mg/kg bw per day; BROD: benzoxyresorufin *O*-debenzylation; CLA = clofibric acid at 300 mg/kg bw per day; PB: phenobarbital at 75 mg/kg bw per day; PROD: pentoxyresorufin *O*-depylation

Source: Chuzel (1999a)

The NOAEL was 450 ppm (equal to 30 mg/kg bw per day) for histopathological findings in the thyroid (follicular cell hypertrophy, smaller follicles and loss of colloid) at 4500 ppm (equal to 299 mg/kg bw per day) (Chuzel, 1999a).

In another non-guideline study, RPA 412708 (purity 98%) was admixed in the diet at concentrations of 0, 200, 500 and 2000 ppm and fed ad libitum to groups of five Wistar (AF) rats RJ: WI (IOPS AF) rats of each sex per dose for 14 days. The achieved doses were, respectively, 0, 15.1, 38.5 and 150.5 for males and 0, 16.1, 37.8 and 147.3 mg/kg bw per day for females.

There were no deaths or treatment-related clinical signs. There were no treatment-related effects on body weight, feed consumption, haematology or clinical chemistry parameters. In rats killed at the end of the study, mean absolute liver weight was significantly higher ($P < 0.05$; 18%) at 2000 ppm than in the controls, with relative liver weight significantly increased at 500 ppm ($P < 0.05$; 8%) and 2000 ppm ($P < 0.01$; 22%). There were no treatment-related macroscopic or histopathological findings. There was no treatment-related induction of cytochrome P450 or increase in BROD, PROD or EROD. In rats killed after 3 days of treatment, an approximate 4.5-fold increase in cell proliferation in the liver occurred at 2000 ppm, but no such effect was evident in rats killed at the end of the study.

The NOAEL was 2000 ppm (equal to 147.3 mg/kg bw per day), the highest dietary concentration tested (Chuzel, 1999b).

In a 90-day study, RPA 412636 (purity 99.8%) was admixed in the diet at concentrations of 0, 100, 500 and 2500 ppm and fed ad libitum to groups of 10 ICO: OFA-SD (IOPS Caw) rats of each sex per dose. The mean achieved doses were 0, 6.4, 33 and 162 mg/kg bw per day for males and 0, 7.7, 39 and 196 mg/kg bw per day for females at 0, 100, 500 and 2500 ppm, respectively. Rats were observed daily for mortalities and clinical signs. Body weight and feed consumption were measured weekly. A neurotoxicity assessment was conducted pretreatment and during week 12, comprising a grasping reflex, righting reflex, corneal reflex, pupillary reflex, auditory startle reflex and head shaking reflex. An ophthalmological examination was performed pretreatment and during week 12. Blood was sampled during weeks 2 and 13 for the analysis of T_3 , T_4 and TSH. Blood sampled during week 12 was also analysed for the standard range of haematology and clinical chemistry parameters. Urine was collected overnight prior to necropsy for urine analysis. Decedents and rats killed at the end of the designated exposure period were necropsied, organs weighed and tissues examined histopathologically.

There were no treatment-related mortalities, clinical signs, neurotoxicity or ophthalmological findings. Body weight was unaffected by treatment. Feed consumption was significantly lower ($P < 0.05$) than in the controls at 2500 ppm at weeks 1 (both sexes, ~9% lower), 6 (males, 12% lower) and 11 (females, 11% lower).

Selected haematology and clinical chemistry findings are summarized in Table 27. Haematological effects included significantly reduced ($P < 0.01$ or 0.05) haematocrit, mean corpuscular volume and mean corpuscular haemoglobin and increased prothrombin time at 2500 ppm, with reduced haematocrit also occurring in 500 ppm males. Treatment-related clinical chemistry findings at the highest dose included significantly increased ($P < 0.01$ or 0.05) ALAT (34% in males), creatinine (31% in males), cholesterol (30% in both sexes), triglycerides (65% in females), albumin (7% in females), phosphorus (17% and 25% in males and females, respectively), potassium and calcium (5% in females). Thyroid hormone analysis was unremarkable. Urine analysis revealed an increase in crystals in 2500 ppm females (1/10, 2/10, 6/10 and 9/10 rats at 0, 100, 500 and 2500 ppm, respectively).

Selected organ weight and histopathological findings are presented in Table 28. Absolute and relative liver weights were significantly increased ($P < 0.01$) at 500 and 2500 ppm in males and at 2500 ppm in females. Macroscopically, livers appeared enlarged at 500 (two males) and 2500 ppm (nine males and one female), with prominent lobulation observed in one, two and five males at 100, 500 and 2500 ppm, respectively; four high-dose females also had prominent lobulation. Microscopically, hypertrophy of centrilobular hepatocytes was observed at 500 and 2500 ppm in both sexes, with vacuolation also observed at these doses. In high-dose males, absolute and relative adrenal weights were significantly higher than in the controls. Minimal diffuse hypertrophy of the zona

fasciculata was observed microscopically in three males at 2500 ppm. Thymus weights were significantly lower than in the controls ($P < 0.01$) in high-dose rats, without any accompanying pathology. In the kidneys, there was a slight increase in eosinophilic droplets (graded as slight) in the proximal tubules in high-dose males (1/10, 0/10, 2/10 and 6/10 at 0, 100, 500 and 2500 ppm, respectively). This finding is consistent with α_{2u} -globulin staining, which was observed in the 14-day study by Bigot (1999c).

Table 27. Haematology and clinical chemistry findings in rats exposed to metabolite RPA 412636 for 90 days

Parameter	0 ppm		100 ppm		500 ppm		2 500 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Haematology^a								
Hct (L/L)	0.48	0.45	0.47	0.44	0.46*	0.44	0.46*	0.44
MCV (fL)	50.3	52.1	49.3	51.9	49.0	51.8	47.1**	50.0*
MCH (pg)	17.18	17.94	16.81	17.85	16.97	17.81	16.29**	17.34**
PT (s)	12.01	12.55	11.78	12.75	12.42	12.54	14.13**	12.33
Clinical chemistry^a								
ALAT (IU/L)	21.0	17.8	21.3	17.5	21.6	17.0	28.1*	20.9
Creatinine (μ mol/L)	22.2	26.8	24.0	29.2	24.9	25.5	29.0**	26.5
Cholesterol (mmol/L)	2.26	2.40	2.33	2.57	2.36	2.50	2.95**	3.10**
Triglycerides (mmol/L)	0.71	0.40	0.80	0.38	0.71	0.41	0.50	0.66**
Albumin (g/L)	32.7	33.0	32.5	34.6	32.8	34.6	34.3	35.2*
Phosphorus (mmol/L)	1.79	1.39	1.82	1.54	1.90	1.49	2.01**	1.74**
Potassium (mmol/L)	3.97	3.51	3.99	3.72	3.92	3.68	3.97	3.77*
Calcium (mmol/L)	2.66	2.58	2.69	2.64	2.71	2.61	2.73	2.70**

ALAT: alanine aminotransferase; Hct: haematocrit; IU: International Units; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; PT: prothrombin time; *: $P < 0.05$; **: $P < 0.01$

^a Results expressed as means.

