SAFLUFENACIL

First draft prepared by
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Explanation

Saflufenacil is the International Organization for Standardization (ISO)–approved name for *N*′-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl) benzoyl]-*N*-isopropyl-*N*-methylsulfamide (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 372137-35-4. Saflufenacil is a new herbicide from the uracil family of herbicides, acting as a protoporphyrinogen IX oxidase (PPO) inhibitor. Saflufenacil has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues.

All pivotal studies with saflufenacil were certified as complying with good laboratory practice unless stated otherwise.

Evaluation for acceptable daily intake

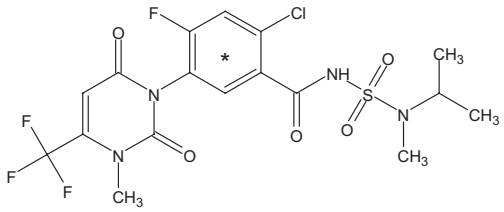
1. Biochemical aspects

The absorption, distribution, metabolism and excretion, as well as the toxicokinetics, of saflufenacil have been investigated in Wistar rats. Summaries of the relevant data are presented below.

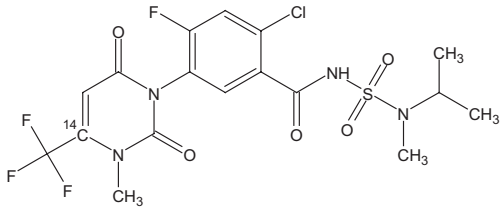
The structure and position of labels on the forms of saflufenacil used in the absorption, distribution, excretion and metabolism studies are shown in Figure 1.

Figure 1. Radiolabelled forms of saflufenacil used in absorption, distribution, metabolism and excretion studies: structure and position of labels

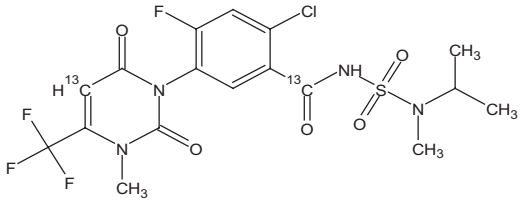
[Phenyl-U-^{14}C]saflufenacil



[Uracil-4-^{14}C]saflufenacil



[Uracil-5-^{13}C, benzamide-carbonyl-^{13}C]saflufenacil



1.1 Absorption, distribution and excretion

The absorption, distribution, excretion and metabolism of orally administered saflufenacil were studied in male and female Wistar rats using (phenyl-U-^{14}C)- and (uracil-4-^{14}C)-labelled saflufenacil. The purity of radioactive saflufenacil used in these studies was greater than 96%. The chemical purity of non-radioactive saflufenacil was greater than 93.9%. In a toxicokinetic study, [phenyl-U-^{14}C]saflufenacil was administered to four Wistar rats of each sex per group via gavage at dose levels of 4, 20 and 100 mg/kg body weight (bw) suspended in an aqueous solution of 0.5% carboxymethylcellulose in plasma kinetics studies and 5 and 100 mg/kg bw for mass balance, tissue distribution and biliary excretion experiments. For plasma kinetics studies, the radioactivity in whole blood and plasma was determined at 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 hours post-dosing for all three dose levels. Animals were sacrificed at 168 hours post-dosing. In mass balance and tissue distribution studies, urine and faeces were collected at 6 (urine only), 12, 24, 48, 72, 96, 120, 144 and 168 hours post-dosing. Animals were sacrificed at 168 hours after oral dosing, and various tissues were collected and analysed for radioactivity. The metabolism cages were rinsed and analysed for the presence of radioactivity. Expired air was collected from two males per dose for up to 48 hours. A time course tissue distribution study was conducted in three rats of each sex following a single gavage administration of 5 and 100 mg/kg bw. In this study, three males per dose were sacrificed at 1, 24, 48 and 72 hours and at 1, 7, 20 and 34 hours post-dosing at 5 and 100 mg/kg bw, respectively, and three females per dose were sacrificed at 1, 4, 20 and 24 hours and at 1, 7, 20 and 34 hours post-dosing at 5 and 100 mg/kg bw, respectively. In a repeated-dose study, four rats of each sex were administered a daily gavage dose of 100 mg/kg bw of non-radioactive saflufenacil for 14 days followed by a single gavage dose of 100 mg/kg bw of radioactive [phenyl-U-^{14}C]saflufenacil. Urine and faeces were collected at 6 (urine only), 12 (urine only), 24, 48, 72, 96, 120, 144 and 168 hours post-dosing with radioactive compound. Animals were sacrificed at 168 hours after the last dose, and selected tissues were sampled and analysed for the radioactive content. The purpose of the study was to evaluate the influence of multiple dosing on the oral absorption, distribution, excretion and metabolism of [phenyl-U-^{14}C]saflufenacil in rats and to compare these data with results from a single-dose gavage metabolism study. For the biliary excretion studies, bile duct–cannulated rats (four of each sex per dose) received a single gavage saflufenacil dose of 5 or 100 mg/kg bw. To facilitate the elucidation of the structure of metabolites formed, ^{13}C-labelled saflufenacil was added to the saflufenacil preparation for the bile excretion experiment at the high dose level. Bile was collected at 3-hour intervals for 48 hours post-dosing. Bile duct–cannulated animals were sacrificed at 48 hours after the dosing. The experimental procedures and results of these studies are presented in a report by Fabian & Landsiedel (2007a).

The mean total radioactive recoveries in these experiments ranged from 91.9% to 113.1% of the administered dose in both sexes. Less than 2% of the administered dose was recovered as carbon dioxide. Orally administered saflufenacil was rapidly absorbed, and the maximum plasma concentration (C_{max}) was reached within 1 hour of dosing (time to reach maximum concentration, T_{max}) for all dose groups (Table 1). Thereafter, the plasma level of saflufenacil declined rapidly, and only residual radioactivity was detected at 168 hours. A comparable time course of radioactivity was found in blood and plasma of both sexes. A relatively constant blood/plasma ratio between 0.1 and 0.4 was generally found, indicating that major parts of the radioactivity were in plasma and not bound to cellular blood constituents. The area under the plasma concentration–time curve (AUC) values indicated a sex difference, with up to 3-fold higher internal exposures for males than for females, most probably due to a higher clearance of ^{14}C-labelled saflufenacil in female rats. Increasing the dose by a factor of 25 resulted in an increase of the AUC values by a factor of 6.1 in males and 12.4 in females.

After single or repeated oral administration of ^{14}C-labelled saflufenacil, mean total recoveries of radioactivity in the urine and faeces were high for all groups (97–110% of the administered dose at 168 hours; Table 2). In general, the majority of radioactivity was excreted in the urine and faeces within the first 24–48 hours, and excretion was essentially complete within 96 hours. After

Table 1. Toxicokinetic parameters of ^{14}C-labelled saflufenacil in the Wistar rat

Sex	Dose (mg/kg bw)	C_{max} (µg eq/g)	T_{max} (h)	Initial half-life (h)	Terminal half-life (h)	AUC (µg eq·h/g)
Male	100	286.0	1	9.1	33.5	4501.5
	20	98.3	1	8.8	22.6	2131.2
	4	23.9	1	20.9	20.9	741.1
Female	100	258.3	1	4.9	59.2	3056.9
	20	84.8	1	6.5	58.1	754.2
	4	23.0	1	8.1	49.5	246.5

From Fabian & Landsiedel (2007a)
AUC, area under the curve; C_{max}, maximum plasma concentration; eq, equivalents; T_{max}, time to reach C_{max}

196 hours, approximately 26% and 96% of the administered dose were excreted in the urine of male and female rats, respectively, at 5 mg/kg bw. About 52.6% and 86.6% of the administered dose were excreted in the urine of male and female rats, respectively, at 100 mg/kg bw in 196 hours. After 196 hours, approximately 81.2% and 12.8% of the administered dose were excreted in the faeces of male and female rats, respectively, at 5 mg/kg bw. About 43.3% and 9.8% of the administered dose were excreted in the faeces of male and female rats, respectively, at 100 mg/kg bw in 196 hours. The higher excretion of radioactivity in the urine of female rats of the 5 mg/kg bw group may be due to contamination of urine samples with faeces. Radioactivity remaining in tissues at 168 hours was very low and occurred mainly in the carcass (0.06–0.10%), liver (0.03–0.06%), skin (0.02–0.03%) and gut content (0.02–0.03%). The data demonstrated a sex-specific excretion pattern for ^{14}C-labelled saflufenacil, with a higher amount of urinary excretion for females than for males. The sex-dependent excretion was more pronounced at the low dose level than at the high dose level.

Within 1 hour after oral administration of ^{14}C-labelled saflufenacil at 5 or 100 mg/kg bw, the highest radioactivity was found in the stomach and gut contents, carcass, liver and skin (Tables 3, 4 and 5). The radioactivity in the gut content generally decreased as the saflufenacil became more bioavailable with time. In general, radioactive residues in tissues were rather low. Radioactivity generally declined continuously in organs and tissues of both sexes during the following hours in parallel to the concentration in plasma. After 20–24 hours, only negligible radioactivity was measured in internal organs except for the liver. By 168 hours, all internal tissues in male and female rats that received a single oral dose of ^{14}C-labelled saflufenacil at 5 or 100 mg/kg bw contained very low radioactivity (< 0.1 and < 1.0 µg equivalent [eq] per gram at 5 and 100 mg/kg bw, respectively; Tables 3, 4 and 5). Similar findings of low radioactivity in internal tissues (< 0.5 µg eq/g except for the liver of females) were also observed in male and female rats that were given 15 daily oral doses of saflufenacil at 100 mg/kg bw and sacrificed 168 hours later.

The amount of radioactivity excreted in the bile duct–cannulated rats following a single gavage dose of 5 or 100 mg/kg bw is shown in Table 6. Within 48 hours after administration of ^{14}C-labelled saflufenacil at the high dose level of 100 mg/kg bw, excretion via bile was found to be about 67.80% and 35.47% of the administered dose in male and female animals, respectively. Within 48 hours after administration of ^{14}C-labelled saflufenacil at a dose of 5 mg/kg bw, excretion via bile was found to be about 52.30% and 18.85% of the administered dose in male and female animals, respectively. There were sex-related differences in the excretion of orally administered saflufenacil, regardless of the dose. Generally, males excreted more radioactivity in the bile compared with females. Based on the amounts of radioactivity excreted via bile and urine, the bioavailability of ^{14}C-labelled saflufenacil in rats was virtually 100% at a dose of 100 mg/kg bw. At 5 mg/kg bw, the bioavailability of ^{14}C-labelled saflufenacil was virtually 100% in female rats and about 79% in male rats. The sum of urinary and biliary excretion is over 100% irrespective of dose in females and over 100% for the high dose level in males, suggesting significant enterohepatic circulation of saflufenacil (Fabian & Landsiedel, 2007a).

Table 2. Mean excretion and tissue retention of radioactivity after oral administration of [phenyl-^{14}C]saflufenacil

Sampling time (h)	% of administered dose					
	Males (n = 4 per dose group)			Females (n = 4 per dose group)		
	Dose (mg/kg bw)					
	5	100	100 (14 + 1)	5	100	100 (14 + 1)
Mean urinary radioactivity						
0–6	6.11 ± 3.31	28.10 ± 12.44	39.78 ± 4.26	30.96 ± 20.07	57.08 ± 6.49	55.99 ± 4.77
6–12	3.61 ± 2.94	9.53 ± 2.89	10.03 ± 4.73	15.38 ± 7.77	8.72 ± 1.90	6.23 ± 1.01
12–24	6.50 ± 5.46	10.26 ± 3.97	8.73 ± 1.09	29.55 ± 11.02	14.11 ± 4.21	10.78 ± 1.33
24–48	5.37 ± 3.97	3.26 ± 1.44	1.91 ± 0.52	14.06 ± 6.90	3.38 ± 1.59	5.25 ± 1.65
48–72	2.40 ± 2.55	0.79 ± 0.25	0.63 ± 0.22	3.56 ± 2.23	1.25 ± 0.64	2.09 ± 0.79
72–96	1.20 ± 1.61	0.34 ± 0.17	0.22 ± 0.05	1.11 ± 0.42	0.71 ± 0.43	1.11 ± 0.40
96–120	0.36 ± 0.28	0.16 ± 0.07	0.13 ± 0.04	0.56 ± 0.31	0.54 ± 0.32	0.63 ± 0.06
120–144	0.17 ± 0.13	0.10 ± 0.05	0.08 ± 0.03	0.45 ± 0.39	0.42 ± 0.09	0.67 ± 0.16
144–168	0.11 ± 0.07	0.06 ± 0.03	0.29 ± 0.45	0.43 ± 0.28	0.36 ± 0.10	0.63 ± 0.33
Urine total	26.02 ± 16.06	52.61 ± 10.11	61.80 ± 3.63	96.06 ± 7.88	86.56 ± 2.68	83.38 ± 2.43
Mean faecal radioactivity						
0–24	30.6 ± 21.98	21.14 ± 6.66	24.31 ± 4.89	6.55 ± 1.46	7.93 ± 2.48	9.64 ± 3.06
24–48	30.05 ± 9.91	18.24 ± 7.43	9.84 ± 2.83	4.71 ± 3.37	1.34 ± 0.47	2.80 ± 0.98
48–72	6.95 ± 3.17	2.92 ± 0.71	1.02 ± 0.19	1.11 ± 0.68	0.24 ± 0.18	0.52 ± 0.24
72–96	11.21 ± 20.04	0.58 ± 0.34	0.30 ± 0.14	0.21 ± 0.15	0.10 ± 0.05	0.14 ± 0.08
96–120	0.72 ± 0.96	0.20 ± 0.12	0.12 ± 0.06	0.09 ± 0.05	0.07 ± 0.03	0.09 ± 0.03
120–144	1.61 ± 2.93	0.18 ± 0.08	0.07 ± 0.03	0.09 ± 0.07	0.05 ± 0.01	0.10 ± 0.06
144–168	0.07 ± 0.02	0.09 ± 0.04	0.10 ± 0.07	0.04 ± 0.03	0.09 ± 0.09	0.09 ± 0.03
Faecal total	81.20 ± 16.41	43.33 ± 8.11	35.76 ± 3.80	12.80 ± 4.38	9.82 ± 2.22	13.38 ± 2.44
Cage wash, 168	0.56 ± 0.59	0.69 ± 0.31	0.58 ± 0.28	1.34 ± 0.65	0.80 ± 0.25	1.69 ± 0.45
Carcass, 168	0.06 ± 0.01	0.07 ± 0.03	0.05 ± 0.01	0.10 ± 0.05	0.08 ± 0.04	0.05 ± 0.02
Skin, 168	0.02 ± 0.0	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Liver, 168	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.02	0.03 ± 0.02	0.05 ± 0.02
Gut content, 168	0.02 ± 0.0	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Kidneys, 168	0.01 ± 0.01	0.0	0.0	0.01 ± 0	0.0	0.0
Total recovery, 168	107.90 ± 3.59	96.77 ± 3.31	98.24 ± 0.15	110.41 ± 3.18	97.32 ± 1.18	98.60 ± 0.09

From Fabian & Landsiedel (2007a). Data taken from tables 1, 8–13, pp. 37, 43–48 of the study report.

Table 3. Distribution of radioactivity in rat tissues/organs after administration of ^{14}C-labelled saflufenacil

Tissue/organ	Mean tissue concentration (µg eq/g)															
	Single low dose (5 mg/kg bw)								Single high dose (100 mg/kg bw)							
	Males				Females				Males				Females			
	Time after dosing (h)															
	1	24	48	72	1	4	20	24	1	7	20	34	1	7	20	34
Blood cells	8.34	1.90	0.36	0.16	5.42	2.37	0.92	0.43	74.02	27.96	8.30	5.80	43.24	12.90	7.70	6.57
Plasma	30.43	9.26	1.71	0.66	18.73	8.07	2.92	1.74	222.22	108.00	49.96	29.86	181.82	69.32	24.40	23.55
Lung	8.16	2.77	0.64	0.19	5.07	2.97	0.97	0.67	205.54	30.51	13.53	8.33	60.07	22.56	9.21	8.24
Heart	5.04	1.50	0.34	0.12	4.45	1.55	0.56	0.35	79.40	31.97	9.39	6.01	40.16	13.61	4.14	4.52
Spleen	1.82	0.55	0.15	0.05	1.26	0.64	0.22	0.14	36.35	10.26	3.62	2.64	18.40	5.28	2.22	2.03
Kidney	8.00	2.05	0.45	0.18	8.71	4.39	0.93	0.72	197.89	79.29	13.75	8.62	142.66	31.41	9.09	7.34
Adrenal glands	5.98	1.51	0.42	0.13	3.30	1.83	0.62	0.49	80.99	23.06	9.20	5.00	46.63	15.72	5.45	4.98
Testes/ovaries	1.27	0.52	0.28	0.04	3.35	1.75	0.63	0.36	26.10	19.56	7.51	4.93	41.85	14.95	5.85	4.61
Uterus	—	—	—	—	1.14	2.02	0.74	0.51	—	—	—	—	54.64	22.35	8.10	7.04
Muscle	1.25	0.39	0.11	0.03	0.86	0.45	0.14	0.09	35.25	9.98	3.49	1.95	14.93	4.24	1.60	1.48
Brain	0.71	0.19	0.04	0.01	0.37	0.17	0.07	0.04	4.68	1.95	1.03	0.61	3.83	1.52	0.58	0.51
Adipose tissue	0.95	0.35	0.10	0.03	0.88	0.42	0.16	0.16	12.54	10.41	3.76	1.67	9.32	3.97	2.75	1.45
Bone	1.01	0.29	0.06	0.03	0.69	0.33	0.14	0.08	18.87	7.44	3.06	1.47	10.15	3.35	1.67	1.14
Bone marrow	3.21	0.99	0.17	0.11	1.93	1.02	0.40	0.24	49.80	21.28	9.27	3.87	37.66	14.25	7.13	4.34
Thyroid	9.72	4.23	0.52	0.29	6.02	2.05	1.00	0.59	79.28	46.16	11.59	8.60	85.62	29.22	11.51	9.54
Pancreas	2.22	0.76	0.24	0.08	1.66	0.85	0.31	0.23	42.07	13.61	5.44	3.00	25.78	8.40	3.56	2.76
Stomach content	66.23	0.13	0.07	0.05	70.23	26.31	0.31	0.25	1213.7	576.83	22.24	2.32	3513.5	422.18	2.54	8.79
Stomach	19.77	0.91	0.22	0.08	18.43	13.55	0.54	0.38	421.61	137.66	12.43	3.69	483.67	157.55	4.30	4.55
Gut content	8.01	21.35	4.03	1.65	6.52	9.47	5.82	3.39	318.65	503.76	277.93	159.87	417.29	299.40	78.83	44.27
Gut	7.94	6.45	1.76	0.65	5.70	3.40	1.07	1.10	130.46	62.00	28.78	8.69	153.13	40.04	14.72	5.14
Liver	34.02	11.45	3.08	0.77	38.32	27.09	6.22	5.76	223.48	116.55	44.87	38.08	187.75	86.74	38.90	35.61
Skin	2.68	1.14	0.29	0.10	1.86	1.23	0.49	0.45	54.99	23.58	9.68	5.73	35.84	13.36	6.70	4.56
Carcass	1.77	0.75	0.20	0.06	1.34	0.72	0.28	0.18	30.66	13.55	5.56	2.86	13.20	4.72	2.11	1.69

From Fabian & Landsiedel (2007a). Data extracted from pp. 39–40 of the study report.