Source: Dange (1999b)

The NOAEL was 100 ppm (equal to 6.4 mg/kg bw per day) for vacuolation of centrilobular hepatocytes in males at 500 ppm (equal to 33 mg/kg bw per day) (Dange, 1999b).

RPA 410193 (purity 99.8%) was admixed in the diet at concentrations of 0, 150, 1500 and 15 000 ppm and fed ad libitum to groups of 10 ICO: OFA-SD (IOPS Caw) rats of each sex per dose for 90 days. The achieved doses were 0, 9.3, 93.3 and 978 mg/kg bw per day for males and 0, 11.5, 115 and 1090 mg/kg bw per day for females at 0, 150, 1500 and 15 000 ppm, respectively. End-points and sampling intervals were consistent with those analysed in the preceding study by Dange (1999b).

There were no deaths, treatment-related clinical signs or effects on body weight or feed consumption. There were no treatment-related ophthalmological or neurotoxicity findings. Selected haematology and clinical chemistry findings are summarized in Table 29. Perturbations in red cell parameters occurred at the highest dose and included significantly lower ($P < 0.01$ or 0.05) red blood cells, haemoglobin, haematocrit and mean corpuscular haemoglobin concentration (both sexes), with

Table 28. Organ weights and histopathological findings in rats exposed to metabolite RPA 412636 for 90 days

Parameter	0 ppm		100 ppm		500 ppm		2 500 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Organ weights^a								
<i>Liver</i>								
Absolute (g)	10.98	6.39	12.00	6.63	13.68**	6.70	16.70**	8.00**
Relative ^b (%)	2.45	2.57	2.56	2.53	2.84*	2.70	3.69**	3.30
<i>Adrenals</i>								
Absolute (g)	0.065	0.072	0.066	0.077	0.068	0.078	0.087**	0.085
Relative (%)	0.015	0.029	0.014	0.030	0.014	0.032	0.019**	0.039
<i>Thymus</i>								
Absolute (g)	0.426	0.297	0.388	0.279	0.377	0.276	0.242**	0.194**
Relative (%)	0.096	0.119	0.083	0.107	0.078	0.112	0.054**	0.081**
Liver histopathology^c								
Hypertrophy of centrilobular hepatocytes								
- Minimal	0	0	0	0	5	3	0	1
- Slight	0	0	0	0	5	0	0	0
- Moderate	0	0	0	0	0	0	10	9
- Total	0	0	0	0	10	3	10	0
Vacuolation of centrilobular hepatocytes								
- Minimal	0	0	0	0	5	0	8	1

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

^a Results expressed as means.

^b Relative to body weight.

^c Results expressed as the absolute number of rats with the finding.

Source: Dange (1999b)

increased mean corpuscular volume in females. Cholesterol was significantly increased ($P < 0.01$) in males at 1500 and 15 000 ppm and in females at 15 000 ppm. Triglycerides were significantly elevated ($P < 0.01$) in high-dose males. Total protein was significantly higher ($P < 0.01$ or 0.05) than in the controls at 15 000 ppm, with elevated albumin also noted at 1500 and 15 000 ppm in both sexes.

Mean urine volume was approximately 2.9-fold higher ($P < 0.01$) than in the controls in high-dose females. Hormone analysis conducted in blood sampled during week 2 detected a significant increase (17% higher than in the controls, $P < 0.05$) in T_4 and a significant increase (50%; $P < 0.05$) in TSH in high-dose males; no differences were noted in females, in T_3 or in either sex at week 12.

The only effect on organ weights was a significant increase ($P < 0.01$ or 0.05) in absolute and relative liver weights at 1500 and 15 000 ppm in males and at 1500 ppm in females. In males, absolute liver weights were approximately 15% and 43% higher than in the controls at 1500 and 15 000 ppm, respectively, whereas relative liver weight was approximately 13% and 51% higher than in the controls, respectively. In females, absolute and relative liver weights were approximately 42% and 43% higher than in the controls at 15 000 ppm. At necropsy, liver enlargement was observed macroscopically (1/10, 5/10 and 9/10 males and 0/10, 0/10 and 9/10 females at 150, 1500 and 15 000

Table 29. Haematology and clinical chemistry findings in rats exposed to metabolite RPA 410193 for 90 days

Parameter	0 ppm		150 ppm		1 500 ppm		15 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Haematology^a								
RBC (1×10^{12})	9.68	8.51	9.30	8.71	9.48	8.52	9.00**	7.70**
Hb (g/100 mL)	15.56	14.91	15.35	15.05	15.58	14.75	14.54**	13.73**
Hct (L/L)	0.47	0.46	0.47	0.45	0.47	0.44	0.45**	0.42*
MCV	48.5	52.3	50.2	51.6	49.6	51.7	50.2	54.4*
MCHC (g/100 mL)	33.21	33.57	32.96	33.55	33.21	33.50	32.31**	32.85*
Clinical chemistry^a								
Cholesterol (mmol/L)	2.03	2.28	2.13	2.12	2.81**	2.45	4.10**	3.69**
Triglycerides (mmol/L)	0.61	0.37	0.74	0.37	0.88	0.43	1.21**	0.59
Total protein (g/L)	70.2	67.4	69.7	68.1	72.8	70.6	73.4*	74.3**
Albumin (g/L)	30.3	31.5	30.1	32.7	32.5**	33.5*	31.7*	33.7*

Hb: haemoglobin; Hct: haematocrit; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; RBC: red blood cells; *, $P < 0.05$; **, $P < 0.01$

^a Results expressed as means.