Table 4. Mean radioactivity in tissues after oral administration of ^{14}C-labelled saflufenacil at 5 mg/kg bw

	Mean radioactivity ± standard deviation (µg eq/g tissue)							
	Males (n = 3 per sacrifice interval)				Females (n = 3 per sacrifice interval)			
	Time (h)							
	1	24	48	72	1	4	20	24
Stomach content	66.2 ± 11.8	—	—	—	70.2 ± 22.6	26.3 ± 12.7	—	—
Liver	34.0 ± 3.01	11.5 ± 0.69	3.08 ± 3.45	—	38.3 ± 9.62	27.1 ± 5.98	6.22 ± 1.44	5.76 ± 2.14
Plasma	30.4 ± 3.42	9.26 ± 7.39	1.71 ± 1.97	—	18.7 ± 11.4	8.07 ± 4.76	2.92 ± 0.01	1.74 ± 2.09
Stomach	19.8 ± 1.53	—	—	—	18.4 ± 4.91	13.6 ± 6.90	—	—
Thyroid	9.72 ± 1.90	4.23 ± 3.20	—	—	6.02 ± 3.21	2.05 ± 1.73	1.00 ± 0.09	—
Blood cells	8.34 ± 2.07	1.90 ± 1.19	—	—	5.42 ± 2.49	2.37 ± 1.01	—	—
Lungs	8.16 ± 1.25	2.77 ± 1.92	—	—	5.07 ± 2.11	2.97 ± 1.41	—	—
Gut content	8.01 ± 0.29	21.4 ± 3.36	4.03 ± 2.01	1.65 ± 1.13	6.52 ± 0.50	9.47 ± 1.30	2.82 ± 1.16	3.39 ± 1.49
Kidneys	8.00 ± 0.58	2.05 ± 0.78	—	—	8.71 ± 1.32	4.39 ± 0.12	—	—
Gut	7.94 ± 0.69	6.45 ± 0.88	1.76 ± 1.06	—	5.70 ± 0.37	3.40 ± 0.52	1.07 ± 0.43	1.10 ± 0.65
Adrenals	5.98 ± 1.44	1.51 ± 1.01	—	—	3.30 ± 1.62	1.83 ± 1.04	—	—
Heart	5.04 ± 0.52	1.50 ± 1.09	—	—	4.45 ± 3.48	1.55 ± 0.72	—	—
Bone marrow	3.21 ± 0.44	—	—	—	1.93 ± 0.89	1.02 ± 0.63	—	—
Skin	2.68 ± 0.35	1.14 ± 0.88	—	—	1.86 ± 0.42	1.23 ± 0.68	—	—
Pancreas	2.22 ± 0.24	—	—	—	1.66 ± 0.69	—	—	—
Spleen	1.82 ± 0.16	—	—	—	1.26 ± 0.44	—	—	—
Carcass	1.77 ± 0.14	—	—	—	1.34 ± 0.47	—	—	—
Testes/ovaries	1.27 ± 0.22	—	—	—	3.35 ± 1.74	1.75 ± 0.72	—	—
Uterus	—	—	—	—	1.14 ± 1.30	2.02 ± 0.66	—	—
Muscle	1.25 ± 0.13	—	—	—	—	—	—	—
Bone	1.01 ± 0.11	—	—	—	—	—	—	—

From Fabian & Landsiedel (2007a). Data taken from tables 5, 30–33, pp. 40, 65–68 of the study report.

[a] Only tissues with ≥ 1.00 µg eq/g are listed.

Table 5. Mean radioactivity in tissues after oral administration of ^{14}C-labelled saflufenacil at 100 mg/kg bw[a]

	Mean radioactivity ± standard deviation (µg eq/g tissue)							
	Males (n = 3 per sacrifice interval)				Females (n = 3 per sacrifice interval)			
	Time (h)							
	1	7	20	34	1	7	20	34
Stomach content	1214 ± 471	577 ± 96.4	22.2 ± 27.7	—	3514 ± 1279	422 ± 258	—	8.79 ± 10.8
Liver	224 ± 45.3	117 ± 35.6	44.9 ± 7.31	38.1 ± 3.9	188 ± 7.87	86.7 ± 7.39	38.9 ± 9.17	35.6 ± 5.35
Plasma	222 ± 40.7	108 ± 43.9	50 ± 35	29.9 ± 7.01	182 ± 14.5	69.3 ± 9.62	24.4 ± 17.4	23.6 ± 9.72
Stomach	421.6 ± 121	138 ± 42.4	12.4 ± 10.4	—	484 ± 184	158 ± 188	—	—
Blood cells	74.0 ± 23.0	28.0 ± 10.2	8.30 ± 5.59	5.80 ± 2.15	43.2 ± 2.66	12.9 ± 5.7	7.70 ± 5.94	6.57 ± 2.44
Thyroid	79.3 ± 21.6	46.2 ± 17.3	11.6 ± 7.33	8.60 ± 1.53	85.6 ± 20.8	29.2 ± 3.70	11.5 ± 8.55	9.54 ± 3.00
Lungs	206 ± 237	30.5 ± 11.8	13.5 ± 9.29	8.33 ± 1.84	60.1 ± 11.2	22.6 ± 5.01	9.21 ± 6.15	8.24 ± 2.51
Kidneys	198 ± 19.2	79.3 ± 36.7	13.8 ± 5.83	8.62 ± 0.67	143 ± 8.90	31.4 ± 3.48	9.09 ± 3.10	7.34 ± 0.48
Gut	131 ± 27.8	62.0 ± 15.1	28.8 ± 15.8	8.69 ± 3.28	153 ± 29.4	40.0 ± 3.35	14.7 ± 3.63	5.14 ± 1.14
Gut content	319 ± 13.9	504 ± 83.0	278 ± 160	160 ± 82.3	417 ± 94.7	299 ± 68.6	78.8 ± 12.4	44.3 ± 13.9
Adrenals	81.0 ± 55.0	23.1 ± 8.81	9.20 ± 5.46	5.00 ± 0.43	46.6 ± 8.43	15.7 ± 2.11	5.45 ± 2.88	—
Heart	79.4 ± 30.2	32.0 ± 20.0	9.39 ± 6.22	6.01 ± 1.78	40.2 ± 6.04	13.6 ± 2.00	—	—
Bone marrow	49.8 ± 24.5	21.3 ± 7.21	9.27 ± 5.82	—	37.7 ± 6.76	14.2 ± 3.61	7.13 ± 5.64	—
Pancreas	42.1 ± 16.2	13.6 ± 5.55	5.44 ± 3.33	—	25.8 ± 6.40	8.40 ± 0.82	—	—
Spleen	36.4 ± 15.2	10.3 ± 3.33	—	—	18.4 ± 2.13	5.28 ± 0.54	—	—
Testes/ovaries	26.1 ± 7.78	19.6 ± 3.76	7.51 ± 4.86	—	41.9 ± 9.00	15.0 ± 3.25	5.85 ± 3.67	—
Uterus	—	—	—	—	54.6 ± 11.4	22.4 ± 5.42	8.10 ± 5.04	7.04 ± 2.01
Skin	55.0 ± 19.9	23.6 ± 9.75	9.68 ± 4.68	5.73 ± 0.92	35.8 ± 6.06	13.4 ± 1.83	6.70 ± 1.65	—
Carcass	30.7 ± 12.5	13.6 ± 3.28	5.56 ± 3.29	—	13.2 ± 0.85	—	—	—

From Fabian & Landsiedel (2007a). Data taken from tables 4, 21–24, pp. 39, 56–59 of the study report.

[a] Only tissues with ≥ 5.00 µg eq/g are listed.

Table 6. Percentages of biliary excretion of radioactivity after a single oral administration of ^{14}C-labelled saflufenacil at 5 or 100 mg/kg bw

Time interval (h)	% of biliary excretion (mean ± standard deviation)			
	Males		Females	
	Dose (mg/kg bw)			
	5	100	5	100
0–3	2.82 ± 1.91	5.01 ± 3.41	1.10 ± 0.34	2.14 ± 1.16
3–6	4.59 ± 2.66	8.15 ± 6.47	1.09 ± 0.21	1.78 ± 0.72
6–9	4.69 ± 2.29	6.59 ± 5.21	0.81 ± 0.21	1.46 ± 0.64
9–12	5.33 ± 2.24	6.24 ± 3.58	0.77 ± 0.14	1.94 ± 1.18
12–15	5.99 ± 2.01	6.94 ± 1.02	0.77 ± 0.07	3.41 ± 2.44
15–18	5.99 ± 1.16	7.60 ± 1.68	0.91 ± 0.15	3.20 ± 1.05
18–21	4.64 ± 0.93	5.29 ± 1.47	1.24 ± 0.21	3.29 ± 0.88
21–24	3.32 ± 0.81	4.28 ± 2.69	1.23 ± 0.47	3.18 ± 0.75
24–27	2.52 ± 0.61	3.36 ± 2.13	0.98 ± 0.71	3.11 ± 0.90
27–30	2.21 ± 0.53	2.64 ± 1.63	0.88 ± 0.88	2.70 ± 1.21
30–33	2.01 ± 0.39	2.26 ± 1.36	1.03 ± 0.55	2.35 ± 1.14
33–36	1.76 ± 0.27	2.41 ± 2.08	1.58 ± 0.45	2.11 ± 0.84
36–39	1.62 ± 0.55	2.44 ± 2.48	1.63 ± 0.42	1.69 ± 0.59
39–42	1.63 ± 0.38	1.88 ± 1.82	1.76 ± 0.36	1.33 ± 0.42
42–45	1.71 ± 0.48	1.47 ± 1.29	1.51 ± 0.24	0.87 ± 0.23
45–48	1.47 ± 0.55	1.18 ± 1.01	1.13 ± 0.43	0.92 ± 0.15
Total	52.3 ± 13.4	67.8 ± 6.16	18.4 ± 3.36	35.5 ± 4.94

From Fabian & Landsiedel (2007a). Data taken from tables 40–41, pp. 75–76 of the study report.

1.2 Biotransformation

Urine, faeces and bile samples collected from the previously conducted absorption, distribution and excretion studies in rats following a single oral dose of 5 or 100 mg/kg bw and a 15-day repeated-dosing study at 100 mg/kg bw were used for metabolic characterization (Fabian & Landsiedel, 2007a). Additional metabolism studies were conducted for the isolation and identification of urinary and faecal metabolites. In these studies, Wistar rats (10 of each sex per dose) were administered a single gavage dose of [uracil-^{14}C]saflufenacil or [phenyl-^{14}C]saflufenacil at 100 mg/kg bw. Urine and faecal samples were collected at several time points for up to 168 hours. Animals were sacrificed 168 hours following dosing. For the analysis of the metabolite patterns in liver, kidney, fat and plasma, Wistar rats (four of each sex per dose) were administered a single gavage dose at 5 or 100 mg/kg bw (uracil and phenyl label), and the organs were removed 1 hour after dosing. Appropriate aliquots of the pooled urine and bile samples were directly subjected to radio-high-performance liquid chromatography (HPLC) without further extraction or other kind of workup. Faecal samples were extracted 3 times with acetonitrile and concentrated with a rotary evaporator. Aliquots of acetonitrile extracts were measured by liquid scintillation counting (LSC) and HPLC. Liver and kidney samples were homogenized and extracted 3 times with acetonitrile and analysed by LSC and HPLC. Aliquots of homogenized fat samples were extracted either 2 or 3 times with a mixture of acetonitrile and isohexane (50:50 volume per volume [v/v]). After centrifugation, the supernatants were transferred to a separatory funnel. The separated phases of acetonitrile and isohexane were collected in volumetric flasks and each adjusted to a defined volume. The acetonitrile extracts were combined, concentrated

with a rotary evaporator and adjusted to a defined volume, and aliquots were measured by LSC and HPLC. Residues were treated with Biolute S and water. The solution was kept at 50 °C until the tissue was completely dissolved. Afterwards, Lumagel Plus was added, and the sample was stored overnight in the refrigerator until chemoluminescence decayed. Radioactivity was determined by LSC. The metabolites were identified using various analytical methods, such as HPLC, mass spectrometry, nuclear magnetic resonance (NMR) spectrometry and co-chromatography (Grosshans, 2007).

The unchanged parent compound was predominant in the urine of all dose groups. In the urine, saflufenacil amounted to 10.9–48.2% of the administered dose for male rats and to 43.9–88.9% of the administered dose for female rats (Tables 7 and 8). The major metabolites identified in urine samples of male rats (Tables 7 and 8) were M800H01 (3.5–9.1% of the administered dose), M800H03 (0.8–2.3% of the administered dose), M800H05 (0.4–4.2% of the administered dose) and M800H07 (0.4–2.2% of the administered dose). In urine samples of female rats (Tables 7 and 8), the metabolite M800H07 (0.6–4.6% of the administered dose) was the predominant one; the other metabolites amounted to 0.05–1.7% of the administered dose. M800H09 occurred in small amounts (< 0.01–0.3% of the administered dose) in urine of both males and females. M800H37 (a phenyl label–specific metabolite), M800H23 (a uracil label–specific metabolite), M800H02 (both phenyl and uracil label) and M800H06 (both phenyl and uracil label) were also detected in small amounts in the urine of male and female rats. Total identified metabolites in urine (both labels) were in the range of 25–62% of the dose for male rats and 53.4–95% of the dose for female rats (Tables 7 and 8).

The predominant metabolites in faeces of male and female rats in all dose groups were M800H01 (18.1–43.9% of the administered dose in males and 1.0–2.5% of the administered dose in females) and the unchanged parent compound (3.8–16.2% and 2.9–9.9% of the administered dose in males and females, respectively) (Tables 7 and 8). Other metabolites identified in faeces were M800H02, M800H03 (male = 2.1–5.2% of the administered dose, female = 0.4–1.6% of the administered dose), M800H04, M800H05 (male = 0.3–3.6% of the administered dose, female = < 0.01–0.2% of the administered dose), M800H06, M800H07 (male = 1.1–2.0% of the administered dose, female = 0.7–1.4% of the administered dose) and M800H08. The HPLC retention behaviour of the metabolites M800H02 and M800H06, as well as of the metabolites M800H04 and M800H08, were very similar; therefore, the peaks were not clearly separated by HPLC. The sum of M800H02 and M800H06 amounted to 2.4–8.6% of the administered dose for male rats and 0.2–0.6% of the administered dose for female rats. The sum of M800H04 and M800H08 amounted to 0.7–2.4% of the administered dose for male rats and to 0.6–1.2% of the administered dose for female rats. The phenyl label–specific metabolite M800H37 was found in amounts of 0.8–4.5% and < 0.01–0.2% of the administered dose for male and female rats, respectively. Metabolite M800H09 was detected only in minor amounts (0.03% of the administered dose) in faeces of female rats. The uracil label–specific metabolite M800H23 was not detected in faeces of both sexes. Total identified metabolites in faeces were in the range of 32–72% of the administered dose for male rats and 10.3–13.4% of the administered dose for female rats (Tables 7 and 8).

For male rats, the major metabolites in the bile (Table 9) were M800H01 (8.7–10.6% of the administered dose), M800H07 (11.1–13.4% of the administered dose), M800H18 (8.9–11.5% of the administered dose) and the unchanged parent compound (4.8–14.5% of the administered dose). The metabolite M800H18 was identified as a derivative of the parent compound in which the uracil ring was cleaved to form the *N*-sulfonylamide group, combined with a demethylation of the *N*-methyl-*N*-isopropyl group. Other metabolites identified in the bile were M800H02 + M800H17 (2.1–4.4% of the administered dose), M800H16 (1.6–2.4% of the administered dose), M800H19 (3.1–3.2% of the administered dose), M800H20 (4.1–4.6% of the administered dose) and M800H21 (0.2–0.3% of the administered dose). M800H17 was identified as a derivative of the parent compound in which the uracil ring was cleaved, but the three-carbon fragment remained attached to the molecule. M800H20 and M800H19 were formed by further degradation of the *N*-methyl-*N*-isopropyl group. Metabolite M800H16

Table 7. Identified metabolites in urine and faeces of rats 72–168 hours after single oral administration of ^{14}C-labelled saflufenacil (phenyl label) at 5 mg/kg bw and 48–168 hours after repeated daily administration of ^{14}C-labelled saflufenacil (phenyl label) at 100 mg/kg bw

Metabolite	% of administered dose							
	Single 5 mg/kg bw				14 + 1 doses; 100 mg/kg bw per day			
	Urine		Faeces		Urine		Faeces	
	Males	Females	Males	Females	Males	Females	Males	Females
	Time assessed (h)							
	0–168	0–168	0–96	0–72	0–168	0–168	0–72	0–48
Saflufenacil	10.9	88.9	3.77	2.93	48.2	78.1	5.55	5.25
M800H01	5.21	0.85	43.9	2.52	7.88	1.70	18.08	1.63
M800H05	4.23	0.09	3.55	0.14	1.45	—	0.867	0.09
M800H03	2.34	0.67	5.22	1.63	1.96	0.43	2.092	0.84
M800H07	1.65	4.62	2.01	1.39	1.86	2.54	1.136	1.12
M800H37	0.54	0.01	4.50	0.13	0.16	—	1.015	0.20
M800H02+06	0.04	—	8.64	0.58	0.04	—	2.387	0.51
M800H09	0.02	—	—	0.03	0.002	0.02	—	—
M800H04+08	nd	—	0.68	0.97	nd	—	1.177	1.23
Total identified	25.0	95.2	72.3	10.3	61.6	82.7	32.3	10.9
Total unidentified	1.05	0.91	3.79	1.08	0.19	0.64	1.31	1.27

From Grosshans (2007). Data taken from tables 10–13, 18–21, pp. 63–65, 67–69 of the study report.
nd, not detectable; —, not reported

could be formed by oxidation of M800H17. M800H21 was an oxidation product of BAS 800 H. In female rats, the predominant biliary metabolite (Table 9) was M800H07 (4.8–8.1% of the administered dose) and the parent compound (6.6–11.4% of the administered dose). Other identified minor metabolites were M800H01 (0.7–1.1% of the administered dose), M800H02 + M800H17 (1.8–3.1% of the administered dose), M800H16 (0.8–1.7% of the administered dose), M800H18 (0.6–1.1% of the administered dose), M800H19 (0.8–2.1% of the administered dose), M800H20 (0.7–1.4% of the administered dose) and M800H21 (0.1–0.3% of the administered dose). Total identified metabolites in bile were in the range of 47–62% and 17–31% of the administered dose in male and female rats, respectively.

In liver, saflufenacil was identified in the range of 16–25% of the dose for the low-dose group and in the range of 4–7% of the dose for the high-dose group (both labels, not sex specific). The metabolite M800H04 amounted to 1.6–4.3% of the dose for the low-dose group and to 1.3–2.1% of the dose for the high-dose group (both labels, not sex specific). The metabolite M800H0l was detected in amounts less than or equal to 1.3% of the dose. The metabolite patterns in kidney and fat were comparable to those in liver. The total radioactivity and the sum of all identified metabolites were below or equal to 1.0% of the dose. In plasma, saflufenacil (1.6–4.1% of the dose) appeared to be predominant throughout all dose groups. M800H01, M800H04 and M800H07 were identified in minor amounts (each below 0.2% of the dose).