Source: Dange (1999c)

Table 30. Incidence of hypertrophy of centrilobular hepatocytes in rats exposed to metabolite RPA 410193 for 90 days

Grading	Incidence of hypertrophy of centrilobular hepatocytes ($n = 10$) ^a							
	0 ppm		150 ppm		1 500 ppm		15 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Minimal	0	0	0	0	9	2	0	0
Slight	0	0	0	0	1	0	8	3
Moderate	0	0	0	0	0	0	2	7
Total	0	0	0	0	10	2	10	10

^a Results expressed as the absolute number of rats with the finding.

Source: Dange (1999c)

ppm, respectively). Hypertrophy of centrilobular hepatocytes was observed histopathologically at 1500 and 15 000 ppm in both sexes (Table 30).

The NOAEL was 1500 ppm (equal to 93.3 mg/kg bw per day) for perturbations in red cell parameters at 15 000 ppm (equal to 978 mg/kg bw per day) (Dange, 1999c).

Genotoxicity

The results of genotoxicity studies on fenamidone metabolites are summarized in Table 31.

Table 31. Results of genotoxicity assays on fenamidone metabolites

End-point	Test object	Concentration	Purity (%)	Results	Reference
RPA 412636					
<i>In vitro studies</i>					
Gene mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535 and TA1537 <i>Escherichia coli</i> WP2uvrA	8–5 000 µg/plate (±S9) DMSO vehicle	99.8	Negative	Beevers (1999a)
Gene mutation	Mouse lymphoma cells (L5178Y)	100–1 600 µg/mL (±S9) DMSO vehicle	99.8	Negative	Fellows (1999d)
<i>In vivo studies</i>					
Micronucleus	CD-1 mice, bone marrow (5 males/group)	0, 75, 150 or 300 mg/kg bw, 2 × ip; killed 24 h after second dose 0.5% (w/v) CMC vehicle	99.8	Negative ^a	Whitewell (1999a)
RPA 410193					
<i>In vitro studies</i>					
Gene mutation	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537 <i>E. coli</i> WP2uvrA	8–5 000 µg/plate (±S9) DMSO vehicle	> 99.8	Negative	Beevers (1999b)
Gene mutation	Mouse lymphoma cells (L5178Y)	50–800 µg/mL (±S9) DMSO vehicle	> 99.8	Negative	Fellows (1999e)
<i>In vivo studies</i>					
Micronucleus	CD-1 mice, bone marrow (8 males/group)	0, 500, 1 000 or 2 000 mg/kg bw, 2 × ip; killed 24 h after second dose 0.5% (w/v) CMC vehicle	99.8	Negative	Whitewell (1999b)
RPA 412708					
<i>In vitro studies</i>					
Gene mutation	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537 <i>E. coli</i> WP2uvrA	8–5 000 µg/plate (±S9) DMSO vehicle	98	Negative	Beevers (1999c)
<i>In vivo studies</i>					
Micronucleus	CD-1 mice, bone marrow (8 males/group)	0, 37.5, 75 or 150 mg/kg bw, 2 × ip; killed 24 h after second dose 1% (w/v) CMC vehicle	98	Negative ^b	Whitewell (1999c)

CMC: carboxymethyl cellulose; DMSO: dimethyl sulfoxide; ip: intraperitoneal, S9, 9000 × g supernatant fraction of rat liver homogenate

^a Clinical signs observed at highest dose (lethargy and piloerection).

^b Clinical signs observed at the middle and high doses (lethargy and eye closure at 75 mg/kg bw; prostration, tremors, eye closure, abnormal breathing, lethargy and piloerection at 150 mg/kg bw).

3. Observations in humans

Steffens (2013) examined the occurrence of occupational medical experiences in 15 workers at a fenamidone processing plant in Mumbai, India, from 2008 until present. It was stated that workers wore personal protective equipment, including a protective suit, chemical-resistant gloves, safety glasses and dust mask. Workers were examined annually. Laboratory examinations included a full blood count, urine examination, fasting blood sugar, lipid profile, liver enzymes, creatinine and TSH. Technical examinations included vision testing, ultrasonography, electrocardiogram and lung function test. Additionally, biomonitoring for methaemoglobin and phenol in urine was conducted every 6 months. No adverse events were reported over the 5-year observation period.

Comments

Biochemical aspects

In experiments conducted in rats using [¹⁴C]fenamidone labelled at either the C-phenyl or N-phenyl part of the molecule, the time to reach the maximum plasma concentration of radioactivity was 2–4 hours after a single oral dose of 3 mg/kg bw and 14–26 hours after a single oral dose of 300 mg/kg bw. Gastrointestinal absorption was greater than 80%. Radioactivity distributed to most tissues, with no evidence of accumulation. Relatively high concentrations of radioactivity (approximately 400 times higher than that in plasma) were detected in the thyroid following dosing with C-phenyl- but not N-phenyl-labelled fenamidone, suggesting the distribution of a radiolabelled metabolite to the thyroid. Fenamidone undergoes extensive metabolism in the rat by phase I (oxidation, reduction and hydrolysis) and phase II reactions (conjugation). More than 20 metabolites were detected in rat excreta. The plasma elimination half-life was at least 60 hours, with the majority of radioactivity excreted in the faeces (up to approximately 90% of the administered dose) and the remainder in urine. Mass balance data indicated that the majority of radioactivity (> 80%) was eliminated within 48 hours of dosing.

Toxicological data

The oral LD₅₀ in rats was greater than 2000 mg/kg bw. The dermal LD₅₀ in rats was greater than 2000 mg/kg bw, and the LC₅₀ was greater than 2.1 mg/L. Fenamidone was neither a skin irritant nor an eye irritant in rabbits. In a guinea-pig maximization test, no skin sensitization occurred.

The target organs for fenamidone are the liver and thyroid. In rats, fenamidone was an inducer of cytochrome P450. Consistent with studies indicating a relatively high distribution of radiolabel to the rat thyroid, increased thyroid weight, follicular cell hypertrophy and hyperplasia were observed. This did not appear to be a secondary effect of liver enzyme induction, as thyroid hormone levels in plasma were not affected by treatment.