In rats, saflufenacil was metabolized by three major transformation steps, which are demethylation of the uracil ring system, degradation of the *N*-methyl-*N*-isopropyl group to amine and cleavage of the uracil ring, forming a sulfonylamide group. The predominant compounds were the metabolites M800H01, M800H03 and M800H07 and the parent compound for male and female rats. In addition, the metabolites M800H05 and M800H18 also occurred in male rats. The metabolites M800H01,

Table 8. Identified metabolites in urine and faeces of rats 24–168 hours after oral administration of ^{14}C-labelled saflufenacil at 100 mg/kg bw

Metabolite	% of administered dose																	
	Urine									Faeces								
	Males					Females				Males					Females			
	Time interval (h)																	
	0–24		0–96		0–168	0–24	0–96	0–168		0–24		0–72	0–96		0–24	0–48	0–72	
	P	U	P	U	P	P	P	P	U	P	U	P	P	U	P	P	P	U
Saflufenacil	21.7	20.9	23.1	22.2	36.6	43.9	48.7	82.1	53.3	14.6	13.7	4.84	16.0	16.2	7.54	3.75	9.94	9.20
M800H01	2.95	3.39	3.53	3.93	9.05	nd	0.06	0.47	0.05	13.2	12.9	23.0	22.3	25.8	0.52	1.16	0.98	1.27
M800H05	0.50	0.29	0.69	0.44	1.76	—	—	nd	—	0.29	nd	0.77	0.59	0.33	—	0.18	—	—
M800H03	0.59	0.63	0.75	0.81	2.21	nd	0.04	0.05	—	1.66	1.52	2.40	2.65	2.71	0.15	0.65	0.36	0.45
M800H07	0.28	—	0.40	—	2.16	0.42	0.63	3.64	—	0.85	—	1.41	1.20	—	0.30	0.75	0.76	—
M800H37	—	—	0.02	—	0.19	—	—	0.01	—	0.47	—	1.79	0.83	—	—	0.10	—	—
M800H02+06	nd	nd	nd	nd	—	nd	nd	—	—	1.32	1.13	2.90	2.68	3.20	nd	0.32	0.16	0.36
M800H09	—	0.30	—	0.34	—	—	—	0.04	0.01	—	nd	—	—	nd	—	—	—	—
M800H04+08	nd	nd	nd	nd	—	nd	nd	—	—	0.97	1.52	0.95	1.38	2.37	0.61	0.59	1.24	0.78
M800H23	—	0.26	—	0.38	—	—	—	—	0.05	—	nd	—	—	nd	—	—	—	—
Total	26.0	25.8	28.5	28.1	52.0	44.3	49.5	86.3	53.4	33.3	30.8	38.1	47.6	50.6	9.12	7.50	13.4	12.1

From Grosshans (2007). Data taken from tables 14–17, 22–26, pp. 65–67, 70–72 of the study report.
nd, not detectable; —, not reported; P, phenyl label; U, uracil label

Table 9. Identified metabolites in the bile of bile duct–cannulated rats 48–72 hours after single administration of [phenyl-^{14}C]saflufenacil at 5 or 100 mg/kg bw

Metabolite	% of administered dose			
	5 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
	Time interval (h)			
	0–48	0–48	0–48	0–48
Saflufenacil	4.78	6.56	14.5	11.4
M800H18	11.5	0.61	8.87	1.14
M800H07	11.1	4.77	13.4	8.08
M800H01	8.69	0.72	10.6	1.13
M800H20	4.11	0.70	4.58	1.43
M800H02+17	2.08	1.75	4.42	3.08
M800H16	1.62	0.82	2.43	1.67
M800H21	0.17	0.14	0.26	0.32
M800H19	3.06	0.83	3.21	2.06
Total	47.0	16.9	62.2	30.9

From Grosshans (2007). Data taken from tables 27–30, pp. 73–76 of the study report.

M800H03 and M800H05 were identified as derivatives of saflufenacil in which the *N*-methyl-*N*-isopropyl group was step by step degraded to unsubstituted sulfonamide. The metabolite M800H07 was identified as a derivative of the parent compound in which the uracil ring was cleaved with loss of three carbons to form a phenyl-*N*-methylurea group. The metabolite M800H18 was identified as a derivative of the parent compound in which the uracil ring was cleaved to form a phenyl-*N*-methyl-urea, combined with demethylation of the *N*-methyl-*N*-isopropylsulfonamide. Metabolites M800H16, M800H17, M800H18, M800M19 and M800M20 were present in bile only at remarkable portions. Further identified metabolites were M800H02, M800H04, M800H06, M800H08, M800H09, M800H10, M800H11, M800H16, M800H17, M800H19, M800H20, M800H21, M800H23, M800H35 and M800H37. The summary of identified metabolites found in urine, faeces, bile, liver, kidney, fat and plasma is shown in Table 10. The proposed metabolic pathway of saflufenacil (BAS 800 H) in rat is shown in Figure 2 (Grosshans, 2007).

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of saflufenacil is summarized in Table 11.

(a) Oral administration

Rats

Three fasted female young adult Wistar rats were treated orally, by gavage, with saflufenacil (purity 93.8%) in 0.5% carboxymethylcellulose in double-distilled water at a single dose of 2000 mg/kg bw. As no morbidity or mortality was observed in the first test group, a second group of three fasted female young adult Wistar rats was similarly treated at a single gavage dose of 2000 mg/kg bw. Animals were observed for mortality and clinical signs several times for the 1st day and once daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. A gross necropsy was performed on all animals. The stability of the test substance was confirmed analytically.

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

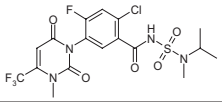
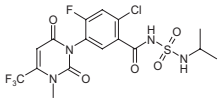
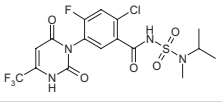
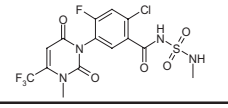
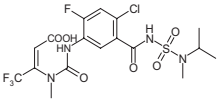
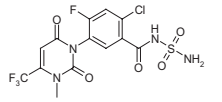
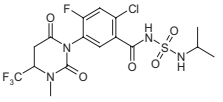
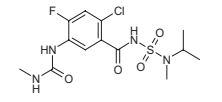
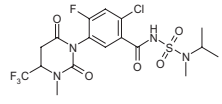
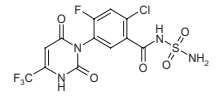
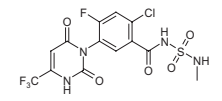
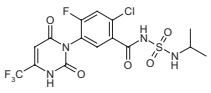
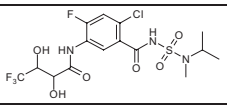
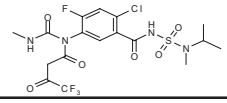
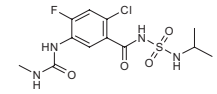
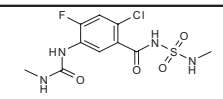
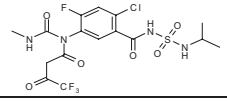
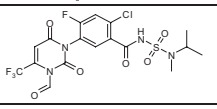
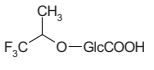
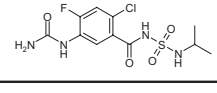
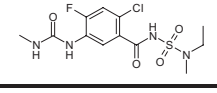
Metabolite Designation	Molecular Mass	Structure	Urine		Feces		Bile	Liver		Kidney		Fat		Plasma	
			phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label
BAS 800 H	500		x	x	x	x	x	x	x	x	x	x	x	x	x
M800H01	486		x	x	x	x	x	x	x	x	x	x	x	x	-
M800H02	486		x	x	x	x	x	-	-	-	-	-	-	-	-
M800H03	458		x	x	x	x	-	x	-	x	-	-	-	-	-
M800H04	518		-	-	x	x	-	x	x	x	x	x	x	x	x
M800H05	444		x	x	x	x	-	-	-	-	-	-	-	-	-
M800H06	488		x	x	x	x	-	-	-	-	-	-	-	-	-
M800H07	380		x	-	x	x[1]	x	-	-	x	-	-	-	-	-
M800H08	502		-	-	x	x	-	-	-	-	-	-	-	-	-
M800H09	430		x	x	x	-	-	-	-	-	-	-	-	-	-
M800H10*	444		-	x	-	-	-	-	-	-	-	-	-	-	-

Table 10 (continued)

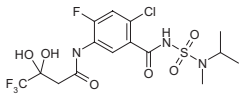
Metabolite Designation	Molecular Mass	Structure	Urine		Feces		Bile	Liver		Kidney		Fat		Plasma	
			phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label	phenyl-label	uracil-label
M800H11*	472		x	-	x	-	x	-	-	-	-	-	-	-	-
M800H16	479		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H17	518		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H18	366		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H19	338		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H20	504		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H21	514		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H23	290		-	x	-	-	-	-	-	-	-	-	-	-	-
M800H35*	352		-	-	-	-	x	-	-	-	-	-	-	-	-
M800H37	366		x	-	x	-	-	-	-	-	-	-	-	-	-

Notes: * Metabolites were identified by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Quantification within the study was not feasible because of negligible amounts.

x = identified

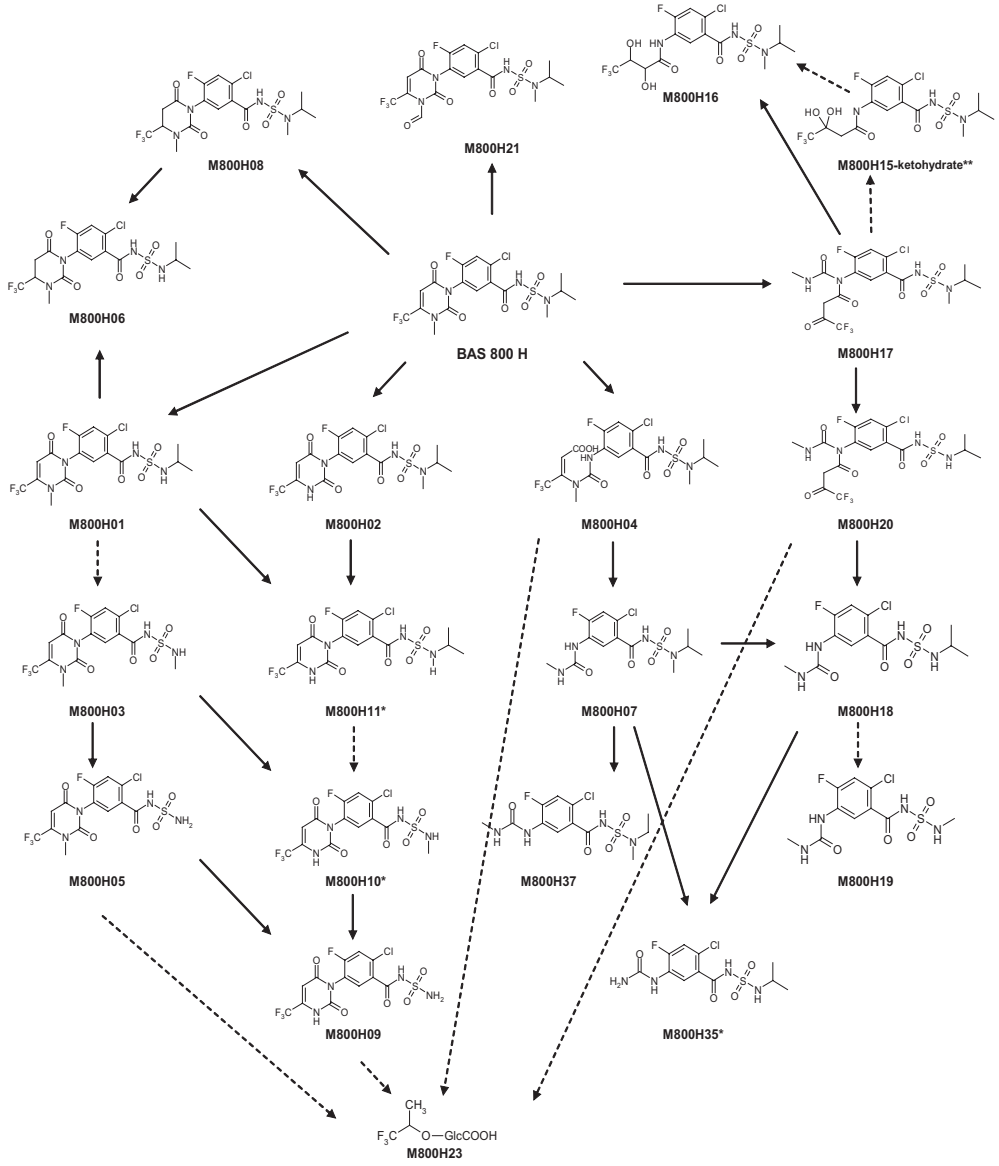
- = not identified

M800H15-ketohydrate is a potential intermediate not identified during the current study.

M800H15-ketohydrate: 

[1] Metabolite M800H07 was detected in the faecal extract from the uracil label by LC-MS/MS only and not by radio-HPLC.

Figure 2. Proposed metabolic pathway of saflufenacil (BAS 800 H) in the rat



* Metabolites were identified by LC-MS/MS. Radio-detection and thus quantification within the study were not feasible because of negligible amounts.

** Metabolite M800H15-ketohydrate is a potential intermediate not identified in the study.

Table 11. Acute toxicity of saflufenacil

Species	Strain	Sex	Route	Purity (%)	Results	Reference
Rat	Wistar	Female	Oral	93.8	LD_{50} > 2000 mg/kg bw	Gamer & Leibold (2005a)
Rat	Wistar	Male and female	Dermal	93.8	LD_{50} > 2000 mg/kg bw	Gamer & Leibold (2005b)
Rat	Wistar	Male and female	Inhalation (nose only)	93.8	LC_{50} > 5.3 mg/l	Ma-Hock & Leibold (2005)
Rabbit	New Zealand White	Male and female	Dermal irritation	93.9	Non-irritating	Remmele & Leibold (2005b)
Rabbit	New Zealand White	Male and female	Ocular irritation	93.9	Minimally irritating	Remmele & Leibold (2005a)
	New Zealand White	Male	Ocular irritation	93.8	Minimally irritating	Remmele & Landsiedel (2007)
Guinea-pig	Dunkin Hartley	Female	Dermal sensitization (maximization test)	93.8	Non-sensitizing	Gamer & Leibold (2005c)

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

No deaths or clinical signs of an adverse reaction to treatment occurred, and there were no macroscopic findings at necropsy in any animal. The mean body weights of the first group were increased more during the 1st week of observation than during the 2nd week. The mean body weights of the second group more uniformly increased over the study period. Based on these results, the acute oral median lethal dose (LD_{50}) was estimated to be greater than 2000 mg/kg bw (Gamer & Leibold, 2005a).

(b) Dermal application

Rats

A group of five male and five female young adult Wistar rats were treated with saflufenacil (purity 93.8%) dispersed in aqueous solution of 0.5% carboxymethylcellulose once for 24 hours by topical, semi-occluded application to a clipped area of intact dorsal skin (40 cm^2; approximately 10% of body surface area) at a dose level of 2000 mg/kg bw. Animals were observed for mortality and clinical signs several times for the 1st day and once daily thereafter for 13 days. Body weights were recorded on days 0, 7 and 14. The animals were observed for 14 days post-treatment and then subjected to necropsy and postmortem examination.

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

(c) Exposure by inhalation

Rats

A group of five male and five female Wistar rats was exposed once for 4 hours by nose-only, flow-past inhalation to a dust aerosol of saflufenacil (purity 93.8%) at an analytically determined mean concentration of 5.3 mg/l. The animals were observed for 14 days post-treatment, during which time clinical signs were recorded twice a day during weekdays and once on weekends. Body weights were recorded on day 0 and weekly thereafter. All animals were subjected to necropsy and postmortem examination.

Cascade impactor measurements resulted in particle size distributions with mass median aerodynamic diameters (MMADs) of 2.3 and 3.2 μm, which were within the respirable range. No deaths occurred during the exposure or observation periods. Clinical signs of toxicity comprised squatting posture, piloerection, visually accelerated respiration and smeared and contaminated fur in all animals. Findings were observed from hour 0 of exposure until and including study day 3. The mean body weights of the male and female animals increased throughout the post-exposure observation period. There were no macroscopic findings at necropsy in any animal. Based on these results, the acute median lethal concentration (4-hour median lethal concentration [LC_{50}]) was estimated to be greater than 5.3 mg/l air (Ma-Hock & Leibold, 2005).

(d) Dermal irritation

Rabbits

In a study of primary dermal irritation, one male and two female young adult New Zealand White rabbits were dermally exposed to 0.5 g of saflufenacil (purity 93.9%) applied to a 2.5 cm × 2.5 cm patch, covered with a semi-occlusive dressing. The test material was in contact with the skin for 4 hours. The adjacent skin area served as the control. After removal of the patch, the treated application site was washed off with Lutrol and Lutrol/water (1:1). Dermal irritation was scored at 1, 24, 48 and 72 hours after the removal of the patch. The animals were observed for 14 days post-treatment, during which time clinical signs were recorded twice a day during weekdays and once on weekends. Body weights were recorded prior to treatment and at termination.

Slight erythema was observed in all animals up to 1 hour after removal of the patch. The cutaneous reactions were reversible in all animals within 24 hours after removal of the patch. The average score (24–72 hours) for irritation was calculated to be 0.0 for erythema and for oedema. Based on the results of this study, saflufenacil was not irritating to the skin of rabbits (Remmele & Leibold, 2005b).

(e) Ocular irritation

Rabbits

In a primary eye irritation study, approximately 0.1 g of saflufenacil (about 32 mg; purity 93.9%) was instilled into the right conjunctival sac of one female and two male New Zealand White rabbits. About 1 hour after application, treated eyes were rinsed with 3–6 ml of warm tap water for 1–2 minutes. The ocular irritation was assessed approximately 1, 24, 48 and 72 hours after application.

Slight or moderate conjunctival redness (grade 1 or 2) was observed in all animals up to 24 hours after application. Slight conjunctival chemosis (grade 1) was noted in one animal, and slight discharge (grade 1) was noted in all animals 1 hour after application. In addition, injected scleral vessels in a circumscribed area were noted in the animals up to 24 hours after application. The ocular reactions were reversible in all animals within 48 hours after application. The average score (24–72 hours) for irritation was calculated to be 0.0 for corneal opacity, iritis and chemosis and 0.3 for conjunctival redness. Based on the results of this study, saflufenacil is minimally irritating to rabbit eyes (Remmele & Leibold, 2005a).

In a second primary eye irritation study, approximately 0.1 g of saflufenacil (about 32 mg; purity 93.8%) was instilled into the right conjunctival sac of three male New Zealand White rabbits. About 24 hours after application, treated eyes were rinsed with 3–6 ml of warm tap water for 1–2 minutes. The ocular irritation was assessed approximately 1, 24, 48 and 72 hours after application.

Slight or moderate conjunctival redness, slight conjunctival chemosis and slight or moderate discharge were observed in the animals within 24 hours after application. In addition, injected

scleral vessels in a circumscribed area were noted in all animals 1 hour after application only. The ocular reactions were reversible in two animals within 24 hours and in one animal within 48 hours after application. The average score (24–72 hours) for eye irritation was calculated to be 0.0 for corneal opacity, iritis and chemosis and 0.1 for conjunctival redness. Based on the results of this study, saflufenacil is minimally irritating to rabbit eyes (Remmele & Landsiedel, 2007).

(f) Dermal sensitization

Guinea-pigs

The skin sensitization potential of saflufenacil (purity 93.8%) was investigated in female Dunkin Hartley guinea-pigs (10 test animals and 5 negative controls) using the maximization test. A concurrent positive control group was not included. Concentrations of 5%, 50% and 25% saflufenacil in aqueous carboxymethylcellulose solution were used for intradermal induction, topical induction and challenge, respectively. Skin reactions to the challenge applications were evaluated 24 and 48 hours after patch removal.