In a non-guideline 90-day toxicity study in mice that tested dietary concentrations of 0, 70, 700 and 7000 ppm (equal to 0, 11.4, 110 and 1102 mg/kg bw per day for males and 0, 14.5, 146 and 1468 mg/kg bw per day for females, respectively), the NOAEL was 700 ppm (equal to 110 mg/kg bw per day), based on clinical signs and deaths in males at 7000 ppm (equal to 1102 mg/kg bw per day). In a second 90-day study in mice that tested dietary concentrations of 0, 50, 200, 1000 and 5000 ppm (equal to 0, 11.3, 44.5, 220 and 1064 mg/kg bw per day for males and 0, 13.7, 54, 274 and 1375 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 220 mg/kg bw per day) for equivocal histopathological findings in the liver at 5000 ppm (equal to 1064 mg/kg bw per day). The overall NOAEL for the two 90-day studies in mice was 1000 ppm (equal to 220 mg/kg bw per day), with an overall LOAEL of 5000 ppm (equal to 1064 mg/kg bw per day).

A 28-day toxicity study in rats tested dietary concentrations of 0, 500, 5000 and 15 000 ppm (equal to 0, 39, 389 and 1203 mg/kg bw per day for males and 0, 42, 405 and 1194 mg/kg bw per day for females, respectively). The NOAEL was 500 ppm (equal to 39 mg/kg bw per day) for reduced red cell parameters and increased spleen weights coincident with hyperplasia of the germinative follicle of the white pulp at and above 5000 ppm (equal to 389 mg/kg bw per day). The NOAEL in a second 28-

day study in rats, which tested dietary concentrations of 0, 60, 150, 1000 and 5000 ppm (equal to 0, 4.9, 12.3, 82.7 and 418.5 mg/kg bw per day for males and 0, 5.3, 13.9, 90.6 and 450.1 mg/kg bw per day for females, respectively), was 1000 ppm (equal to 82.7 mg/kg bw per day) for thyroid follicular cell hypertrophy in males at 5000 ppm (equal to 418.5 mg/kg bw per day).

In a 90-day toxicity study in rats, which tested dietary concentrations of 0, 50, 150, 500 and 5000 ppm (equal to 0, 3, 9, 30 and 305 mg/kg bw per day for males and 0, 3, 11, 35 and 337 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 30 mg/kg bw per day) for reduced body weight gain, reduced feed consumption and changes in red cell parameters at 5000 ppm (equal to 305 mg/kg bw per day). The NOAEL in a second 90-day rat study, which tested dietary concentrations of 0, 60, 150, 1000 and 5000 ppm (equal to 0, 4.1, 10.4, 68.3 and 344 mg/kg bw per day for males and 0, 4.8, 12.0, 83.3 and 381 mg/kg bw per day for females, respectively), was 1000 ppm (equal to 68.3 mg/kg bw per day) for reduced body weight gain at 5000 ppm (equal to 344 mg/kg bw per day).

In a 28-day toxicity study in dogs, which tested doses of 0, 3, 10 and 100 mg/kg bw per day given by capsule, the NOAEL was 100 mg/kg bw per day, the highest dose tested. In a 13-week study in dogs, which tested doses of 0, 10, 100 and 500 mg/kg bw per day given by capsule, the NOAEL was 500 mg/kg bw per day, the highest dose tested. In a 52-week study in dogs, which tested doses of 0, 10, 100 and 1000 mg/kg bw per day given by capsule, the NOAEL was 100 mg/kg bw per day for clinical signs (hypersalivation and vomiting), increased AP activity, increased liver weight (males) and increased bile duct hyperplasia (males) at 1000 mg/kg bw per day. The overall NOAEL for the 13- and 52-week studies was 500 mg/kg bw per day, with an overall LOAEL of 1000 mg/kg bw per day.

In a long-term study of toxicity and carcinogenicity in mice, which tested dietary concentrations of 0, 70, 350, 3500 and 7000 ppm (equal to 0, 9.5, 47.5, 525.5 and 1100 mg/kg bw per day for males and 0, 12.6, 63.8, 690.5 and 1393 mg/kg bw per day for females, respectively), the NOAEL was 350 ppm (equal to 47.5 mg/kg bw per day) for lower body weight, increased liver weight and histopathological findings in the liver at 3500 ppm (equal to 525.5 mg/kg bw per day). No treatment-related neoplastic lesions were detected at dietary concentrations up to 7000 ppm (equal to 1100 mg/kg bw per day).

In a long-term toxicity and carcinogenicity study in rats that tested dietary concentrations of 0, 60, 150, 1000 and 5000 ppm (equal to 0, 2.8, 7.1, 47.7 and 260 mg/kg bw per day for males and 0, 3.6, 9.2, 60.9 and 335 mg/kg bw per day for females, respectively), the NOAEL was 60 ppm (equal to 2.8 mg/kg bw per day) for thyroid follicular cell hypertrophy and hyperplasia at 150 ppm (equal to 7.1 mg/kg bw per day). No treatment-related neoplastic lesions were detected at dietary concentrations up to 5000 ppm (equal to 260 mg/kg bw per day).

The Meeting concluded that fenamidone is not carcinogenic in mice or rats.

Fenamidone was negative in the Ames test and in an in vitro unscheduled DNA synthesis assay in rat hepatocytes. Fenamidone was positive in a mouse lymphoma forward mutation assay and caused chromosomal aberrations in cultured human peripheral blood lymphocytes in the presence of metabolic activation. Negative responses were obtained in vivo in the mouse micronucleus test and unscheduled DNA synthesis assay in rat hepatocytes.

The Meeting concluded that fenamidone is unlikely to be genotoxic in vivo.

Considering the lack of in vivo genotoxicity potential and the absence of a carcinogenic response in mice and rats, the Meeting concluded that fenamidone is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation study in rats, which tested dietary concentrations of 0, 60, 1000 and 5000 ppm (equal to 0, 3.9, 63.8 and 328.3 mg/kg bw per day for males and 0, 5.15, 84.4 and 459.6 mg/kg bw per day for females, respectively), there was no evidence of reproductive toxicity up to the highest tested dietary concentration of 5000 ppm (equal to 328.3 mg/kg bw per day). The NOAEL for parental toxicity was 60 ppm (equal to 3.9 mg/kg bw per day) for lower body weight, reduced body

weight gain and reduced feed consumption at 1000 ppm (equal to 63.8 mg/kg bw per day). The NOAEL for offspring toxicity was 1000 ppm (equal to 63.8 mg/kg bw per day) for lower F₁ and F₂ pup weights at 5000 ppm (equal to 328.3 mg/kg bw per day).