The intradermal induction caused moderate and confluent to intense erythema and swelling at the injection sites of the test substance preparation in all test group animals. After the epicutaneous induction, incrustation, partially open (caused by the intradermal induction), could be observed in addition to moderate and confluent erythema and swelling in all test group animals. Saflufenacil was not sensitizing to skin in the maximization test in guinea-pigs (Gamer & Leibold, 2005c).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 28-day toxicity study, saflufenacil (purity 94.2%) was administered daily in the diet to C57BL/6NCrl mice (five of each sex per dose) at 0, 50, 150, 450, 1350 or 4050 parts per million (ppm) (equal to 0, 12.8, 36.6, 112, 335 and 882 mg/kg bw per day in males and 0, 17.9, 63.4, 153, 446 and 1621 mg/kg bw per day in females). The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, the body weight was determined on day 0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. An ophthalmoscopic examination was not conducted. Urine parameters were not analysed in this study. At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (98.2–102.8% of the nominal concentrations). No clinical signs of toxicity were observed. One female animal of the 150 ppm dose group was found dead on day 28 of the study, which was considered not related to the treatment. There was no treatment-related effect on body weight or body weight gain in females. The body weight and body weight gains of males at 1350 and 4050 ppm were adversely affected. At termination, the body weight gains of males were 63% and 31% of the control values at 1350 and 4050 ppm, respectively. There were no statistically significant findings for feed consumption, although males at 4050 ppm

consistently consumed less feed during the study period. In males, significantly lower values for red blood cells, haemoglobin, haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were observed in males at 1350 and 4050 ppm. In addition, haemoglobin and haematocrit values were significantly lower in the male mice at 150 and 450 ppm, and MCV and MCH were significantly decreased in the males at 450 ppm. Examination of red blood cell morphology in males revealed increased anisocytosis, microcytosis and polychromasia at 4050 ppm. Increased polychromasia was also seen in the erythrocytes of the males at 1350 ppm. In females, significantly reduced values for haemoglobin, haematocrit, MCV, MCH and mean corpuscular haemoglobin concentration (MCHC) were found at 4050 ppm. Increased anisocytosis and polychromasia were measured in erythrocytes of females at 4050 ppm. Serum enzyme examinations revealed dose-dependent, significant increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in males given 150, 450, 1350 and 4050 ppm of the test compound. Significantly higher alkaline phosphatase activities were also seen in males at 4050 ppm. In the serum of the females receiving 1350 and 4050 ppm of the test substance, ALT activities were significantly increased. No treatment-related changes were seen in the other serum enzymes or in cyanide-insensitive palmitoyl coenzyme A oxidation in the liver. Significantly increased urea and total bilirubin concentrations were found in the serum of males receiving 150, 450, 1350 and 4050 ppm of the test substance, and decreased glucose levels were noted in male mice at 450, 1350 and 4050 ppm. However, the fall in glucose level in the 1350 ppm group appeared only as a tendency towards reduced values. In males, there were statistically significant increases in absolute and relative liver weights at and above 150 ppm and an increase in spleen weight at 4050 ppm. All other statistically significant weight changes (kidneys, thymus and brain) were either secondary to the significant terminal body weight decrease in the high-dose group or of no biological significance. In females, the only treatment-related findings on organ weights were statistically significant increases of absolute and relative liver weights at 1350 and 4050 ppm. There were no treatment-related gross pathological findings. Substance-induced findings were observed in the liver and spleen. In the liver, changes included centrilobular fatty changes (males $\geq$ 150 ppm; females $\geq$ 450 ppm), minimal lymphoid infiltration (males $\geq$ 150 ppm; females $\geq$ 1350 ppm) and extramedullary haematopoiesis (males $\geq$ 450 ppm). In the spleen, the only finding was extramedullary haematopoiesis (males $\geq$ 1350 ppm; females 4050 ppm). A slight increase of apoptotic necrosis of lymphocytes was observed in the thymus of treated males at and above 150 ppm, which was regarded as a secondary effect of treatment.

The lowest-observed-adverse-effect level (LOAEL) established in males was 150 ppm (equal to 36.6 mg/kg bw per day), based on haematological parameters (decreased haemoglobin and haematocrit), altered clinical chemistry (increased ALT, AST, urea and total bilirubin) and liver pathology (increased weight and centrilobular fatty change). The no-observed-adverse-effect level (NOAEL) in males was 50 ppm (equal to 12.8 mg/kg bw per day). The LOAEL established in females was 450 ppm (equal to 153 mg/kg bw per day), based on moderate centrilobular fatty change in the liver. The NOAEL in females was 150 ppm (equal to 63.4 mg/kg bw per day) (Kaspers et al., 2007a).

In a 90-day toxicity study, saflufenacil (purity 93.9%) was administered daily in the diet to C57BL/6NCrl mice (10 of each sex per dose) at 0, 15 (males only), 50, 150, 450 or 1350 ppm (females only). The mean doses for males were 0, 3.6, 12.5, 36.7 and 109.1 mg/kg bw per day at 0, 15, 50, 150 and 450 ppm, respectively. The mean doses for females were 0, 17.6, 51.8, 156.7 and 471.2 mg/kg bw per day at 0, 50, 150, 450 and 1350 ppm, respectively. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed in all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, the body weight was determined on day

0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. No ophthalmoscopic examination was performed. Urine parameters were not analysed in this study. At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (92.3–104.2% of the nominal concentrations). There were no premature deaths during the study. There were no treatment-related clinical signs of toxicity. Body weight and body weight changes were not statistically significantly different from control. However, body weight in the males at 450 ppm was decreased ($P < 0.05$) on day 14. The overall body weight gains for the males at 150 and 450 ppm were lower than for the control males. These findings in the males were considered substance related. There were no treatment-related effects on feed consumption. At the end of the study, statistically significantly decreased values for haemoglobin, haematocrit, MCV, MCH and MCHC as well as increased platelet counts were found in the peripheral blood of male mice at 150 and 450 ppm. Moreover, decreases in MCV and MCH were observed in male mice treated with 15 and 50 ppm. Although the decreases in MCV and MCH values were statistically significant and consistent with the mechanism of action of the test substance, the changes were small and not accompanied by any other effects. As such, the differences in calculated values observed at the 15 and 50 ppm levels were considered not to be biologically relevant. In female mice, haematological examinations revealed statistically significantly decreased values for haemoglobin, haematocrit, MCV and MCH at the 150, 450 and 1350 ppm levels. MCHC was decreased at 1350 ppm and platelets were increased at 450 and 1350 ppm in female mice. These changes were considered biologically relevant and adverse. In males, most of the changes in clinical chemistry parameters were minor, although a few changes were statistically significant. The values could represent normal biological variations. At 150 and 450 ppm, increased levels of serum ALT and AST activities and possibly increased blood urea levels might be related to liver pathology. There were no treatment-related clinical chemistry effects in females. The only treatment-related effect on organ weight was the increase in liver weights (absolute and relative to body weight) of males at 150 and 450 ppm and in females at 450 and 1350 ppm. Other findings were not considered relevant because of the inconsistencies, the lack of a dose–response relationship and the absence of associated histopathological findings. There were no treatment-related gross pathological findings. Substance-induced histopathological findings were observed in the liver. The findings included an increase in the occurrence and severity of a diffuse (males) or central (females) fatty change of hepatocytes, as well as increased lymphoid infiltration (both sexes), at and above 150 ppm. All other findings noted are considered spontaneous or incidental in origin and not related to treatment.

The LOAEL was 150 ppm (equal to 36.7 mg/kg bw per day in males and 51.8 mg/kg bw per day in females), based upon decreased body weight and body weight gain in males, multiple haematological changes (haemoglobin, haematocrit, MCV, MCH, MCHC and/or platelet counts) in both sexes and liver weight increases in males with centrilobular fatty change in 8 of 10 males and 1 of 10 females. The NOAEL was 50 ppm (equal to 12.5 mg/kg bw per day in males and 17.6 mg/kg bw per day in females) (Kaspers et al., 2007e).

Rats

In a 28-day toxicity study, saflufenacil (purity 94.2%) was administered daily in the diet to Wistar rats (five of each sex per dose) at 0, 50, 150, 450, 1350 or 4050 ppm (equal to 0, 4.5, 13.4, 39.2, 117 and 357 mg/kg bw per day in males and 0, 5.0, 15.0, 43.6, 130 and 376 mg/kg bw per day in females). The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays,

Sundays and public holidays. Detailed clinical observations were performed in all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, body weight was determined on day 0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. A functional observational battery (FOB) was conducted for all rats at the end of the administration period. Motor activity was measured on the same day as the FOB. The eyes of all animals prior to the start of the administration period and the eyes of the control and high-dose animals on days 23 (males) and 22 (females) were examined for any changes using an ophthalmoscope after administration of a mydriatic. Urinalysis was conducted on samples from all study animals on day 25 (females) or 26 (males). At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (95.7–103.1% of the nominal concentration). There was no mortality in the study. Motor activity and FOB parameters were not affected in males or females. Several males at 450 ppm and all animals at 1350 and 4050 ppm showed dark, discoloured urine. The discoloration of the urine was considered a compensatory response to the altered porphyrin metabolism. This response is treatment related, but not adverse. Skin paleness was observed in all rats at 1350 and 4050 ppm. Urine-smeared anogenital region was observed with all males and three females at 4050 ppm and one male at 1350 ppm. There were statistically significant decreases in body weight and body weight gain in males at 1350 and 4050 ppm (76% and 75% of the control values at 1350 and 4050 ppm, respectively). Body weights and body weight changes in females were not affected. Feed consumption was reduced in males at 1350 and 4050 ppm throughout most of the study period. Isolated increases in feed consumption in females were not considered treatment related due to the lack of a clear dose–response relationship. The ophthalmoscopic examination of the eyes indicated no treatment-related findings. Dietary exposure to saflufenacil at 1350 and 4050 ppm resulted in changes in several haematological parameters in both sexes. The affected parameters were erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, reticulocytes, white blood cells, lymphocytes, neutrophils, anisocytosis and/or polychromasia. In males at 450 ppm, statistically significantly lower haemoglobin, MCV and MCH were also reported. Compound-related differences in serum enzyme activities were not evident at any dose level in either males or females. No treatment-related effects on serum hormone levels (triiodothyronine [T_3], thyroxine [T_4], thyroid stimulating hormone [TSH]) were found in either sex. Blood chemistry examinations revealed statistically significantly decreased total protein, albumin and globulin concentrations and increased total bilirubin levels in the males at 4050 ppm and reduced globulin concentrations as well as high total bilirubin concentrations in the serum of the males at 1350 ppm. Similar decreases in total protein, albumin and globulin levels as well as increases in total bilirubin concentrations were observed in the females at 4050 ppm. With the exception of the increase in total bilirubin values, the magnitude of changes in most of these values was small and could represent normal biological variations. Urine specimens of the males given 450, 1350 and 4050 ppm of the test compound and of the females receiving 4050 ppm were discoloured, from light yellow orange to maize yellow. In addition, urinalyses revealed statistically significantly increased urobilinogen levels in the males at 150, 450, 1350 and 4050 ppm and in the females at 4050 ppm. Slightly, but not statistically significantly, increased urinary urobilinogen concentrations were also found in the females given 1350 ppm of the test compound. No treatment-related effects were seen in the other urine parameters. The only treatment-related findings were the increases in absolute and relative spleen weights in males at 1350 and 4050 ppm and in females at 4050 ppm. The increase was associated with significant extramedullary haematopoiesis in the spleen.

The gross examinations revealed the enlarged spleens in males at 1350 and 4050 ppm and in females at 4050 ppm. Substance-induced findings were observed in the liver, spleen and bone marrow. In the liver and spleen of males at 1350 and 4050 ppm and of females at 4050 ppm, extramedullary haematopoiesis was evident. Erythroid hyperplasia was the microscopic finding in the bone marrow of males at 1350 and 4050 ppm and of females at 4050 ppm. The microscopic findings in these tissues were directly related to anaemia and porphyria. All other findings noted were considered to be spontaneous or incidental in origin and not related to treatment.

The LOAEL established in males was 450 ppm (equal to 39.2 mg/kg bw per day), based on decreased haemoglobin, MCV and MCH. Increased polychromasia and anisocytosis were also observed at this dose. The NOAEL in males was 150 ppm (equal to 13.4 mg/kg bw per day). The LOAEL established in females was 1350 ppm (equal to 130 mg/kg bw per day), based on decreased haemoglobin, haematocrit, MCV and MCH. The NOAEL in females was 450 ppm (equal to 43.6 mg/kg bw per day) (Kaspers et al., 2007b).

In a 90-day toxicity study, saflufenacil (purity 93.9%) was administered in the diet to groups of 10 male and 10 female Wistar rats of each sex per dose at dose levels of 0, 50, 150, 450 (males only), 1350 and 4050 (females only) ppm for a 3-month period. Owing to severe signs of general toxicity, the 4050 ppm dose group (females only) was terminated on day 53. The mean doses for males were 0, 3.5, 10.5, 32.3 and 94.7 mg/kg bw day at 0, 50, 150, 450 and 1350 ppm, respectively. The mean doses for females were 0, 4.3, 12.6, 110.5 and 344.7 mg/kg bw day at 0, 50, 150, 1350 and 4050 ppm, respectively. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period, body weight was determined on day 0 and thereafter at weekly intervals. Individual feed consumption was determined weekly over a period of 7 days and calculated as mean feed consumption in grams per animal per day. A FOB was conducted for all rats at the end of the administration period. Motor activity was measured on the same day as the FOB. The eyes of all animals prior to the start of the administration period and the eyes of the control and high-dose animals (1350 ppm) on day 91 were examined for any changes using an ophthalmoscope after administration of a mydriatic. Urinalysis was conducted from all study animals at termination. At the end of the study, blood was collected for haematology and clinical chemistry measurements. All rats that died and those sacrificed at study termination were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

The test compound was stable in the diets for 49 days at room temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable (91.3–103.9% of the nominal concentrations). Owing to severe signs of general toxicity and two mortalities, the 4050 ppm dose group (females only) was terminated on day 53. At 1350 ppm, urine-smeared anogenital region was observed in two females. Two males showed skin paleness (slight) on day 91, and one showed piloerection on day 91. At 4050 ppm, body weight and body weight changes were statistically significantly decreased in females. At 1350 ppm, body weight and body weight gains of the males were decreased during the whole study period; the decrease was statistically significant during days 21–49. The body weight gain of 1350 ppm males was reduced by 14.1% of the control values at termination. In male animals of the 1350 ppm dose group, feed consumption was reduced, most of the time statistically significantly, during the whole study period (up to 10.7% on day 21). At the end of the study, statistically significantly decreased values for haemoglobin, haematocrit, MCV, MCH and MCHC and increased reticulocytes and polychromasia were found in the peripheral blood of male and female rats in the 1350 ppm dose group. In addition,

higher platelet counts and shortened prothrombin times were measured in females at this dose level. Haemoglobin, haematocrit, MCV and MCH values were also decreased in males at 450 ppm. Increased numbers of white blood cells were measured in the circulation of the male and female rats at 1350 ppm. In females, there were no treatment-related changes in the blood chemistry parameters. In males, blood chemistry examinations revealed statistically significantly increased chloride and total bilirubin concentrations in males at 1350 ppm and reduced total protein and globulin levels in males at 450 and 1350 ppm. All ophthalmoscopic findings were incidental in nature, because of their occurrence in single animals only and/or the lack of a dose–response relationship. Some males given 150, 450 and 1350 ppm of the test compound and some females receiving 1350 ppm of the test substance excreted urine that was discoloured, from maize yellow to orange. Furthermore, urinalyses revealed statistically significantly increased urobilinogen levels in males at 150, 450 and 1350 ppm and in females at 1350 ppm. Urinary bilirubin concentrations were also elevated in males given 450 and 1350 ppm of the test compound and in females exposed to 1350 ppm of the test substance. An increased number of transitional epithelial cells was also found in the urine sediments of the males at 450 and 1350 ppm, and granulated casts were detected in the urine specimens of males at 1350 ppm. In males at 1350 ppm, treatment-related effects on organ weights were noted for the spleen and heart, organs associated with treatment-related anaemia. Other statistically significant organ weight values were regarded to be secondary to the lower terminal body weight. For females at 1350 ppm, a trend towards an increase in spleen weight was regarded as a treatment-related effect. The gross macroscopic examination indicated enlarged spleens in the males of the 1350 ppm dose group. Extramedullary haematopoiesis was the main treatment-related histological finding affecting the liver of males at 1350 ppm and the spleen of males at 450 and 1350 ppm and of females at 150 and 1350 ppm. When compared with the control females, the incidence and magnitude of the effects for the females at 150 ppm were comparable, and the findings might not be considered toxicologically significant. Increased iron storage in the liver in males and females at 1350 ppm was also related to the test material. All other findings noted were considered as spontaneous or incidental in origin and were not related to treatment. No treatment-related findings were observed in the FOB parameters except for urogenital staining in two females at 1350 ppm. Statistically significantly decreased values in motor activity (intervals 2, 6 and overall) were measured in males at 1350 ppm. These findings were assessed as being related to the test article and caused by systemic toxicity and reduced body weight. A few isolated statistically significant single intervals were considered incidental.

The LOAEL established in the males was 450 ppm (equal to 32.3 mg/kg bw per day), based upon multiple clinical chemistry end-points typical of microcytic hypochromic anaemia (MHA) (decreased haemoglobin, haematocrit, MCV, MCH, total protein and globulins). Histopathological findings at this LOAEL included spleen weight increases with extramedullary haematopoiesis. The LOAEL established in the females was 1350 ppm (equal to 110.5 mg/kg bw per day), based upon multiple clinical chemistry end-points typical of MHA (decreased haemoglobin, haematocrit, MCV, MCH and MCHC). Histopathological findings at this LOAEL included spleen weight increases with extramedullary haematopoiesis.

The resulting NOAEL in both sexes was 150 ppm (equal to 10.5 mg/kg bw per day in males and 12.6 mg/kg bw per day in females) (Kaspers et al., 2007d).

Dogs

In a 4-week oral toxicity study, saflufenacil (purity 93.8%) was administered daily via gelatine capsules to purebred Beagle dogs (four of each sex per group) at 0, 30, 100 or 300 mg/kg bw per day. The dogs were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Detailed clinical observations were conducted for all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The

weights were then determined on day 0 and weekly thereafter. The feed consumption of the animals was determined each working day, starting on day −7 (beginning of the adaptation period), and calculated as mean feed consumption in grams per animal. Ophthalmoscopic examination of the eyes was not conducted. Blood was removed from the vena cephalica antebrachii of non-anaesthetized, fasted animals for haematological and clinical chemistry measurements. Blood sampling occurred prior to dosing (on study day −14 or 13) and on study day 27. Urinalysis was conducted prior to the dosing period (study day −12 or −11) and at the end of the study (study day 23 or 24). All dogs that died and those sacrificed on schedule were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

Treatment had no effects on mortality, body weight and body weight gain, feed consumption and feed efficiency, or gross pathology. Dark brown discoloured faeces were observed in male and female dogs in the 100 and 300 mg/kg bw per day groups. Treatment-related haematological findings were decreased erythrocyte counts, haemoglobin concentration and haematocrit values in both males and females at 300 mg/kg bw per day (Table 12). Decreased values for MCV, MCH and MCHC (males only) were also recorded in males and females at 100 and 300 mg/kg bw per day. Although the magnitude of the decreases was small and there was no clear dose–response relationship, the effects were considered biologically significant because the blood was known to be the target for saflufenacil. Alkaline phosphatase activity was higher in males and females at 300 mg/kg bw per day. Examination of porphyrin levels in the plasma, urine and faeces showed significant increases in all test groups (Table 13). The increase at 30 mg/kg bw per day, in the absence of any other adverse effects, was not considered toxicologically important. At terminal sacrifice, absolute and relative weights of the liver and spleen of males and females at 300 mg/kg bw per day were significantly higher than those of control animals. Histological examination revealed increased iron storage in the liver, extramedullary haematopoiesis in the spleen and hypertrophy of the bone marrow of male and female dogs at 300 mg/kg bw per day.

The LOAEL in both male and female dogs was 100 mg/kg bw per day, based upon MHA resulting from altered porphyrin metabolism. The NOAEL was 30 mg/kg bw per day (Kaspers et al., 2007c).

In a 90-day toxicity study, saflufenacil (purity 93.8%) was administered daily via gelatine capsules to purebred Beagle dogs (five of each sex per group) at 0, 10, 50 or 150 mg/kg bw per day. The dogs were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Detailed clinical observations were conducted for all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter. The feed consumption of the animals was determined each working day, starting on day −7 (beginning of the adaptation period), and calculated as mean feed consumption in grams per animal. Blood was removed from the vena cephalica antebrachii of non-anaesthetized, fasted animals for haematological and clinical chemistry measurements. The blood was withdrawn at three separate time points in the study: prior to the beginning of the experiment (day −14 to day −13); at the middle of the experiment (days 41–43); and at the end of the study (days 93–94). Urine was collected at days 11–12, 44–45 and 86–87 for urinalysis. All dogs were examined with an ophthalmoscope prior to and at the end of the administration period. All dogs that died and those sacrificed on schedule were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

There were no treatment-related effects on mortality, ophthalmoscopy, urinalysis or gross pathology. Dark brown/dark red brown discoloured faeces were seen in all dogs at 150 mg/kg bw per day. This finding was likely caused by excretion of porphyrins via faeces, owing to the mode of action of saflufenacil as a PPO inhibitor. There were no other treatment-related clinical findings.