In a rat developmental toxicity study that tested doses of 0, 25, 150 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity was 150 mg/kg bw per day for reduced body weight gain and feed consumption at 1000 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 150 mg/kg bw per day for lower fetal weight at 1000 mg/kg bw per day.

In a rabbit developmental toxicity study that tested doses of 0, 10, 30 and 100 mg/kg bw per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day for decreased body weight gain at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested.

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

Acute and subchronic neurotoxicity studies were conducted in rats. In the acute gavage study, which tested doses of 0, 125, 500 and 2000 mg/kg bw per day, the NOAEL was 125 mg/kg bw for nonspecific findings in the functional observational battery at 4 hours after dosing at 500 mg/kg bw per day and above. In the subchronic study, which tested dietary concentrations of 0, 150, 1000 and 5000 ppm (equal to 0, 11.2, 73.5 and 392.3 mg/kg bw per day for males and 0, 12.7, 83.4 and 414.2 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 73.5 mg/kg bw per day) for reduced body weight gain at 5000 ppm (equal to 392.3 mg/kg bw per day).

There was no evidence of developmental neurotoxicity in a dietary study conducted in female rats, which tested dietary concentrations of 0, 60, 250, 1000 and 4700 ppm (equal to 0, 5.5, 23, 92.3 and 429 mg/kg bw per day, respectively). The NOAEL for maternal toxicity was 1000 ppm (equal to 92.3 mg/kg bw per day) for reduced body weight gain at 4700 ppm (equal to 429 mg/kg bw per day). The NOAEL for offspring toxicity was also 1000 ppm (equal to 92.3 mg/kg bw per day), for lower absolute body weight and reduced body weight gain at 4700 ppm (equal to 429 mg/kg bw per day).

The Meeting concluded that fenamidone is not neurotoxic.

Toxicological data on metabolites and/or degradates

Toxicity studies were conducted on three fenamidone metabolites: (1) (S)-5-methyl-5-phenyl-2,4-imidazolidine-dione (RPA 412636), which is a metabolite in rat urine present at less than 1% of an administered dose; (2) (5S)-5-methyl-5-phenyl-3-(phenylamino)-2,4-imidazolidine-dione (RPA 410193), which is a novel plant metabolite; and (3) (5S)-5-methyl-2-(methylthio)-5-phenyl-3,5-dihydro-4H-imidazol-4-one (RPA 412708), which is a metabolite in rat bile present at greater than 10% of an administered dose.

The oral LD₅₀ in rats was 1520 mg/kg bw for RPA 412636, greater than 2000 mg/kg bw for RPA 410193 and greater than 100 mg/kg bw for RPA 412708.

Short-term studies of toxicity were performed on RPA 412636, RPA 410193 and RPA 412708 in rats. As with the parent compound, liver enzyme induction and liver hypertrophy occurred with RPA 412636 and RPA 410193, but not RPA 412708. In a non-guideline 14-day study on RPA 412636 that tested dietary concentrations of 0, 300, 1200 and 3000 ppm (equal to 0, 23, 90 and 215 mg/kg bw per day for males and 0, 24.5, 96.7 and 233 mg/kg bw per day for females, respectively), the NOAEL was 1200 ppm (equal to 90 mg/kg bw per day) for reduced body weight gain and feed consumption at 3000 ppm (equal to 215 mg/kg bw per day). In a similar non-guideline study on RPA 410193, which tested dietary concentrations of 0, 450, 4500 and 15 000 ppm (equal to 0, 30, 299 and 1098 mg/kg bw per day for males and 0, 37, 374 and 1133 mg/kg bw per day for females, respectively), the NOAEL was 450 ppm (equal to 30 mg/kg bw per day) for histopathological findings in the thyroid at 4500 ppm (equal to 299 mg/kg bw per day). In the non-guideline 14-day study on RPA 412708, which tested dietary concentrations of 0, 200, 500 and 2000 ppm (equal to 0, 15.1, 38.5 and 150.5 mg/kg bw per day for males and 0, 16.1, 37.8 and 147.3 mg/kg bw per day for

females, respectively), the NOAEL was 2000 ppm (equal to 147.3 mg/kg bw per day), the highest dietary concentration tested.

Ninety-day dietary studies were conducted on RPA 412636 and RPA 410193 in rats. The study on RPA 412636 tested dietary concentrations of 0, 100, 500 and 2500 ppm (equal to 0, 6.4, 33 and 162 mg/kg bw per day for males and 0, 7.7, 39 and 196 mg/kg bw per day for females, respectively); the NOAEL was 100 ppm (equal to 6.4 mg/kg bw per day) for vacuolation of centrilobular hepatocytes at 500 ppm (equal to 33 mg/kg bw per day) in males. In the study conducted on RPA 410193, which tested dietary concentrations of 0, 150, 1500 and 15 000 ppm (equal to 0, 9.3, 93.3 and 978 mg/kg bw per day for males and 0, 11.5, 115 and 1090 mg/kg bw per day for females, respectively), the NOAEL was 1500 ppm (equal to 93.3 mg/kg bw per day), based on perturbations in red cell parameters at 15 000 ppm (equal to 978 mg/kg bw per day).

There was no evidence of genotoxicity in vitro or in vivo for RPA 412636, RPA 410193 and RPA 412708.

Human data

In a cohort of 15 workers involved in the processing of fenamidone over a 5-year period, no adverse medical events were reported, and there were no detectable effects following physical and biochemical examinations.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.03 mg/kg bw per day, based on the NOAEL of 2.8 mg/kg bw per day for a dose-related increase in thyroid follicular cell hypertrophy and hyperplasia in both sexes after 52 weeks in the 2-year rat study. A 100-fold safety factor was applied.

The Meeting established an acute reference dose (ARfD) of 1 mg/kg bw, based on the NOAEL of 125 mg/kg bw per day for nonspecific findings in the functional observational battery in the acute neurotoxicity study in rats, and using a 100-fold safety factor. The Meeting noted that decreased maternal body weight gain in the developmental toxicity study in rabbits with a lower NOAEL of 30 mg/kg bw per day was not an acute effect and was therefore not a suitable basis for establishing an ARfD for fenamidone.

Levels relevant to risk assessment of fenamidone

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day studies of toxicity ^{a,b}	Toxicity	1 000 ppm, equal to 220 mg/kg bw per day	5 000 ppm, equal to 1 064 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity ^a	Toxicity	350 ppm, equal to 47.5 mg/kg bw per day	3 500 ppm, equal to 525.5 mg/kg bw per day
		Carcinogenicity	7 000 ppm, equal to 1 100 mg/kg bw per day ^c	–

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity study ^d	Toxicity	125 mg/kg bw per day	500 mg/kg bw per day
	Thirteen-week studies of toxicity or neurotoxicity ^{a,b}	Toxicity	1 000 ppm, equal to 73.5 mg/kg bw per day	5 000 ppm, equal to 305 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity ^a	Toxicity	60 ppm, equal to 2.8 mg/kg bw per day	150 ppm, equal to 7.1 mg/kg bw per day
		Carcinogenicity	5 000 ppm, equal to 260 mg/kg bw per day ^c	–
	Two-generation study of reproductive toxicity ^a	Reproductive toxicity	5 000 ppm, equal to 328.3 mg/kg bw per day ^c	–
		Parental toxicity	60 ppm, equal to 3.9 mg/kg bw per day	1 000 ppm, equal to 63.8 mg/kg bw per day
		Offspring toxicity	1 000 ppm, equal to 63.8 mg/kg bw per day	5 000 ppm, equal to 328.3 mg/kg bw per day
	Developmental toxicity study ^d	Maternal toxicity	150 mg/kg bw per day	1 000 mg/kg bw per day
		Embryo and fetal toxicity	150 mg/kg bw per day	1 000 mg/kg bw per day
Rabbit	Developmental toxicity study ^d	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day ^c	–
Dog	Thirteen- and 52-week studies of toxicity ^{b,e}	Toxicity	500 mg/kg bw per day	1 000 mg/kg bw per day

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

^a Dietary administration.

^b Two or more studies combined. For the rat, two 90-day toxicity studies and a subchronic neurotoxicity study were combined.

^c Highest dose tested.

^d Gavage administration.

^e Capsule administration.

Levels relevant to risk assessment of fenamidone metabolites based on studies conducted in rats

Metabolite	Study	Effect	NOAEL	LOAEL
RPA 412636 ^a	Ninety-day study of toxicity ^b	Toxicity	100 ppm, equal to 6.4 mg/kg bw per day	500 ppm, equal to 33 mg/kg bw per day
RPA 410193 ^c	Ninety-day study of toxicity ^b	Toxicity	1 500 ppm, equal to 93.3 mg/kg bw per day	15 000 ppm, equal to 978 mg/kg bw per day
RPA 412708 ^d	Fourteen-day study of toxicity ^b	Toxicity	2 000 ppm, equal to 147.3 mg/kg bw per day ^e	–

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

^a (S)-5-Methyl-5-phenyl-2,4-imidazolidine-dione.

^b Dietary administration.

^c (5S)-5-Methyl-5-phenyl-3-(phenylamino)-2,4-imidazolidine-dione.

^d (5S)-5-methyl-2-(methylthio)-5-phenyl-3,5-dihydro-4H-imidazol-4-one.

^e Highest dose tested.

Estimate of acceptable daily intake

0–0.03 mg/kg bw

Estimate of acute reference dose

1 mg/kg bw

Information that would be useful for the continued evaluation of fenamidone

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

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

<i>Absorption, distribution, excretion and metabolism in mammals</i>	
Rate and extent of oral absorption	Rats: $T_{\max}$ = 2–26 h; extensive, > 80%
Distribution	Widespread tissue distribution; preferential distribution of radiolabel to the thyroid
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Excretion via faeces and urine; half-life > 60 h
Metabolism in animals	Extensive
Toxicologically significant compounds in animals, plants and the environment	Fenamidone, RPA 412636, RPA 410193, RPA 412708
<i>Acute toxicity</i>	
Rat, LD ₅₀ , oral	> 2 000 mg/kg bw
Rat, LD ₅₀ , dermal	> 2 000 mg/kg bw
Rat, LC ₅₀ , inhalation	> 2.1 mg/L
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Non-irritating
Dermal sensitization	Non-sensitizing (guinea-pigs)
<i>Short-term studies of toxicity</i>	
Target/critical effect	Liver, thyroid
Lowest relevant oral NOAEL	68.3 mg/kg bw per day (90 days, rat)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
<i>Long-term studies of toxicity and carcinogenicity</i>	
Target/critical effect	Thyroid
Lowest relevant NOAEL	2.8 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans from the diet
<i>Genotoxicity</i>	
	Not genotoxic in vivo
<i>Reproductive toxicity</i>	
Reproduction target/critical effect	No evidence of reproductive toxicity (rat)
Lowest relevant parental NOAEL	3.9 mg/kg bw per day
Lowest relevant offspring NOAEL	63.8 mg/kg bw per day
Lowest relevant reproduction NOAEL	328.3 mg/kg bw per day, the highest dose tested

<i>Developmental toxicity</i>	
Developmental target/critical effect	Decreased pup weights at maternally toxic doses (rat)
Lowest maternal NOAEL	30 mg/kg bw per day (rabbit)
Lowest embryo/fetal NOAEL	100 mg/kg bw per day, the highest dose tested (rabbit); 150 mg/kg bw per day (rat)
<i>Neurotoxicity</i>	
Neurotoxicity	Not neurotoxic
Developmental neurotoxicity	No developmental neurotoxicity
<i>Toxicological studies on RPA 412636</i>	
Rat, LD ₅₀ , oral	1 520 mg/kg bw
Lowest relevant short-term oral NOAEL	6.4 mg/kg bw per day (90 days, rat)
Genotoxicity	Not genotoxic
<i>Toxicological studies on RPA 410193</i>	
Rat, LD ₅₀ , oral	> 2 000 mg/kg bw
Lowest relevant short-term oral NOAEL	93.3 mg/kg bw per day (90 days, rat)
Genotoxicity	Not genotoxic
<i>Toxicological studies on RPA 412708</i>	
Rat, LD ₅₀ , oral	100 mg/kg bw
Lowest relevant short-term oral NOAEL	147.3 mg/kg bw per day (14 days, rat)
Genotoxicity	Not genotoxic
<i>Medical data</i>	
	No adverse effects in workers involved with manufacturing fenamidone

LC₅₀: median lethal concentration; LD₅₀: median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level