Table 12. Selected haematological values in a 28-day dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 4 per group)				Females (n = 4 per group)			
	Dose (mg/kg bw per day)							
	0	30	100	300	0	30	100	300
Red blood cells (10^{12}/l)	6.94 ± 0.12	6.68 ± 0.45	7.32 ± 0.47	**5.75 ± 1.00**	7.00 ± 0.34	7.08 ± 0.35	7.97 ± 0.34*	**6.33 ± 0.71**
Haemoglobin (mmol/l)	9.7 ± 0.2	9.3 ± 0.7	9.3 ± 0.7	**7.4 ± 1.1**	10.3 ± 0.6	9.9 ± 0.5	10.3 ± 0.6	**8.4 ± 1.0***
Haematocrit (%)	46.6 ± 0.6	45.1 ± 3.6	45.8 ± 3.3	**36.5 ± 5.2**	49.3 ± 3.4	47.8 ± 2.3	50.0 ± 2.4	**41.1 ± 4.4***
MCV (fl)	67.2 ± 1.1	67.5 ± 2.1	**62.6 ± 0.5***	**63.6 ± 1.7***	70.3 ± 1.8	67.5 ± 2.3	**62.7 ± 1.3***	**65.0 ± 0.8***
MCH (fmol)	1.40 ± 0.03	1.40 ± 0.04	**1.27 ± 0.02***	**1.29 ± 0.03***	1.47 ± 0.03	1.40 ± 0.05	**1.30 ± 0.04***	**1.33 ± 0.02***
MCHC (mmol/l)	20.8 ± 0.20	20.7 ± 0.18	**20.3 ± 0.19***	**20.3 ± 0.38**	20.9 ± 0.24	20.8 ± 0.17	20.7 ± 0.37	20.5 ± 0.24
White blood cells (10^9/l)	11.8 ± 2.38	12.2 ± 1.27	12.9 ± 2.71	**15.2 ± 3.83**	11.6 ± 2.05	12.1 ± 2.58	12.5 ± 2.00	**17.2 ± 3.50**
Neutrophils (10^9/l)	7.02 ± 1.58	7.21 ± 0.61	8.34 ± 2.40	**9.33 ± 2.92**	6.80 ± 1.73	7.21 ± 1.85	7.38 ± 0.96	**10.39 ± 2.3**
Lymphocytes (10^9/l)	3.91 ± 0.65	4.05 ± 0.65	3.69 ± 0.27	**4.63 ± 0.90**	3.93 ± 0.40	4.06 ± 0.59	4.32 ± 0.97	**5.67 ± 0.92**
Platelets (10^9/l)	333 ± 38	332 ± 41	424 ± 17*	560 ± 102*	372 ± 44	332 ± 61	368 ± 12	552 ± 97*
Partial thromboplastin time (s)	11.9 ± 0.4	11.6 ± 0.6	10.9 ± 0.4*	10.3 ± 0.3*	11.3 ± 0.7	12.0 ± 0.5	10.5 ± 0.7	10.4 ± 0.4

From Kaspers et al. (2007c). Data taken from table 1B, pp. 72–87, 138, 142 of the study report.
* $P \leq 0.05$; **bolded** values are considered treatment related

Table 13. Porphyrin values in a 28-day dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 4 per group)				Females (n = 4 per group)			
	Dose (mg/kg bw per day)							
	0	30	100	300	0	30	100	300
Plasma porphyrin (nmol/l)	3.9 ± 0.6	**16.5 ± 3.4***	**49.2 ± 22.9***	**121.5 ± 59.7***	4.8 ± 1.0	**16.5 ± 5.1***	**43.8 ± 26.0***	**109.8 ± 52.3***
Urinary porphyrin (µg/l)	13.1 ± 4.8	35.4 ± 21.4	**152.8 ± 14.4***	**383.8 ± 215.1***	3.1 ± 3.9	16.5 ± 11.6	**197.1 ± 107.6***	**463.8 ± 311.7***
Faecal porphyrin (µmol/kg dry faeces)	35.9 ± 28.1	**333.3 ± 151.4***	**1147.7 ± 730***	**951.7 ± 635.9***	51.5 ± 29.4	**302.0 ± 90.9***	**1247.4 ± 926.7**	**1563.6 ± 312.6***

From Kaspers et al. (2007c). Data taken from table 1B, pp. 100–101 of the study report.
* $P \leq 0.05$; **bolded** values are considered treatment related

There was no statistically significant deviation in body weight in any test group (males and females) in comparison with the control groups. Body weights of high-dose dogs were consistently lower than those of the control dogs, from day 35 to day 91 in males and for the entire dosing period in females. The lower body weights of the high-dose groups were considered treatment-induced adverse effects. Body weights and body weight gains of dogs in the 50 and 10 mg/kg bw per day groups were not affected. Feed consumption in male dogs was not affected. For the females, mean feed consumption of the high-dose dogs was lower than the control value during most of the dosing period. Mean feed efficiency data were highly variable, with large standard deviations. Consequently, there were rarely treatment-related statistically significant findings, although the high-dose dogs appeared to have lower feed efficiencies when compared with the control animals. Throughout the study period, statistically significantly decreased values for MCV, MCH and MCHC were recorded (assessed at days 41/43 and 93/94) in dogs at 150 mg/kg bw per day. It was stated in the study report that red blood cell morphology showed increased microcytosis and polychromasia at both time intervals in these dogs, and increased anisocytosis was also noted in males at 150 mg/kg bw per day on day 41 and in females at 150 mg/kg bw per day on days 43 and 94. Moreover, in males at 150 mg/kg bw per day, haemoglobin concentrations were significantly decreased on days 41 and 93, and haematocrit values were reduced on day 93. Statistically significantly increased platelet counts were observed at 150 mg/kg bw per day (males on days 41 and 93; females on day 94). However, assessment of blood clotting parameters did not show any treatment-related effects. At the end of the study, an increase in red blood cells was measured in high-dose females only. This isolated finding was considered incidental and unrelated to administered saflufenacil, because it was inconsistent with the mode of action of saflufenacil. At 50 mg/kg bw per day, significantly decreased MCH was noted on days 41 and 93, and reduced MCV in males and significantly lower values for MCV and MCH were found in females on day 94. Throughout the administration period, statistically significantly increased alkaline phosphatase activities were recorded in males at 150 mg/kg bw per day and in females at 50 and 150 mg/kg bw per day. Blood chemistry examinations revealed significantly lower total protein and albumin levels at 50 (male) and 150 (both sexes) mg/kg bw per day throughout the study. Lower albumin and bilirubin levels were also seen in males at 10 mg/kg bw per day. Total bilirubin concentrations were decreased when assessed on days 41/43 (all males and females at 50 and 150 mg/kg bw per day), but not at the end of the dosing period on days 93/94. The decreased bilirubin and albumin concentrations in males at 10 mg/kg bw per day were not considered to be treatment related because the values of both parameters were within or near the lower limit of the historical control range (Hempel, 2010). With the exception of a slight increase in relative liver weights of high-dose males (14% of control values), there were no obvious treatment-related effects on organ weights. There were no treatment-related gross pathological findings. Substance-induced microscopic findings were observed in the liver (iron storage), spleen (extramedullary haematopoiesis) and bone marrow (hypertrophy) of male and female dogs at 150 mg/kg bw per day. Iron storage in the liver and kidneys was also observed in one low-dose male and two mid-dose males. The authors considered the findings secondary to microcytic hypochromic anaemia. The defect in haem synthesis led to an excess of iron or iron-containing intermediate products, which then was intracytoplasmatically stored in liver and spleen cells. Extramedullary haematopoiesis and bone marrow hyperplasia are typical findings associated with anaemia and are considered a compensatory response.

The NOAEL was 10 mg/kg bw per day, based on lower MCV and MCH values in both sexes seen at 50 mg/kg bw per day (Kaspers et al., 2006a).

In a 1-year toxicity study, saflufenacil (purity 93.8%) was administered daily via gelatine capsules to purebred Beagle dogs (five of each sex per group) at 0, 5, 20 or 80 mg/kg bw per day. The dogs were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Detailed clinical observations were conducted for all animals

prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter. The feed consumption of the animals was determined each working day, starting on day −7 (beginning of the adaptation period), and calculated as mean feed consumption in grams per animal. Blood was removed from the vena cephalica antebrachii of fasted anaesthetized animals. Blood sampling for haematological and clinical analyses was carried out on days −14/15, 89/90, 180/181 and 362/363. Urine was collected on days −12/13, 92/93, 183/184 and 358/359 for urinalysis. All dogs used in the study were examined with a Kowa fundus camera after administration of a mydriatic prior to and at the end of the administration period. All dogs that died and those sacrificed on schedule were subjected to gross pathological examination, and the selected tissues were collected for histological examination. The selected organs were weighed.

There were no treatment-related effects on mortality, ophthalmoscopy, urinalysis, organ weight or gross pathology. Signs of systemic toxicity were evident at 80 mg/kg bw per day only. Test substance–related findings consisted of discoloured faeces seen in four male and three female animals in the 80 mg/kg bw per day dose group. This finding was likely caused by excretion of porphyrins via faeces, due to the mode of action of the test substance as a PPO inhibitor. There were no other treatment-related findings. There was no statistically significant deviation in body weight in any test group (males and females) in comparison with the control groups. Body weights of high-dose males were consistently lower than those of the control males. The body weight gain of the high-dose males was 23% less than that of control males for the duration of the study period. The lower body weights of high-dose males were considered treatment-induced adverse effects. Body weights and body weight gains of dogs in other groups were not affected. The overall body weight gains of low- and mid-dose females were also markedly less than that of control females. The lower body weight gains of these females were not considered treatment induced, because the body weight gain of high-dose females was similar to that of the control females. Feed consumption of males was not affected. For females at 80 mg/kg bw per day, the mean feed consumption was decreased during most of the study period. The lower mean value was caused mainly by one female. This female was considered to have substance-related reduced feed consumption, along with substance-related body weight loss or retarded body weight gain and impairment of feed efficiency. The mean feed consumption over the entire administration period for this female was about 81%. In contrast, feed intake of the remaining four females at 80 mg/kg bw per day was not impaired. Throughout the study period, statistically significantly decreased values for MCV and MCH were recorded in dogs at 80 mg/kg bw per day (Table 14). The females of the 20 mg/kg bw per day dose group showed a decrease of the MCH values at the 3rd study month and a decrease of the MCV at the 6th study month. At these time points, the parameter values were isolated changes without any other haematological changes that would indicate a possible morphological dysfunction of the red blood cells in the dogs of this dose group. Furthermore, both median values were within the range of the historical controls; therefore, these deviations were not regarded as adverse effects in this dose group. At the end of the study, the high-dose males had a marginally decreased partial thromboplastin time, but other blood clotting parameters were not affected. This isolated incidence might be considered incidental.

At the 3rd, 6th and 12th study months, the alkaline phosphatase activity of the dogs at 80 mg/kg bw per day was increased (Table 15). The total protein and albumin levels were decreased at 80 mg/kg bw per day throughout the study period, statistically significantly in males as well as in females after 3 months. No treatment-related changes were found in the other blood chemistry parameters examined.

A few marginally statistically significant changes were noted. The changes were considered to be incidental and of no biological relevance because of the lack of a dose–response relationship and the absence of corresponding histopathology. There were no treatment-related gross pathological findings. Substance-induced microscopic findings were observed only in the liver. The findings were

Table 14. Selected haematological values in a 1-year dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 5 per group)				Females (n = 5 per group)			
	Dose (mg/kg bw per day)							
	0	5	20	80	0	5	20	80
MCV (fl)								
Day 89/90	65.8 ± 1.6	66.2 ± 1.9	64.9 ± 1.3	**59.5 ± 2.1****	67.5 ± 2.0	67.1 ± 1.3	65.6 ± 1.4	**59.2 ± 1.8****
Day 180/181	66.0 ± 2.0	65.6 ± 1.6	64.3 ± 1.3	**58.5 ± 2.9****	68.2 ± 2.0	67.8 ± 1.2	66.0 ± 1.7*	**57.8 ± 2.9****
Day 362/363	64.3 ± 1.8	64.4 ± 1.3	62.4 ± 2.2	**56.2 ± 3.4****	65.9 ± 1.7	65.6 ± 1.9	63.5 ± 1.7	**55.1 ± 3.4****
MCH (fmol)								
Day 89/90	1.43 ± 0.04	1.42 ± 0.04	1.39 ± 0.03	**1.27 ± 0.06****	1.47 ± 0.04	1.45 ± 0.04	1.42 ± 0.04*	**1.27 ± 0.06****
Day 180/181	1.45 ± 0.04	1.43 ± 0.04	1.40 ± 0.03	**1.28 ± 0.08***	1.50 ± 0.04	1.49 ± 0.03	1.44 ± 0.04	**1.25 ± 0.07****
Day 362/363	1.37 ± 0.04	1.36 ± 0.03	1.32 ± 0.05	**1.19 ± 0.07****	1.41 ± 0.04	1.41 ± 0.05	1.36 ± 0.04	**1.16 ± 0.09****

From Hempel et al. (2007). Data taken from table 1B, pp. 167–192 of the study report.
MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; * $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 15. Selected clinical chemistry values in a 1-year dog study with saflufenacil

	Mean values ± standard deviation							
	Males (n = 5 per group)				Females (n = 5 per group)			
	Dose (mg/kg bw per day)							
	0	5	20	80	0	5	20	80
Alkaline phosphatase (µkat/l)								
Day 89/90	1.22 ± 0.30	1.18 ± 0.38	1.75 ± 0.57	**3.04 ± 1.15****	1.38 ± 0.45	1.37 ± 0.23	1.68 ± 0.71	**3.19 ± 1.37***
Day 180/181	1.15 ± 0.30	1.37 ± 0.40	1.53 ± 0.21	**3.77 ± 1.32****	1.83 ± 0.72	1.66 ± 0.58	2.26 ± 0.68	**3.72 ± 0.95****
Day 362/363	0.95 ± 0.25	1.06 ± 0.36	1.41 ± 0.15**	**3.34 ± 1.20****	2.42 ± 1.57	1.55 ± 0.51	2.29 ± 1.74	3.50 ± 0.99
Total protein (g/l)								
Day 89/90	61.5 ± 2.14	61.9 ± 1.75	60.3 ± 1.05	**57.9 ± 1.32***	61.8 ± 2.07	60.4 ± 1.55	59.7 ± 1.39	**57.9 ± 1.93***
Day 180/181	61.3 ± 1.96	62.0 ± 1.88	59.7 ± 1.82	**56.9 ± 2.09***	61.8 ± 1.79	60.1 ± 2.29	59.0 ± 1.08	58.1 ± 2.87
Day 362/363	60.1 ± 1.63	60.7 ± 0.91	58.1 ± 1.86	**56.3 ± 1.63***	60.1 ± 1.96	59.9 ± 2.09	57.6 ± 1.24	57.0 ± 2.84
Albumin (g/l)								
Day 89/90	36.2 ± 1.54	34.9 ± 0.86	34.2 ± 1.54	**32.4 ± 1.12****	36.0 ± 1.12	36.3 ± 1.07	34.3 ± 1.41	**32.8 ± 1.84***
Day 180/181	35.7 ± 1.54	34.0 ± 1.00	34.3 ± 1.43	**31.6 ± 1.04****	35.5 ± 0.60	35.5 ± 1.59	34.4 ± 1.25	32.3 ± 2.51
Day 362/363	35.9 ± 1.61	34.7 ± 0.64	34.1 ± 0.95	**31.2 ± 0.52****	34.7 ± 0.95	35.6 ± 1.72	34.5 ± 1.24	32.8 ± 2.76

From Hempel et al. (2007). Data taken from table 1B, pp. 193–216 of the study report.
kat, katal, a Système international d'unités (SI) unit of catalytic activity; * $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

slightly more pronounced for iron storage in Kupffer cells and hepatocytes of males at 20 and 80 mg/kg bw per day and of females at 80 mg/kg bw per day. This increase in iron storage was considered to be an adaptive treatment-related effect and non-adverse.

The NOAEL was 20 mg/kg bw per day, based on discoloured faeces, lower body weight in males, decreased feed consumption, lower MCV and MHC, increased serum alkaline phosphatase activity, lowered total blood protein and albumin levels, and slightly more pronounced iron storage in Kupffer cells and hepatocytes at 80 mg/kg bw per day (Hempel et al., 2007).

The overall NOAEL for the 90-day and 1-year toxicity studies in dogs was 20 mg/kg bw per day.

(b) Dermal application

Rats

In a 28-day repeated-dose dermal toxicity study, saflufenacil (purity 93.8%) was applied to the shaved skin of 10 Wistar rats of each sex per dose at 0, 100, 300 or 1000 mg/kg bw per day, 6 hours/day, 5 days/week. The test substance was suspended in an aqueous solution of 0.5% carboxymethylcellulose. The test substance was administered uniformly to the clipped dorsal skin (dorsal and dorsolateral parts of the trunk; at least 10% of the body surface area) using 3 ml syringes. The administration volume was 4 ml/kg bw, based on the latest individual body weight determination. The skin was covered for 6 hours after application using a semi-occlusive dressing, consisting of four layers of porous gauze dressing and held in place with an elastic top dressing. After removal of the dressing, the treated skin was washed with lukewarm water. Control animals received only the vehicle. Dosing preparations were analysed for stability, homogeneity and concentration. Animals were checked for mortality and clinical signs of toxicity twice daily on working days and once daily on Saturdays, Sundays and public holidays. Body weights were recorded before the start of the study (day 0) and thereafter at weekly intervals. Individual feed consumption was determined weekly. A FOB was performed in all animals at the end of the study. Motor activity was measured with all animals on the same day on which the FOB was performed. A blood sample was collected for haematological and clinical chemistry parameters at the end of the study. Urinalysis was conducted at the end of the study. The eyes of all animals prior to the start of the administration period and the eyes of the control and high-dose animals on day 27/28 (females/males) were examined for any changes using an ophthalmoscope after administration of a mydriatic. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues from the control and high-dose rats were collected for histological examination. The selected organs were weighed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. All rats survived to study termination. There were no treatment-related effects on clinical signs, feed consumption and feed efficiency, skin reaction, ophthalmoscopy, FOB, motor activity, clinical chemistry, gross pathology or histopathology. The only treatment-related finding was a slight decrease in haemoglobin concentration in males at 1000 mg/kg bw per day (97% of the controls). The marginal change in the haematological parameter was not considered adverse by the Meeting because the magnitude of the change was small and statistical significance was not achieved. Urinalyses revealed significantly increased urobilinogen levels in males at 1000 mg/kg bw per day and in females at 300 and 1000 mg/kg bw per day. Moreover, urine specimens of the high-dose males were discoloured (light orange), which was likely related to increased urinary excretion of porphyrins. The changes in urine parameters were considered to be compensatory responses to the altered porphyrin metabolism. As these changes in urinary parameters were not associated with other pathological alterations, they were not considered adverse.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested. The study authors concluded that, under the conditions of the study, the NOAEL was 300 mg/kg bw per day for males due to a mild

anaemic process, based on the mode of action of the test substance, and 1000 mg/kg bw per day for females (Kaspers et al., 2006b).

(c) Exposure by inhalation

No studies were submitted.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a carcinogenicity study, saflufenacil (purity 93.8%) was administered daily in the diet to groups of C57BL/6NCrl mice (50 of each sex per group) at 0, 1 (males only), 5, 25, 75 or 150 (females only) ppm for an 18-month period. The mean doses for males were 0, 0.2, 0.9, 4.6 and 13.8 mg/kg bw per day at 0, 1, 5, 25 and 75 ppm, respectively. The mean doses for females were 0, 1.2, 6.4, 18.9 and 38.1 mg/kg bw per day at 0, 5, 25, 75 and 150 ppm, respectively. In satellite groups of 10 mice of each sex per group, saflufenacil was administered daily in the diet at 0, 75 (males only) and 150 (females only) ppm (equal to 0 and 14.2 mg/kg bw per day for males and 0 and 39.0 mg/kg bw per day for females) over a period of 10 months. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and thereafter at weekly intervals. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0, weekly during the first 13 weeks, at 4-week intervals thereafter and prior to the start of necropsy. Feed consumption was determined once a week over a period of 7 days during the first 13 weeks of the administration period, at 4-week intervals thereafter and prior to the start of necropsy. The values were calculated as feed consumption in grams of feed per mouse per day. Haematology was assessed in satellite mice only and on day 303/304. No clinical parameters were assessed. Urine parameters were not assessed. The eyes were not examined. Faeces were collected overnight from all satellite animals. At scheduled sacrifice, parts of the liver of the satellite mice were removed. The collected faecal and liver samples were analysed for porphyrin levels. All mice that died and those sacrificed at study termination were subject to gross pathological examination, and the selected tissues were collected for histological examination from the controls and the high-dose animals. In addition, gross lesions, liver, bone marrow (femur) and spleen from all mice were subjected to histopathological examination. The selected organs were weighed.

The test substance was stable in the diets for a period of 49 days. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the mice was acceptable (91.2–110.3% of the nominal). There were no treatment-related effects on clinical signs, mortality, body weight and body weight gain, feed consumption and feed efficiency, gross pathology or organ weights. As a result of the high mortality observed in test and control males (Table 16), all surviving males were sacrificed on study days 527 and 528 (approximately 20 days before the originally scheduled sacrifice).

In the 10th month of the study, statistically significantly reduced red blood cell counts, haemoglobin concentrations and haematocrit values were recorded for the males at 75 ppm and for the females at 150 ppm in the satellite group (Table 17). The red blood cell indices (MCH, MCHC, MCV) as well as the reticulocyte counts were not changed compared with the controls. Also, the total white blood cell counts, as well as the differential blood cell counts, showed no significant differences between the treated mice and the controls of both sexes. There were no treatment-related findings in the differential blood cell count and the red or white blood cell morphology of the high-dose mice (males 75 ppm; females 150 ppm) at 10 months of the treatment and at termination in the main study groups.

Table 16. Mortality rates of mice in the carcinogenicity study with saflufenacil

Dietary concentration (ppm)	Mortality (%)	
	Males	Females
0 (satellite)	0	0
0	72	58
1	62	—
5	72	56
25	60	68
75 (satellite)	0	—
75	56	60
150 (satellite)	—	10
150	—	58

From Kamp et al. (2007)

Table 17. Selected haematological values from satellite mice, day 304, in the carcinogenicity study with saflufenacil

	Mean values ± standard deviation			
	Males		Females	
	Dietary concentration (ppm)			
	0	75	0	150
	$n = 10$	$n = 10$	$n = 10$	$n = 9$
Red blood cells (10^{12}/l)	8.94 ± 0.26	**8.33 ± 0.23****	8.61 ± 0.40	**8.01 ± 0.73***
Haemoglobin (mmol/l)	8.1 ± 0.3	**7.4 ± 0.3****	7.8 ± 0.4	**7.3 ± 0.6***
Haematocrit (%)	40.3 ± 1.4	**37.1 ± 1.5****	38.4 ± 1.5	**35.9 ± 2.7***

From Kamp et al. (2007); data taken from table 1B, pp. 164–165 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

The mean porphyrin values in the faeces and liver were statistically significantly increased in the treated animals, although there were large interindividual variations (Table 18).

The only notable microscopic finding was in the liver. There was a significant increase in lipogenic pigment in the liver of males at 25 and 75 ppm and in females at 75 and 150 ppm (Table 19). Although the deposition of lipogenic pigment was considered to be treatment related, it is a common finding in older mice and might be accelerated by treatment with certain chemicals. As this finding was not associated with other histological changes in the liver and was not considered a precancerous lesion, the finding was not considered adverse. Enlarged nuclei of variable size (karyomegaly) occurred in centrilobular hepatocytes, especially in treated males. Karyomegaly was a common age-related finding in mice, the biological significance of which is unclear. As this finding was not associated with other adverse effects (e.g. hyperplasia) and was not considered to be a carcinogenic precursor, the finding was not considered adverse. All other non-neoplastic findings (including those in the decedents) either occurred singly or were equally distributed among control and test groups. They were considered to be incidental or spontaneous in origin and unrelated to treatment.

All neoplastic findings either occurred singly or were equally distributed among control and test groups. They were considered spontaneous or incidental in nature and unrelated to treatment. Under the conditions of the study, saflufenacil demonstrated no carcinogenic potential up to the highest dose levels tested.

Table 18. Faecal and liver porphyrin values from satellite mice, day 304, in the carcinogenicity study with saflufenacil

	Mean values ± standard deviation			
	Males		Females	
	Dietary concentration (ppm)			
	0	75	0	150
	n = 10	*n* = 10	*n* = 10	*n* = 9
Faecal total porphyrin (µmol/kg dry faeces)	15.2 ± 5.2	**432 ± 75****	9.2 ± 8.8	**463 ± 273***
Liver total porphyrin (pmol/g protein)	273 ± 104	**8146 ± 5546****	139 ± 46	**14 332 ± 14 159****

From Kamp et al. (2007); data taken from table 1B, pp. 170–171 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 19. Selected microscopic findings in the carcinogenicity study with saflufenacil

	No. of mice affected									
	Males (*n* = 50 per group)					Females (*n* = 50 per group)				
	Dietary concentration (ppm)									
	0	1	5	25	75	0	5	25	75	150
Liver, focal lipogenic pigment	0	0	0	9**	37**	4	2	4	15**	21**
Liver, karyomegaly	2	0	0	6	16**	1	0	0	1	0

From Kamp et al. (2007). Data taken from table 1C, pp. 193–248 of the study report.
** $P \leq 0.01$; **bolded** values are considered treatment related

The NOAELs for systemic toxicity were 25 ppm (equal to 4.6 mg/kg bw per day) in males and 75 ppm (equal to 18.9 mg/kg bw per day) in females, based on anaemia and porphyria observed in the satellite group at 75 ppm (equal to 13.8 mg/kg bw per day) in males and 150 ppm (equal to 38.1 mg/kg bw per day) in females. Saflufenacil was not carcinogenic at the dose levels tested. The NOAELs for carcinogenicity were the highest doses tested (75 ppm, equal to 13.8 mg/kg bw per day, for males and 150 ppm, equal to 38.1 mg/kg bw per day, for females) (Kamp et al., 2007).

Rats

In a combined 2-year toxicity and carcinogenicity study, saflufenacil (purity 93.8%) was administered in the diet to Wistar rats (50 of each sex per group; satellite groups of 10 of each sex per group) at 0, 20, 100, 250 (males only), 500 or 1000 (females only) ppm for 24 months. The satellite groups were dosed for 12 months. The mean doses for males were 0, 0.9, 4.8, 12.0 and 24.2 mg/kg bw per day at 0, 20, 100, 250 and 500 ppm, respectively. The mean doses for females were 0, 1.3, 6.2, 31.4 and 63.0 mg/kg bw per day at 0, 20, 100, 500 and 1000 ppm, respectively. The diets were analysed for stability, homogeneity and concentration. The animals were examined for clinical signs and mortality twice a day on working days and once daily on Saturdays, Sundays and public holidays. Detailed clinical observations were performed on all animals prior to the administration period and at weekly intervals thereafter. Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0, weekly during the first 13 weeks, at 4-week intervals thereafter and prior to the start of necropsy. Feed consumption was determined once a week over a period of 7 days during the first 13 weeks of the administration period, at 4-week intervals thereafter and prior to the start of necropsy. The values were calculated as feed consumption in grams feed per rat per day. Prior to the administration period, the eyes of all rats were

examined with an ophthalmoscope. The eyes of the control and high-dose rats were also examined on day 353/354. Haematological and clinical chemistry parameters were examined on days 92, 176 and 358. Urinalysis was conducted on days 88, 178/179 and 360/361. All rats that died and those sacrificed at study termination were subject to gross pathological examination, and selected tissues were collected for histological examination. For the rats in the two lowest-dose groups (i.e. 20 and 100 ppm), only the liver, lungs, bone marrow, spleen, kidneys and gross lesions were processed for histopathological examination. Selected organs were weighed.

The test substance was stable in the diets for a period of 49 days. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable (91.4–109.6% of the nominal). Compared with the control group (6/60), higher incidences of urine-smeared anogenital region were observed in females at 500 ppm (9/60) and 1000 ppm (21/60). These findings also occurred at earlier times than those seen in control rats and were assessed as treatment related. There was no excessive mortality in any group (Table 20).

Body weight and body weight gain of the females were not affected. In males, mean body weight at 500 ppm was slightly decreased during the first 10–30 weeks of dosing. The decreases were rarely higher than 5% of the control values. Weekly body weight changes varied considerably, but did not show a clear treatment-related adverse effect. The decreases in body weight and body weight gains in the 500 ppm males are considered treatment-related effects. There were no treatment-related effects on feed consumption or feed efficiency. All ophthalmoscopic findings were considered to be incidental in nature, because they were observed only in single animals and/or occurred without a dose–response relationship. Treatment-induced haematological effects were lower haemoglobin concentration, haematocrit, MCV and MCH in males at 500 ppm and females at 1000 ppm at all time points assessed. In addition, decreases in haemoglobin and haematocrit values were also recorded in females at 500 ppm on day 176. No significant changes in the differential blood count were noted in any dose group. There were no treatment-related adverse effects on serum enzyme activities. The only noted findings in clinical chemistry parameters were the slight decreases in protein (on day 176 at 250 and 500 ppm) and albumin (on day 176 at 500 ppm) levels in males. Significant changes in these clinical chemistry parameters, in any dose group, were not noted after 1 year of treatment. The marginally statistically significant findings might be transient in nature, although an association with dietary exposure to saflufenacil could not be ruled out, because lower protein levels were recorded in other toxicity studies after oral administration of saflufenacil. No changes in any clinical chemistry parameters were noted in females of any dose group at any time point. Treatment-related urinalysis findings were generally higher urobilinogen values in males (250 and 500 ppm) and females (500 and 1000 ppm). The authors considered that the increase in urinary urobilinogen represented heightened porphyrin metabolism in the liver, resulting in higher accumulation in the circulation, which was effectively excreted in the urine and not considered as an adverse effect. There were no significant absolute organ weight deviations. In males, the mean relative weights of epididymides were significantly increased at 250 (+10.2%, $P < 0.05$) and 500 ppm (+10.7%, $P < 0.01$). The increased relative weights of epididymides were related to the slightly decreased terminal body weights in these animals (−7.6% and −9.4%, respectively). Statistically significant changes in organ weights in males were lower absolute and relative spleen and kidney weights at 500 ppm and higher absolute brain weights at 100, 250 and 500 ppm. The increase of the mean absolute brain weights was not dose related. The mean relative brain weights did not show significant changes; in addition, there were no histopathological correlates. Therefore, the increased brain weights were considered incidental. The decreased absolute and relative kidney and spleen weights did not show a clear dose–response relationship, and there were no pathological findings that explain the weight changes. Additionally, saflufenacil, at toxic doses, causes increased, rather than decreased, spleen weights as a compensatory response to anaemia. Therefore, the spleen weight decreases were not considered a treatment-related finding. In females, no significant weight deviations were recorded. There were no treatment-related gross necropsy findings in either the satellite or the main study groups. All non-neoplastic findings in the

Table 20. Mortality rate in a carcinogenicity study in rats

Test group	Dietary concentration (ppm)	Mortality (%)	
		Males	Females
0	0	22	30
1	20	25	28
2	100	22	33
3	250	30	—
4	500	17	23
5	1000	—	22

From Kaspers et al. (2007f)

satellite or the main study groups either occurred singly or were biologically equally distributed over the control group and the treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment. All neoplastic findings in the satellite or the main study groups either occurred singly or were biologically equally distributed over the control group and the treatment groups. As such, all neoplastic lesions were considered spontaneous or incidental in nature and not related to treatment. Saflufenacil was not considered oncogenic in rats. There were small differences in the incidence of uterine and thyroid tumours between control and high-dose animals. The incidence of uterine adenocarcinoma was higher in high-dose females (10/50) than in the control females (5/50). The incidence of thyroid C-cell adenoma was higher in high-dose males (10/50) than in control males (3/49). However, both tumour types in the high-dose animals were within the historical control range. The historical control values for uterine adenocarcinoma in six studies (n = 50 per study) were 7, 1, 1, 15, 12 and 9. The historical control values for thyroid C-cell adenoma in six studies (n = 50 per study) were 10, 9, 10, 7, 11 and 11. Thus, the observation of a slight increase in uterine adenocarcinoma and thyroid C-cell adenoma in high-dose rats in the present study was not considered treatment related. In addition, the incidence of the potentially preneoplastic focal/multifocal C-cell hyperplasia is very similar for males at 500 ppm (6/50) compared with controls (6/49). Therefore, for the reasons given above, the increased incidence (10/50) (20%) of C-cell adenoma for males receiving saflufenacil at 500 ppm (highest concentration tested), compared with the control incidence of 3/49 (approximately 6%), should not be considered treatment related.

The NOAELs for oncogenicity were the highest dose tested: for males, 500 ppm (equal to 24.2 mg/kg bw per day), and for females, 1000 ppm (equal to 63.0 mg/kg bw per day). The NOAELs for systemic toxicity were 250 and 100 ppm for the male and female rats (equal to 12.0 and 6.2 mg/kg bw per day for males and females, respectively), based on decreased body weight and body weight gain in males, anogenital region smeared with urine in females, and lower haemoglobin concentration, haematocrit, MCV and MCH in males and females at 500 ppm (equal to 24.2 and 31.4 mg/kg bw per day for males and females, respectively) (Kaspers et al., 2007f).

2.4 Genotoxicity

A battery of studies of mutagenicity with saflufenacil was conducted to assess potential for inducing gene mutation, chromosomal aberration and unscheduled deoxyribonucleic acid (DNA) synthesis. The study results (summarized in Table 21) were negative except for positive results in the in vitro mammalian clastogenicity assay in the presence of a metabolic activation system. In this study, the test substance saflufenacil led to a statistically significant increase in the number of structural chromosomal aberrations, including and excluding gaps, after the addition of a metabolizing system in two experiments performed independently of each other after an exposure time of 4 hours

Table 21. Mutagenicity studies with saflufenacil

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Bacterial reverse mutation (Ames test)	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2*uvrA*	0, 55, 174, 550, 1740 or 5500 µg/plate (with and without S9 mix)	93.8	Negative	Engelhardt & Leibold (2005a)
Bacterial reverse mutation[a] (Ames test)	*S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* WP2*uvrA*	0, 20, 100, 500, 2500 or 5500 µg/plate (with and without S9 mix)	99.0	Negative	Engelhardt & Leibold (2005e)
Mammalian clastogenicity	Chinese hamster lung (V79) cells	1st: 0, 156.25, 312.5, 625, 1250, 2500 or 5000 µg/ml 2nd: 0, 250, 500, 1000, 2000 or 4000 µg/ml (with and without S9 mix)	93.8	−S9: Negative +S9: Positive	Engelhardt & Leibold (2005f)
Forward mutation assay in mammalian cells (HPRT test)	Chinese hamster ovary cells	0, 312.5, 625, 1250, 2500 or 5000 µg/ml (with and without S9 mix)	93.8	Negative	Engelhardt & Leibold (2005c)
In vivo					
Mouse micronucleus	NMRI mice, male	0, 500, 1000 or 2000 mg/kg bw (oral gavage in 0.5% carboxymethylcellulose) Sampling time: 24 h for 500 and 1000 mg/kg bw group; 48 h for 2000 mg/kg bw group	93.8	Negative	Engelhardt & Leibold (2005b)
Unscheduled DNA synthesis	Wistar rats, male	0, 1000 or 2000 mg/kg bw (oral gavage in 0.5% carboxymethylcellulose) Sampling times: 3 h, 14 h	93.8	Negative	Engelhardt & Leibold (2005d)

S9, 9000 × *g* rat liver supernatant

[a] Saflufenacil anhydrate form.

and a sampling time of 28 hours. Saflufenacil did not induce an increase in chromosomally damaged cells, including and excluding gaps, at other time points with metabolic activation or under any treatment conditions without addition of a metabolic activation system. However, it was negative for clastogenicity in the mouse micronucleus assay. Overall, saflufenacil is not likely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study, saflufenacil (purity 93.8%) was administered in the diet to groups of 25 male and 25 female healthy young Wistar rats (F_0 parental generation) at 0, 5, 15 or 50 mg/kg bw per day. At least 75 days after the beginning of treatment, F_0 animals were mated to produce a litter (F_1). Mating pairs were from the same dose group, and F_1 animals selected for breeding were continued in the same dose group as their parents. Groups of 25 males and 25 females, selected from

F_1 pups to become the F_1 parental generation, were offered diets containing the test substance at 0, 5, 15 or 50 mg/kg bw per day post-weaning, and the breeding programme was repeated to produce an F_2 litter. The study was terminated with the terminal sacrifice of the F_2 weanlings and F_1 parental animals. Test diets containing saflufenacil were adjusted regularly to obtain the desired doses throughout the study. Test diets were analysed for stability, homogeneity and concentration. The rats were observed for clinical signs and mortality twice daily on weekdays and once on Saturdays, Sundays and public holidays. In general, body weights of the male and female parents were determined on the first day of the pre-mating period and then once a week. Feed consumption was determined once a week (each time for a period of at least 6 days) for the F_0 and F_1 parents. Feed consumption of the females during pregnancy was determined weekly for days 0–7, 7–14 and 14–20 post-coitus and during lactation for days 1–4, 4–7 and 7–14 postpartum. Estrous cycle length and normality were evaluated for females. Preputial separation and sperm parameters were evaluated for males. Blood samples were withdrawn from all non-fasted F_0 and F_1 parents before sacrifice. Blood samples were taken on lactation days 4 (post-culling) and 21 from F_1 and F_2 pups (10 of each sex per group). All surviving parental males and females were subjected to gross pathological examination (external and internal examinations, including the cervical, thoracic and abdominal viscera). The culled F_1 and F_2 offspring were sacrificed on lactation day 4. The F_2 offspring carried through the lactation period were sacrificed at day 21 postpartum. The pups were examined externally and eviscerated, and their organs were assessed macroscopically.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable (89.7–107.7% of the nominal values). There were no deaths. No treatment-related clinical signs of toxicity were observed in males or females in any dose group. During pre-mating, mean body weights and body weight gains of the F_0 parents of all test groups were generally comparable to those of the controls. Mean body weights of F_0 females of all test groups were comparable to those of the controls during gestation, lactation and post-weaning periods. However, mean body weight gain in high-dose F_0 females was statistically significantly below the control value on gestation days 7–20 and the beginning of lactation (days 1–4). Thereafter, body weight gains of high-dose females recovered. Mean body weights of high-dose F_1 males were statistically significantly lower (up to approximately 10%) from week 5 onwards until the end of the study. For the entire dosing period (weeks 0–15), mean body weight gain of high-dose males was about 11% below the concurrent control value. Mean body weights and body weight gain of F_1 females were comparable to those of the control group during the pre-mating period. Mean body weights of F_1 parental females in the 50 mg/kg bw per day dose group were statistically significantly lower (approximately 6%) than those of controls on gestation day 20. Mean body weight gain was statistically significantly decreased in these females on days 14–20 post-coitus (about 21%) and days 0–20 post-coitus (about 12%) and was regarded as a treatment-related effect. At the beginning of lactation (days 1–4 postpartum), the high-dose F_1 females still gained significantly less weight (−49%) than the concurrent controls, but recovered afterwards. Feed consumption of the F_0 male rats of all test groups was generally comparable to that of the controls throughout the entire study. Feed consumption of the F_0 females of the 50 mg/kg bw per day dose group was also comparable to that of the controls during premating, gestation and post-weaning. However, during the entire lactation period, the feed consumption of the high-dose F_0 females was statistically significantly below that of controls (as high as about −19%) on days 1–4. Feed intake of high-dose F_1 males was consistently lower than that of the control males during weeks 4–15 of the pre-mating period. Feed consumption of high-dose F_1 females was statistically significantly decreased during pre-mating weeks 4–7 and during lactation days 1–14. These were considered treatment related. The body weights and feed consumption of F_0 parental and F_1 parental animals at the middle and low doses were comparable to those of concurrent controls. After dietary exposure to saflufenacil for about 17 weeks for the males and about 19 weeks for the females (including pregnancy), the rats showed signs of MHA. Haemoglobin concentrations and other indices of the

red blood cell (haematocrit, MCV, MCH and/or MCHC) were decreased in both sexes at 50 mg/kg bw per day. The decreases in haemoglobin, haematocrit, red blood cells, MCH and/or MCV in F_0 and F_1 males and in haemoglobin in F_0 females at 15 mg/kg bw per day were marginal (≤ 5%) and were not considered toxicologically significant. The effects of dietary exposure to saflufenacil on clinical parameters were highly consistent in F_0 and F_1 parents. When compared with the concurrent controls, serum protein levels were marginally (3–6%) but statistically significantly lower in high-dose F_0 and F_1 rats. In high-dose males, this decrease was due to both protein fractions (albumin and globulin). The triglyceride values were also decreased in high-dose males of both generations. At 15 mg/kg bw per day, the decreases in total protein and albumin levels in F_1 males were marginal and were not considered toxicologically significant. Evaluation of the estrous cycle data for 3 weeks prior to mating for the F_1 and F_2 litters revealed very regular cycles (mean of 4.0–4.8 days) in the F_0 and F_1 females of all groups. There were no treatment-related effects noted for the sperm parameters examined at or after the sacrifice of the F_0 and F_1 males. No treatment-related effects on the mating and fertility of the F_0 and F_1 males (96–100%) were observed. All sperm-positive F_0 females delivered pups or had implants in utero. Thus, the fertility index was 100% for all groups. The mean duration of gestation of F_0 females was similar among all groups (21.8–22.2 days) and was within the historical control range of 21.5–22.3 days. The gestation indices of the F_0 females were comparable among all groups. The mean number of implantation sites of the F_0 females was comparable among all dose groups (12.2, 12.3, 12.2 and 11.8 implants per dam at 0, 5, 15 and 50 mg/kg bw per day, respectively). There were no statistically significant differences in post-implantation loss among dose groups. However, the high-dose post-implantation loss of 14.6% was above the historical control range of the test facility (2.5–9.0%). As no such effects were seen in the subsequent F_1 generation, this finding was regarded as incidental and not treatment related. The number of liveborn pups was, however, lowest, and the number of stillborn pups and pups that died after birth was highest, at 50 mg/kg bw per day in both F_0 and F_1 generations, and the changes were both statistically significant (Table 22). The statistically significant decreases in the liveborn pups and the live birth index, as well as the significant increase in stillborn pups and pups that died after birth, in the high-dose group were considered treatment related.