Summary

	Value	Studies	Safety factor
ADI	0–0.03 mg/kg bw	Two-year dietary study in rats	100
ARfD	1 mg/kg bw	Acute neurotoxicity study in rats	100

ADI: acceptable daily intake; ARfD: acute reference dose

References

- Beevers C (1999a). RPA 412636 (*S*-enantiomer of RPA 717879): reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli*. Unpublished study (198/129-D5140) by Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Report No. R006728. Submitted to WHO by Bayer CropScience.
- Beevers C (1999b). RPA 410193 (*S*-enantiomer of RPA 405862): reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli*. Unpublished study (198/128-D5140) by Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Report No. R006729. Submitted to WHO by Bayer CropScience.
- Beevers C (1999c). RPA 412708 (*S*-enantiomer of RPA 408056): reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli*. Unpublished study (198/130-D5140) by Covance Laboratories Ltd, North Yorkshire, England, United Kingdom. Report No. R006724. Submitted to WHO by Bayer CropScience.
- Berthe P (1995). RPA 407213: 90 day toxicity study in mouse by dietary administration. Unpublished study (SA 94357) by Rhône-Poulenc Agro, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005301. Submitted to WHO by Bayer CropScience.

- Bigot D (1997). RPA 407213: 90-day toxicity study in the rat by dietary administration. Unpublished study (SA 96562) by Rhône-Poulenc Agro, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005625. Submitted to WHO by Bayer CropScience.
- Bigot D (1999a). RPA 407213: chronic toxicity and carcinogenicity study of RPA 407213 in Sprague Dawley rat by dietary administration. Part A. Unpublished study (SA 96188) by Rhône-Poulenc Agro, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006352. Submitted to WHO by Bayer CropScience.
- Bigot D (1999b). RPA 407213: chronic toxicity and carcinogenicity study of RPA 407213 in Sprague Dawley rat by dietary administration. Part B. Unpublished study (SA 96426) by Rhône-Poulenc Agro, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006349. Submitted to WHO by Bayer CropScience.
- Bigot D (1999c). RPA 412636 (*S*-enantiomer of RPA 717879): exploratory 14-day toxicity study in the rat by dietary administration. Unpublished study (SA 98576) by Rhône-Poulenc Agro, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006337. Submitted to WHO by Bayer CropScience.
- Bigot D, Tassel JP (1997). RPA 407213: 90-day toxicity study in the mouse by dietary administration. Unpublished study (SA 96183) by Rhône-Poulenc Agro, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005615. Submitted to WHO by Bayer CropScience.
- Bussi R (1999). RPA 407213: two-generation reproduction toxicity study by diet route in male and female rats. Unpublished study (SA 960608) by Istituto di Ricerche Biomediche, Colletterto Giacosa, Torino, Italy. Report No. R006732. Submitted to WHO by Bayer CropScience.
- Chuzel F (1997a). RPA 407213: acute dermal irritation test in the rabbit. Unpublished study (SA 97013) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005576. Submitted to WHO by Bayer CropScience.
- Chuzel F (1997b). RPA 407213: acute eye irritation test in the rabbit. Unpublished study (SA 97014) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005588. Submitted to WHO by Bayer CropScience.
- Chuzel F (1999a). RPA 410193 (*S*-enantiomer of RPA 405862): exploratory 14-day toxicity study in the rat by dietary administration. Unpublished study (SA 98506) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005987. Submitted to WHO by Bayer CropScience.
- Chuzel F (1999b). RPA 412708 (*S*-enantiomer of RPA 408056): exploratory 14-day toxicity study in the rat by dietary administration. Unpublished study (SA 99007) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006196. Submitted to WHO by Bayer CropScience.
- Coombs DW, Horrell PJ, Bannerman M (1998). RPA 407213: acute (4-hour) inhalation toxicity study in rats. Unpublished study (RNP 553/974516) by Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England, United Kingdom. Report No. R006117. Submitted to WHO by Bayer CropScience.
- Dange M (1994). RPA 407213: exploratory 14-day toxicity study in the mouse by gavage. Unpublished study (SA 94007) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005193. Submitted to WHO by Bayer CropScience.
- Dange M (1995a). RPA 407213: exploratory 14-day toxicity study in the rat by gavage. Unpublished study (SA 94072) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005223. Submitted to WHO by Bayer CropScience.
- Dange M (1995b). RPA 407213: preliminary 28-day toxicity study in the rat by dietary administration. Unpublished study (SA 94120) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005236. Submitted to WHO by Bayer CropScience.
- Dange M (1995c). RPA 407213: 90-day toxicity study in the rat by dietary administration. Unpublished study (SA 94292) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005381. Submitted to WHO by Bayer CropScience.
- Dange M (1997). RPA 407213: dermal limit test in the rat. Unpublished study (SA 97007) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005569. Submitted to WHO by Bayer CropScience.
- Dange M (1999a). RPA 407213: 28-day toxicity study in the rat by dietary administration. Unpublished study (SA 98407) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006357. Submitted to WHO by Bayer CropScience.