Organ weight changes of the F_0 and F_1 male rats are shown in Table 23. The weights of several organs from the high-dose males were statistically significantly different from the concurrent control values. The findings were mostly attributed to the significant reduction in terminal body weight of these rats. The increased absolute and relative spleen weights in high-dose males were consistent with the findings in the F_0 males and were probably related to dietary exposure to saflufenacil. The increased absolute and relative mean thyroid weights and the increased relative weights of ovaries in low-dose females (Table 24) as well as the decreased relative liver weight in mid-dose males are considered incidental.

All gross lesions observed in F_0 and F_1 test animals occurred singularly. They are considered to be spontaneous in origin and are not related to treatment. The F_0 female rat that was not pregnant did not show relevant gross lesions. The F_0 male mating partner showed a severe reduction in sizes of testes and epididymides. The non-pregnant F_1 females and their F_1 male mating partners did not show gross lesions explaining the infertility. All histopathological findings noted either were single observations or were similar in distribution pattern and severity in control and test rats. All findings were considered incidental and unrelated to dietary exposure to saflufenacil. The non-pregnant low-dose F_0 female did not show histopathological findings explaining the infertility. The male mating partner showed, corresponding to gross lesions, a moderate diffuse tubular degeneration in the left testicle resulting in epididymis aspermia, causing the impaired fertility. The findings in the testes and epididymides were considered incidental. For two of the non-pregnant F_1 females, a dilatation of uterus horns was observed. One of the mating partners showed a moderate chronic inflammation of the prostate. These findings did not explain the infertility and were considered incidental. The other non-pregnant F_1 female and two of the male mating partners did not show histopathological findings explaining the infertility.

Table 22. Summary of selected F_0 and F_1 female reproductive data in a two-generation study in rats with saflufenacil

	Mean values ± standard deviation							
	F_0 (n = 25 per group)				F_1 (n = 25 per group)			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
Number mated	25	24	25	25	25	25	25	25
Number pregnant	25	24	25	25	24	24	25	24
Number with liveborn	25	24	25	24	24	24	25	24
Number with stillborn pups	2	1	3	**7**	4	0	1	**9**
Duration of gestation (days)	21.8 ± 0.5	21.9 ± 0.45	22.0 ± 0.2	22.2 ± 0.51**	21.7 ± 0.46	22.0 ± 0.42	21.9 ± 0.33	22.0 ± 0.51
Post-implantation loss								
- total	21	21	15	35	12	16	13	21
- %	6.8 ± 8.6	7.1 ± 8.1	5.0 ± 7.0	**14.6 ± 20.7**	5.0 ± 8.66	6.7 ± 8.25	4.6 ± 6.02	8.3 ± 10.3
- per dam	0.8 ± 1.03	0.9 ± 0.99	0.6 ± 0.87	**1.4 ± 1.32**	0.5 ± 0.78	0.7 ± 0.82	0.5 ± 0.65	0.9 ± 1.12
Pups delivered								
- total	285	273	291	259	247	254	279	227
- per dam	11.4 ± 1.96	11.4 ± 2.08	11.6 ± 2.04	10.8 ± 2.02	10.3 ± 2.22	10.6 ± 2.22	11.2 ± 1.75	9.5 ± 2.32
- liveborn								
- total	283	272	288	**240****	243	254	278	**211****
- per dam	11.3	11.3	11.5	**10**	10.1	10.6	11.1	**8.8**
- stillborn								
- total	2	1	3	**19****	4	0	1	**16****
- per dam	0.08	0.003	0.12	**0.79**	0.17	0	0.04	**0.67**
- died								
- total	0	5	0	**9****	1	1	1	**12****
- per dam	0	0.21	0	**0.38**	0.04	0.04	0.04	**0.5**

From Schneider et al. (2007a). Data taken from table 1A, pp. 159–161, 205–207 of the study report.
** $P \leq 0.01$; **bolded** values are considered treatment related

Table 23. Selected organ weights of F_0 and F_1 male rats in a two-generation study with saflufenacil

Organ	Mean weights ± standard deviation			
	Dose (mg/kg bw per day)			
	0	5	15	50
F_0 (*n* = 25 per group)				
Body weight (g)	*365.2 ± 32.8*	*370.2 ± 29.9*	*369.1 ± 31.5*	*351.1 ± 40.0*
Adrenal				
- absolute (mg)	61.1 ± 8.13	55.1 ± 6.27**	55.8 ± 6.16*	56.9 ± 5.08*
- relative (/bw)	0.017 ± 0.002	0.015 ± 0.002**	0.015 ± 0.002*	0.016 ± 0.002
Spleen				
- absolute (g)	0.628 ± 0.119	0.614 ± 0.091	0.601 ± 0.063	**0.703 ± 0.112***
- relative (/bw)	0.172 ± 0.031	0.166 ± 0.022	0.164 ± 0.019	**0.201 ± 0.032****
Epididymides				
- absolute (g)	1.122 ± 0.076	1.125 ± 0.156	1.126 ± 0.103	1.163 ± 0.103
- relative (/bw)	0.309 ± 0.024	0.305 ± 0.039	0.307 ± 0.032	0.334 ± 0.031**
Seminal vesicle				
- absolute (g)	0.943 ± 0.174	1.028 ± 0.200	1.050 ± 0.115	1.041 ± 0.176
- relative (/bw)	0.258 ± 0.042	0.279 ± 0.054	0.287 ± 0.043*	0.297 ± 0.044*
F_1 (*n* = 25 per group)				
Body weight (g)	*372.6 ± 31.1*	*366.4 ± 42.0*	*373.1 ± 35.5*	***331.7 ± 32.9****
Brain				
- absolute (g)	2.059 ± 0.103	2.024 ± 0.092	2.022 ± 0.101	**1.977 ± 0.084****
- relative (/bw)	0.556 ± 0.048	0.558 ± 0.057	0.546 ± 0.05	**0.6 ± 0.043****
Adrenal				
- absolute (mg)	64.6 ± 11.1	58.7 ± 8.11	59.5 ± 6.77	59.0 ± 6.45
- relative (/bw)	0.017 ± 0.002	0.016 ± 0.002*	0.016 ± 0.002	0.018 ± 0.002
Spleen				
- absolute (g)	0.619 ± 0.1	0.608 ± 0.105	0.609 ± 0.065	**0.945 ± 1.0****
- relative (/bw)	0.167 ± 0.029	0.167 ± 0.028	0.164 ± 0.019	**0.28 ± 0.264****
Liver				
- absolute (g)	8.602 ± 0.855	8.176 ± 1.047	8.300 ± 1.018	7.581 ± 0.831**
- relative (/bw)	2.308 ± 0.111	2.231 ± 0.124	2.221 ± 0.109*	2.285 ± 0.106
Kidneys				
- absolute (g)	2.361 ± 0.255	2.248 ± 0.222	2.275 ± 0.239	2.093 ± 0.204**
- relative (/bw)	0.634 ± 0.051	0.616 ± 0.048	0.61 ± 0.04	0.633 ± 0.051
Pituitary gland				
- absolute (mg)	9.12 ± 2.068	9.08 ± 1.913	9.2 ± 1.893	8.8 ± 1.633
- relative (/bw)	0.002 ± 0.01	0.002 ± 0.0	0.002 ± 0.01	0.003 ± 0.0
Testes				
- absolute (g)	3.697 ± 0.344	3.67 ± 0.332	3.65 ± 0.275	3.512 ± 0.284
- relative (/bw)	0.998 ± 0.11	1.01 ± 0.112	0.984 ± 0.098	1.063 ± 0.073**
Epididymides				
- absolute (g)	1.11 ± 0.115	1.1 ± 0.097	1.082 ± 0.068	1.073 ± 0.118
- relative (/bw)	0.299 ± 0.036	0.303 ± 0.031	0.292 ± 0.027	0.323 ± 0.029**
Cauda epididymides				
- absolute (g)	0.422 ± 0.039	0.418 ± 0.041	0.423 ± 0.039	0.419 ± 0.042
- relative (/bw)	0.114 ± 0.014	0.115 ± 0.012	0.114 ± 0.012	0.127 ± 0.01**
Seminal vesicle				
- absolute (g)	1.152 ± 0.185	1.117 ± 0.186	1.113 ± 0.198	1.117 ± 0.223
- relative (/bw)	0.31 ± 0.051	0.306 ± 0.047	0.298 ± 0.042	0.337 ± 0.061

From Schneider et al. (2007a). Data taken from table 1C, pp. 280–285, 289–294 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 24. Selected organ weights of F_0 and F_1 female rats in a two-generation study with saflufenacil

Organ	Mean weights ± standard deviation			
	Dose (mg/kg bw per day)			
	0	5	15	50
F_0 (*n* = 25 per group)				
Body weight (g)	*226.8 ± 10.7*	*223.3 ± 15.9*	*229.6 ± 13.6*	*228.7 ± 14.6*
Adrenal				
- absolute (mg)	72.6 ± 8.66	71.7 ± 8.72	72.0 ± 7.27	70.9 ± 7.52
- relative (/bw)	0.032 ± 0.004	0.032 ± 0.004	0.031 ± 0.003	0.031 ± 0.003
Spleen				
- absolute (g)	39.5 ± 1.49	38.9 ± 2.20	38.6 ± 1.35	38.3 ± 1.89
- relative (/bw)	0.22 ± 0.041	0.238 ± 0.048	0.232 ± 0.037	0.203 ± 0.027
Uterus				
- absolute (g)	0.798 ± 0.242	0.689 ± 0.178	0.742 ± 0.181	0.652 ± 0.318*
- relative (/bw)	0.353 ± 0.11	0.309 ± 0.081	0.324 ± 0.081	0.286 ± 0.142**
F_1 (*n* = 25 per group)				
Body weight (g)	*220.3 ± 16.8*	*218.0 ± 12.7*	*221.0 ± 18.9*	*213.0 ± 13.1*
Brain				
- absolute (g)	1.889 ± 0.098	1.87 ± 0.061	1.888 ± 0.09	1.866 ± 0.068
- relative (/bw)	0.861 ± 0.059	0.86 ± 0.053	0.858 ± 0.058	0.878 ± 0.041
Adrenal				
- absolute (mg)	75.9 ± 8.38	74.3 ± 8.35	72.0 ± 7.62	69.0 ± 9.72**
- relative (/bw)	0.035 ± 0.004	0.034 ± 0.004	0.033 ± 0.004	0.032 ± 0.004
Spleen				
- absolute (g)	0.481 ± 0.05	0.505 ± 0.059	0.499 ± 0.051	0.479 ± 0.816
- relative (/bw)	0.219 ± 0.024	0.232 ± 0.029	0.222 ± 0.025	0.225 ± 0.03
Liver				
- absolute (g)	6.679 ± 0.774	6.6 ± 0.638	6.918 ± 0.873	6.257 ± 0.816*
- relative (/bw)	3.032 ± 0.26	3.03 ± 0.27	3.129 ± 0.279	2.939 ± 0.343
Kidneys				
- absolute (g)	1.705 ± 0.127	1.691 ± 0.126	1.732 ± 0.16	1.652 ± 0.103
- relative (/bw)	0.775 ± 0.044	0.776 ± 0.049	0.785 ± 0.056	0.777 ± 0.043
Pituitary gland				
- absolute (mg)	12.12 ± 2.44	11.8 ± 1.94	11.64 ± 1.32	10.48 ± 2.29**
- relative (/bw)	0.006 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001
Uterus				
- absolute (g)	0.607 ± 0.236	0.696 ± 0.276	0.61 ± 0.187	0.662 ± 0.248
- relative (/bw)	0.276 ± 0.106	0.32 ± 0.127	0.276 ± 0.079	0.311 ± 0.116
Ovaries				
- absolute (mg)	93.9 ± 14.33	101.3 ± 10.7	96.4 ± 14.8	99.9 ± 13.3
- relative (/bw)	0.043 ± 0.006	0.047 ± 0.005*	0.044 ± 0.006	0.047 ± 0.006*

From Schneider et al. (2007a). Data taken from table 1C, pp. 280–285, 289–294 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$

In F_1 pups, several litter and pup parameters at 50 mg/kg bw per day were adversely (statistically significant) affected by dietary exposure to saflufenacil (Table 25). The effects included a decrease in the number of liveborn pups, a higher number of stillborn pups, a reduced viability index and a lower lactation index when compared with the concurrent control values. The marginally higher number of dead pups and lower viability index in the low-dose group were regarded as spontaneous in nature and not related to treatment. There were no treatment-related effects on clinical signs or on sex ratios of live F_1 pups at birth or on lactation day 21. In F_2 pups, the number of dead pups was statistically significantly increased at 50 mg/kg bw per day. Consequently, the viability index was statistically significantly reduced in the high-dose group (87% versus 99–100% for other groups). The increased pup mortality in the high-dose group during this early stage of pre-weaning development was considered to be treatment related.

Mean body weights of F_1 male and female pups at 50 mg/kg bw per day were statistically significantly lower than those of controls during lactation days 1–14 (approximately 16% of control value), but the pups gained weight steadily (Table 26). Mean body weights of high-dose F_1 pups were about 5% less than those of the concurrent controls on lactation day 21; however, the decrease was not statistically significant. Mean body weights of F_2 male and female pups at 50 mg/kg bw per day were statistically significantly lower than those of controls during the entire lactation period. Starting with a body weight of approximately 20% less than that of the concurrent control on day 1 postpartum, the high-dose pups weighed about 9% less than the concurrent controls on lactation day 21. Body weight gain was statistically significantly decreased in high-dose pups on lactation days 1–7 (approximately 22%). Thereafter, body weight gain of these pups was essentially similar to that of the controls, although, throughout lactation, high-dose male pups on average still gained 7% less weight than control pups. No treatment-related effects on F_1 and F_2 pup body weights were noted at 5 or 15 mg/kg bw per day.

Each F_1 female pup that was selected to become the F_1 parent was evaluated for commencement of sexual maturity based on the observation of vaginal opening. The first and the last days when vaginal opening occurred were day 28 and day 36 postpartum, respectively. The mean numbers of days to reach the criterion were 31.3 ± 1.17, 31.3 ± 1.18, 32.5 ± 1.64 (statistically significant: $P \leq 0.05$) and 31.4 ± 1.96 at 0, 5, 15 and 50 mg/kg bw per day, respectively. Thus, based on the lack of a dose–response relationship, dietary exposure to saflufenacil had no adverse effects on female pup maturation. The values were also within the historical control range. Each F_1 male pup that was selected to become the F_1 parent was evaluated for commencement of sexual maturity based on the observation of preputial separation. The first and the last days when preputial separation occurred were day 40 and day 46 post-partum, respectively. The mean numbers of days to reach the criterion were 42.4 ± 1.64, 42.0 ± 1.41, 42.1 ± 1.44 and 41.7 ± 0.95 at 0, 5, 15 and 50 mg/kg bw per day, respectively. Thus, dietary exposure to saflufenacil had no adverse effects on male pup maturation. F_1 pups, on lactation day 4 but not on day 21, exhibited a dose-dependent reduction in mean platelet counts (statistically significant only in female pups at 50 mg/kg bw per day). The day 21 male pups at 50 mg/kg bw per day had decreased MCV values and increased MCHC values; both effects were marginal. The increased MCHC values were inconsistent with the kind of anaemia that was expected (i.e. hypochromic anaemia) and therefore were regarded as incidental. The decreased MCV values as the solely changed parameter of red blood cells could not be regarded as an indicator of an anaemic situation and therefore was not considered toxicologically relevant. Compared with the control, the F_2 female pups at 50 mg/kg bw per day had statistically significantly lower haemoglobin and haematocrit values. As the red blood cells were the targets of saflufenacil, the lower haemoglobin and haematocrit values in high-dose female pups were considered to be treatment related and adverse. Decreases in platelet counts were observed on lactation day 4 in male and female F_2 pups at 50 mg/kg bw per day. When assessed on lactation day 21, there were no treatment-related effects on haematology parameters. There were no treatment-related effects on serum enzyme activities in male and female F_1 and F_2 pups. The statistically significantly decreased absolute brain, thymus and/or spleen weights and relative thymus weights of the high-dose F_1 and/or F_2

Table 25. Summary of litter/pup data in a two-generation study in rats with saflufenacil

	Mean values ± standard deviation							
	F_1				F_2			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
No. of litters with live pups	25	24	25	24	24	24	25	24
No. of pups delivered	285	273	291	**259**	247	254	279	**227**
No. of liveborn pups	283	272	288	**240****	243	254	278	**211****
No. of stillborn pups	2	1	3	**19****	4	0	1	**16****
No. of pups that died	0	5*	0	**9****	1	1	1	**12****
Sex ratio (% males)								
- day 0	51.2	46.7	46.9	53.3	46.9	46.9	53.2	49.3
- day 21	51.3	49.2	47.7	52.3	46.5	50.3	52.5	48.0
No. of pups died, days 0–4 (%)	0 (0)	5* (2)	1 (0)	**23** (10)**	0 (0)	2 (1)	1 (0)	**27** (13)**
No. of pups died, days 4–21 (%)	1 (0)	1 (1)	1 (1)	**5* (3)**	0 (0)	0 (0)	0 (0)	1 (1)
No. of pups surviving								
- days 0–4 (viability index, %)	283 (100)	267 (98)	287 (100)	**217** (90)**	242 (100)	252 (99)	277 (100)	**183** (87)**
- days 4–21 (lactation index, %)	199 (100)	189 (99)	197 (99)	**172*** (97)	187 (100)	187 (100)	200 (100)	152 (99)
Mean litter size								
- day 1	11.3 ± 1.97	11.2 ± 2.06	11.5 ± 2.04	**9.3 ± 2.82**	10.1 ± 2.23	10.5 ± 2.17	11.1 ± 1.73	**7.7 ± 3.71**
- day 4 (pre-cull)	11.3 ± 1.97	11.1 ± 2.09	11.5 ± 2.04	**9.0 ± 2.97**	10.1 ± 2.24	10.5 ± 2.21	11.1 ± 1.73	**7.6 ± 3.77**
- day 4 (post-cull)	8.0 ± 0.20	7.9 ± 0.41	7.9 ± 0.40	7.4 ± 1.71	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.4 ± 2.76
- day 7	8.0 ± 0.20	7.9 ± 0.41	7.9 ± 0.40	7.3 ± 1.71	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.3 ± 2.82
- day 14	8.0 ± 0.20	7.9 ± 0.41	7.9 ± 0.44	7.2 ± 1.74	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.3 ± 2.82
- day 21	8.0 ± 0.20	7.9 ± 0.45	7.9 ± 0.44	7.2 ± 1.74	7.8 ± 0.72	7.8 ± 1.02	8.0 ± 0	6.3 ± 2.82