- Dange M (1999b). RPA 412636 (*S*-enantiomer of RPA 717879): 90-day toxicity study in the rat by dietary administration. Unpublished study (SA 98619) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006355. Submitted to WHO by Bayer CropScience.
- Dange M (1999c). RPA 410193 (*S*-enantiomer of RPA 405862): 90-day toxicity study in the rat by dietary administration. Unpublished study (SA 98620) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006347. Submitted to WHO by Bayer CropScience.
- Dange M, Foulon O (1997). RPA 407213: oral LD₅₀ in the rat. Unpublished study (SA 97005) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005600. Submitted to WHO by Bayer CropScience.
- Dange M, Katchadourian P (1995). RPA 407213: preliminary 28-day toxicity study in the dog by dietary administration. Unpublished study (SA 94113) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005225. Submitted to WHO by Bayer CropScience.
- De Jouffrey S (1997). RPA 407213: skin sensitization test in the guinea-pigs maximization method of Magnusson B and Kligman AM. Unpublished study (15347 TSG) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005617. Submitted to WHO by Bayer CropScience.
- Fellows M (1999a). RPA 407213: mutation at the thymidine kinase (*tk*) locus of mouse lymphomas L5178Y cells (MLA) using the Microtitre® fluctuation technique. Unpublished study (198/104-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006723. Submitted to WHO by Bayer CropScience.
- Fellows M (1999b). RPA 407213: measurement of unscheduled DNA synthesis in isolated rat hepatocytes in vitro. Unpublished study (198/104-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006720. Submitted to WHO by Bayer CropScience.
- Fellows M (1999c). RPA 407213: measurement of unscheduled DNA synthesis in isolated rat hepatocytes in vivo/in vitro procedure. Unpublished study (198/104-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006719. Submitted to WHO by Bayer CropScience.
- Fellows M (1999d). RPA 412636 (*S*-enantiomer of RPA 717879): mutation at the *hprt* locus of L5178Y mouse lymphoma cells using the Microtitre fluctuation technique. Unpublished study (198/148-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006730. Submitted to WHO by Bayer CropScience.
- Fellows M (1999e). RPA 410193 (*S*-enantiomer of RPA 405862): mutation at the *hprt* locus of L5178Y mouse lymphoma cells using the Microtitre fluctuation technique. Unpublished study (198/148-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R011579. Submitted to WHO by Bayer CropScience.
- Fisch C (1999a). RPA 407213: 4-week toxicity study by oral route (capsules) in Beagle dogs. Unpublished study (12650 TSR) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006272. Submitted to WHO by Bayer CropScience.
- Fisch C (1999b). RPA 407213: 13-week toxicity study by oral route (capsules) in Beagle dogs. Unpublished study (12800 TCC) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Sophia Antipolis, France. Report No. R006274. Submitted to WHO by Bayer CropScience.
- Fisch C (1999c). RPA 407213: 52-week toxicity study by oral route (capsules) in Beagle dogs. Unpublished study (14075 TCC) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Sophia Antipolis, France. Report No. R006276. Submitted to WHO by Bayer CropScience.
- Foulon O (1999). RPA 407213: developmental toxicology study in the rabbit by gavage. Unpublished study (SA 98390) by Rhône-Poulenc Agro, Sophia Antipolis, France. Report No. R006194. Submitted to WHO by Bayer CropScience.
- Foulon O, Wason S (1997). RPA 407213: developmental toxicology study in the rat by gavage. Unpublished study (SA 96319) by Bayer CropScience, Sophia Antipolis, France. Report No. R005598. Submitted to WHO by Bayer CropScience.
- Hooks WN (2001). RPA 407213: neurotoxicity study by dietary administration to CD rats for 13 weeks. Unpublished study (RNP 604/994541) by Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England, United Kingdom. Report No. B003411. Submitted to WHO by Bayer CropScience.

- Hughes EW, Crome SJ (1999). RPA 407213: neurotoxicity study by a single oral gavage administration to CD rats followed by a 14-day observation period. Unpublished study (RNP 656/993507) by Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England, United Kingdom. Report No. R006704. Submitted to WHO by Bayer CropScience.
- Katchadourian P, Berniaud F, Fourmer A (1996). RPA 407213: *Salmonella typhimurium* reverse mutation assay (Ames test). Unpublished study (SA 96421) by Rhône-Poulenc Agrochimie, Centre de Recherche de la Dargaire, Lyon, France. Report No. R005506. Submitted to WHO by Bayer CropScience.
- Manciaux X (1999a). RPA 412636 (*S*-enantiomer of RPA 717879): acute oral toxicity in rats. Unpublished study (18110 TAR) by Centre International de Toxicologie, Evreux, France. Report No. R006735. Submitted to WHO by Bayer CropScience.
- Manciaux X (1999b). RPA 410193 (*S*-enantiomer of RPA 717879): acute oral toxicity in rats. Unpublished study (18109 TAR) by Centre International de Toxicologie, Evreux, France. Report No. R006734. Submitted to WHO by Bayer CropScience.
- Manciaux X (1999c). RPA 412708 (*S*-enantiomer of RPA 408056): acute oral toxicity in rats. Unpublished study (18111 TAR) by Centre International de Toxicologie, Evreux, France. Report No. R006736. Submitted to WHO by Bayer CropScience.
- Marshall R (1999a). RPA 407213: induction of chromosomal aberration in cultured human peripheral blood lymphocytes. Unpublished study (198/106-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006722. Submitted to WHO by Bayer CropScience.
- Marshall R (1999b). RPA 407213: induction of micronuclei in the bone marrow of treated mice. Unpublished study (198/106-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006721. Submitted to WHO by Bayer CropScience.
- Milburn GM (1999). RPA 407213: 80 week oncogenicity study in mice. Unpublished study (PM1080, CTL/P/6139) by Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England, United Kingdom. Report No. R006733. Submitted to WHO by Bayer CropScience.
- Sheets LP, Gilmore RG, Hoss HE (2005). A developmental neurotoxicity screening study with technical grade fenamidone in Wistar rats. Unpublished study (04-D72-UM) by Bayer CropScience LP, Toxicology, Stilwell, KS, USA. Report No. 201299. Submitted to WHO by Bayer CropScience.
- Steffens W (2013). Occupational medical experiences with fenamidone. Unpublished study by Bayer CropScience AG, Monheim, Germany. Submitted to WHO by Bayer CropScience.
- Totis M (1999). RPA 407213: rat absorption, distribution, metabolism and elimination study. Unpublished study (SA 96413) by Rhône-Poulenc Agro, Centre de Recherche de la Dargaire, Lyon, France. Report No. R006718.27. Submitted to WHO by Bayer CropScience.
- Totis M (2000). Fenamidone: additional information on the metabolism in the rat. Unpublished expert summary by Aventis CropScience, ADME, Sophia Antipolis, France. Report No. C030267. Submitted to WHO by Bayer CropScience.
- Whitewell J (1999a). RPA 412636 (*S*-enantiomer of RPA 717879): induction of micronuclei in the bone marrow of treated mice. Unpublished study (198/132-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006727. Submitted to WHO by Bayer CropScience.
- Whitewell J (1999b). RPA 410193 (*S*-enantiomer of RPA 405862): induction of micronuclei in the bone marrow of treated mice. Unpublished study (198/131-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006725. Submitted to WHO by Bayer CropScience.
- Whitewell J (1999c). RPA 412708 (*S*-enantiomer of RPA 408056): induction of micronuclei in the bone marrow of treated mice. Unpublished study (198/133-D5140) by Covance Laboratories Ltd, Harrogate, North Yorkshire, England, United Kingdom. Report No. R006726. Submitted to WHO by Bayer CropScience.