From Schneider et al. (2007a). Data taken from table 1A, pp. 161–163, 208–209 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

Table 26. Summary of litter/pup body weight data in a two-generation study in rats with saflufenacil

	Mean weights ± standard deviation							
	F_1				F_2			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
No. of litters	*25*	*24*	*25*	*23*	*24*	*24*	*25*	*22*
Pup weight (g)								
Day 1								
- Males	6.4 ± 0.61	6.3 ± 0.58	6.2 ± 0.51	**5.4 ± 0.78****	6.6 ± 0.63	6.5 ± 0.50	6.3 ± 0.62	**5.2 ± 0.82****
- Females	6.1 ± 0.56	6.0 ± 0.50	6.0 ± 0.61	**5.0 ± 0.65****	6.3 ± 0.61	6.3 ± 0.47	6.0 ± 0.63	**4.9 ± 0.72****
Day 4 (pre-cull)								
- Males	9.7 ± 1.10	9.7 ± 1.12	9.6 ± 0.92	**8.3 ± 1.44****	10.3 ± 1.08	10.1 ± 0.93	9.8 ± 1.02	**8.1 ± 1.61****
- Females	9.3 ± 0.97	9.3 ± 1.08	9.4 ± 1.05	**7.9 ± 1.19****	9.8 ± 1.03	9.8 ± 0.89	9.4 ± 0.95	**7.8 ± 1.47****
Day 4 (post-cull)								
- Males	9.7 ± 1.10	9.8 ± 1.09	9.6 ± 0.90	**8.3 ± 1.45****	10.3 ± 1.09	10.1 ± 0.95	9.8 ± 1.03	**8.1 ± 1.61****
- Females	9.3 ± 0.93	9.3 ± 1.03	9.5 ± 0.98	**8.0 ± 1.22****	9.8 ± 1.05	9.9 ± 0.88	9.5 ± 0.94	**7.8 ± 1.51****
Day 7								
- Males	15.7 ± 1.30	15.7 ± 1.58	15.8 ± 1.25	**13.4 ± 2.09****	16.3 ± 1.32	16.1 ± 1.16	15.6 ± 1.48	**13.1 ± 2.33****
- Females	15.0 ± 1.03	15.2 ± 1.47	15.5 ± 1.28	**13.0 ± 1.87****	15.6 ± 1.33	15.7 ± 1.13	15.2 ± 1.26	**12.6 ± 2.14****
Day 14								
- Males	31.3 ± 2.25	31.6 ± 2.80	31.7 ± 2.43	**29.2 ± 2.73***	32.2 ± 2.53	32.0 ± 1.65	31.3 ± 2.76	**28.3 ± 4.14***
- Females	30.4 ± 1.78	30.5 ± 2.75	31.4 ± 2.47	**28.2 ± 2.60****	31.1 ± 2.48	31.6 ± 1.76	30.4 ± 2.42	**27.5 ± 3.86****
Day 21								
- Males	49.2 ± 2.87	49.2 ± 3.64	49.8 ± 3.51	47.0 ± 4.50	50.4 ± 3.72	50.2 ± 2.48	49.4 ± 4.14	**45.4 ± 5.90****
- Females	47.7 ± 2.37	47.4 ± 3.26	48.5 ± 3.43	45.5 ± 3.69	48.2 ± 3.88	49.0 ± 2.75	47.9 ± 3.66	**43.8 ± 5.38****
Pup weight change (g)								
Days 1–4								
- Males	3.3 ± 0.59	3.4 ± 0.63	3.4 ± 0.47	2.9 ± 0.75	3.7 ± 0.54	3.5 ± 0.56	3.5 ± 0.50	**2.8 ± 0.99****

Table 26 (continued)

	Mean weights ± standard deviation							
	F_1				F_2			
	Dose (mg/kg bw per day)							
	0	5	15	50	0	5	15	50
- Females	3.2 ± 0.52	3.3 ± 0.66	3.4 ± 0.49	2.8 ± 0.66	3.5 ± 0.54	3.6 ± 0.49	3.5 ± 0.43	**2.8 ± 0.83****
Days 4–7								
- Males	6.0 ± 0.53	6.0 ± 0.67	6.1 ± 0.77	**5.1 ± 0.85****	6.0 ± 0.69	5.9 ± 0.57	5.8 ± 0.74	**4.9 ± 0.84****
- Females	5.7 ± 0.51	5.9 ± 0.65	6.0 ± 0.73	**5.0 ± 0.87****	5.8 ± 0.70	5.9 ± 0.57	5.7 ± 0.63	**4.8 ± 1.08****
Days 7–14								
- Males	15.6 ± 1.47	15.8 ± 1.52	16.0 ± 1.52	15.8 ± 2.00	16.0 ± 1.68	16.0 ± 1.09	15.6 ± 1.56	15.2 ± 2.24
- Females	15.4 ± 1.31	15.2 ± 1.56	15.8 ± 1.59	15.2 ± 1.30	15.5 ± 1.42	15.8 ± 1.24	15.2 ± 1.49	14.9 ± 1.78
Days 14–21								
- Males	17.9 ± 1.74	17.7 ± 1.51	18.1 ± 1.51	17.8 ± 2.05	18.1 ± 1.75	18.1 ± 1.21	18.1 ± 1.69	17.1 ± 2.07
- Females	17.3 ± 1.38	16.9 ± 1.45	17.1 ± 1.39	17.3 ± 1.49	17.2 ± 1.90	17.4 ± 1.46	17.5 ± 1.60	16.4 ± 1.89
Days 4–21								
- Males	39.5 ± 2.54	39.6 ± 2.79	40.2 ± 2.98	38.7 ± 3.65	40.1 ± 3.19	40.1 ± 2.24	39.6 ± 3.61	**37.3 ± 4.56***
- Females	38.4 ± 1.99	38.0 ± 2.49	39.1 ± 2.71	37.6 ± 2.88	38.4 ± 3.34	39.2 ± 2.44	38.5 ± 3.25	36.1 ± 4.21

From Schneider et al. (2007a). Data taken from table 1A, pp. 164–167, 210–213 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered treatment related

pups were considered secondary to the lower pup body weights in this group. They were not considered to be adverse or toxicologically relevant. A number of F_1 pups showed findings at gross necropsy, such as postmortem autolysis, sloped incisors, anasarca, small thymus, abnormal lung lobulation, infarct of liver, yellowish discoloured liver, empty stomach, hydronephrosis, infarct of kidney, dilated renal pelvis, distended urinary bladder, dilated ureter, small testis, anorchia and haemorrhagic testis. However, these findings were not considered treatment related, as findings were observed only in single animals and/or can be found in the historical control data at comparable or even higher incidences. Discoloured liver was observed in three animals from two litters in the F_1 generation and five animals from four litters in the F_2 generation. A number of F_2 pups showed findings at gross necropsy, such as postmortem autolysis, haemorrhagic thymus, diaphragmatic hernia, misshapen spleen, infarct of liver, dilated renal pelvis, small testis, malpositioned testis and haemorrhagic epididymis. All of these pup necropsy findings occurred without any relation to dosing, and most can be found in the historical control data at comparable or even higher incidences. The number of affected pups per litter showing yellowish discoloured livers or intestines was increased at 50 mg/kg bw per day (significant increase in intestines only). Discoloured liver was observed in three pups from two litters in the F_1 generation and five pups from four litters in the F_2 generation. Based on the mode of action of saflufenacil for which the liver is a target of toxicity due to porphyrin accumulation, the significance of this finding is unclear in the absence of other evidence of hepatotoxicity. There were no treatment-related microscopic findings noted in any of the dose groups of any generation.

The NOAEL for parental systemic toxicity was 15 mg/kg bw per day, based on adverse effects on feed intake, body weight gain and MHA seen at 50 mg/kg bw per day. The NOAEL for reproductive toxicity was 50 mg/kg bw per day, the highest dose tested. The NOAEL for offspring toxicity was 15 mg/kg bw per day, based on the increased number of stillborn pups, increased pup mortality during the early phase of lactation, reduced pup weight gains and indications of anaemia seen at 50 mg/kg bw per day. The study authors (Schneider et al., 2007b) concluded that the NOAEL for parental systemic toxicity was 5 mg/kg bw per day. However, marginally decreased haemoglobin (F_0 and F_1 males and females), haematocrit and MCV (F_0 and F_1 males) and decreased MCH, protein and albumin (F_1 males) were seen at 15 mg/kg bw per day. These effects were not considered adverse, as the magnitude of the decreases was small and there were no other adverse effects observed in the F_0 and F_1 parental animals.

(b) Developmental toxicity

Rats

In a developmental toxicity study, saflufenacil (purity 93.8%) was administered to 25 female Wistar rats per dose by gavage at a dose level of 0, 5, 20 or 60 mg/kg bw per day from days 6 through 19 of gestation. The test substance suspension for gavage administration was prepared in 1.0% carboxymethylcellulose in doubly distilled water. The dosing solution was analysed for stability, homogeneity and concentration. The animals were checked for mortality or clinical signs at least daily. Body weight data were recorded on gestation days 0, 1, 3, 6, 8, 10, 13, 15, 17, 19 and 20. With the exception of day 0, feed consumption was determined on the same days as body weight. Dams were sacrificed on day 20 of gestation. Blood was taken from all females prior to sacrifice for haematological and clinical chemistry measurements. Gross pathology was conducted on all females. Livers and spleens were weighed and prepared for histopathological examination. At necropsy, each fetus was weighed, sexed and examined macroscopically for any external findings. The viability of the fetuses and the condition of the placenta, umbilical cords, fetal membranes and fluids were examined. Individual placental weights were recorded. Thereafter, the fetuses were sacrificed by subcutaneous injection of a pentobarbital solution. After these examinations, approximately half of the fetuses per dam were eviscerated, skinned and placed in ethyl alcohol, and the remaining fetuses were placed in Harrison's fluid for fixation and further evaluation.

The dosing solution was stable for 96 hours at room temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable (90.7–103.2% of the nominal concentrations). There were no treatment-related effects on body weight, body weight gain, feed consumption or gross necropsy. At the end of the administration period, significantly decreased values for haemoglobin (94% of control), haematocrit (95% of control), MCV (95% of control) and MCH (95% of control) were found in the peripheral blood of the high-dose dams (Table 27). Statistically significantly decreased haematocrit (95% of control) and decreased haemoglobin concentration (96% of control; not statistically significant but dose related) were also observed in the mid-dose group (20 mg/kg bw per day). The changes in haematological parameters seen at 20 and 60 mg/kg bw per day are small in magnitude but considered to be adverse effects because of the primary mode of action of saflufenacil. No treatment-related changes were seen in the other red blood cell parameters of treated animals. Haematology examinations also revealed slightly, but statistically significantly ($P < 0.05$), increased white blood cell counts (117% of control) in the circulation of the high-dose dams. Because of the low magnitude of change, this finding was considered not to be of toxicological or biological relevance. There were no treatment-related changes in clinical chemistry parameters. There was an increase in liver porphyrins at and above 5 mg/kg bw per day, but the adverse nature of the change in porphyrins is unknown in the absence of other liver changes. There were no treatment-related changes at gross necropsy and no organ weight changes in the liver or spleen.

There was a decrease in fetal body weights at 20 mg/kg bw per day, as summarized in Table 28. Although there was an increase in live births per dam at the same dose, it does not entirely account for the reduced fetal weights and is considered treatment related and adverse.

Only one external malformation (cleft palate) was seen in one fetus of the 20 mg/kg bw per day test group, which was not considered to be treatment related. There was no increase in visceral malformations or variations. There was a treatment-related increase in the number of skeletal malformations (anomalies) at 60 mg/kg bw per day, with 10 individuals and seven litters affected (Table 29). The malformations consisted of bent scapulae, radii, ulnas and femurs, malpositioned and bipartite sternebrae and thick humeri. At 20 mg/kg bw per day, there was a single incidence of bent scapula. As this occurred in the absence of historical control findings, the malformation was considered treatment related. However, the incidence of bent scapula was considered delayed ossification and therefore unlikely to occur following a single dose. The litters of every dose group, including control, had skeletal variations. However, the proportions of individuals affected were higher in the 60 mg/kg bw per day dose groups, with all individuals affected, a finding above that of historical controls. At doses of 20 mg/kg bw per day and above, there were increases in incomplete ossification of the skull, nasal area and thoracic centrum and wavy ribs. At 60 mg/kg bw per day, there were increases in supraoccipital holes, bassioccipital holes, incomplete ossification of the hyoid and thoracic centrum, dumbbell ossification of the lumbar centrum and bipartite ossification of the sternebrae.

The maternal toxicity LOAEL is 60 mg/kg bw per day, based on statistically significantly decreased haematocrit (95% of control). The maternal toxicity NOAEL is 20 mg/kg bw per day. The developmental toxicity LOAEL is 20 mg/kg bw per day, based on decreased fetal body weights in males and females and increased skeletal anomalies (only one finding of bent scapula; unlikely due to single dose) and variations. The developmental toxicity NOAEL is 5 mg/kg bw per day (Schneider et al., 2007b; Schneider, 2008).

Rabbit

In a developmental toxicity study, saflufenacil (purity 93.8%) was administered to 25 female Himalayan rabbits per dose by gavage at a dose level of 0, 50, 200 or 600 mg/kg bw per day from days 6 through 28 of gestation. The test substance was given via gavage as an aqueous suspension in 1% carboxymethylcellulose. The dosing solution was analysed for stability, homogeneity and concentration.

Table 27. Select haematological parameters in the dams

	Mean values ± standard deviation			
	Dose (mg/kg bw per day)			
	0	5	20	60
Number of dams	*24*	*23*	*22*	*24*
White blood cells (10^9/l)	4.38 ± 1.21	4.57 ± 0.96 (↑4.33)	4.50 ± 0.96 (↑2.74)	5.14 ± 1.06* (↑17.35)
Haemoglobin (mmol/l)	6.9 ± 0.7	6.9 ± 0.5 (0.0)	6.6 ± 0.3 (↓4.35)	**6.5 ± 0.6* (↓5.80)**
Haematocrit (l/l)	0.320 ± 0.030	0.321 ± 0.024 (↑0.31)	0.303 ± 0.015* (↓5.31)	**0.304 ± 0.027* (↓5.00)**
MCV (fl)	55.0 ± 1.5	54.6 ± 1.5 (↓0.73)	54.6 ± 1.4 (↓0.73)	**52.5 ± 1.5** (↓4.55)**
MCH (fmol)	1.19 ± 0.04	1.17 ± 0.04 (↓1.68)	1.19 ± 0.04 (0.0)	**1.13 ± 0.04** (↓5.04)**
Platelets (10^9/l)	983 ± 172	984 ± 132 (↑0.10)	960 ± 143 (↓2.34)	945 ± 161 (↓3.87)

From Schneider et al. (2007b). Data extracted from pp. 82–83 of the study report.
MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; * $P < 0.05$; ** $P < 0.01$; **bolded** values are considered to be treatment related

Table 28. Caesarean section observations in rat developmental toxicity study with saflufenacil

Observation	Dose (mg/kg bw per day)			
	0	5	20	60
No. of animals assigned (mated)	25	25	25	25
No. of animals pregnant	24	23	22	24
- pregnancy rate (%)	96	92	88	96
No. non-pregnant	1	2	3	1
Maternal wastage				
- no. died	0	0	0	0
- no. died pregnant	0	0	0	0
- no. died non-pregnant	0	0	0	0
- no. aborted	0	0	0	0
- no. premature delivery	0	0	0	0
Total no. of corpora lutea	231	218	221	240
- no. of corpora lutea/dam	9.6 ± 176	9.5 ± 195	10.0 ± 1.46	10.0 ± 1.640
Total no. of implantations	185	191	207	203
- no. of implantations/dam	7.7 ± 3.26	8.3 ± 3.07	9.4 ± 1.71	8.5 ± 2.69
Total no. of litters	22	23	22	22
Total no. of live fetuses	174	179	196	187
- no. of live fetuses/dam	7.9 ± 2.67	7.8 ± 2.91	8.9 ± 1.97	8.5 ± 1.74
Total no. of dead fetuses	0	0	0	0
- no. of dead fetuses/dam	0	0	0	0
Total no. of resorptions	11	12	11	16
- early	11	11	11	14
- late	0	1	0	2
No. of resorptions/dam	0.5 ± 0.66	0.5 ± 0.59	0.5 ± 1.22	0.7 ± 1.05
- early	0.5 ± 0.66	0.5 ± 0.51	0.5 ± 1.22	0.6 ± 1.06
- late	0.0 ± 0.00	0.0 ± 0.21	0.0 ± 0.00	0.1 ± 0.28
No. of litters with total resorptions	2	0	0	2
Mean fetal weight (g)	3.7 ± 0.44	3.7 ± 0.21 (0)	**3.4 ± 0.32* (↓8.1)**	**3.1 ± 0.25** (↓16.2)**
- males	3.8 ± 0.49	3.8 ± 0.19 (0)	**3.5 ± 0.31 (↓7.9)**	**3.2 ± 0.30** (↓15.8)**
- females	3.6 ± 0.44	3.7 ± 0.21 (↑2.8)	**3.4 ± 0.34* (↓5.6)**	**3.0 ± 0.30** (↓16.7)**
Sex ratio (% male)	52.9	49.2	47.4	54.5
Pre-implantation loss (%)	19.6 ± 29.93	16.0 ± 24.11	6.1 ± 11.51	14.1 ± 26.34
Post-implantation loss (%)	14.0 ± 28.86	5.7 ± 6.40	5.1 ± 12.30	14.8 ± 28.73

From Schneider et al. (2007b). Data obtained from pp. 79–81 of the study report.
* $P < 0.05$; ** $P < 0.01$; **bolded** values are considered to be treatment related

Table 29. Skeletal examinations in rat developmental toxicity study with saflufenacil

Observations[a]	Dose (mg/kg bw per day)			
	0	5	20	60
No. of fetuses (litters) examined	94 (22)[b]	97 (23)	106 (22)	98 (22)
Malformations				
No. of fetuses (litters) affected	1 (1)	0 (0)	4 (4) [3.8 (18.2)]	10 (7)* [10.2 (31.8)]
HC: Total skeletal malformations	0.0–5.1 (0.0–25.0)			
Bent scapula: Cartilage present	0 (0)	0 (0)	1 (1)	5 (3)*
HC: Bent scapula	No historical findings			
Malpositioned and bipartite sternebra: Unchanged cartilage	0 (0)	0 (0)	1 (1) [0.9 (4.5)]	2 (2) [2.0 (9.0)]
HC: Malpositioned and bipartite sternebra: Unchanged cartilage	0.0–1.7 (0.0–8.0)			
Thick humerus: Cartilage present	0 (0)	0 (0)	0 (0)	5 (3)*
HC: Thick humerus	No historical findings			
Bent radius: Cartilage present	0 (0)	0 (0)	0 (0)	4 (2)
HC: Bent radius	No historical findings			
Bent ulna: Cartilage present	0 (0)	0 (0)	0 (0)	3 (2)
HC: Bent ulna	No historical findings			
Bent femur: Cartilage present	0 (0)	0 (0)	0 (0)	3 (2)
HC: Bent femur	No historical findings			
Misshapen lumbar vertebrae	0 (0)	0 (0)	1 (1)	0 (0)
Notched scapula: Cartilage present	0 (0)	0 (0)	1 (1)	0 (0)
Cleft sternum: Split cartilage	0 (0)	0 (0)	1 (1)	0 (0)
Variations				
No. of fetuses (litters) affected	90 (22)	94 (23)	105 (22) [99.0 (100.0)]	98 (22)* [100.0 (100.0)]
HC: Total skeletal variations	88.0–99.2 (100.0–100.0)			
Supraoccipital hole(s)	26 (13)	30 (15)	33 (18) [31.4 (81.8)]	49 (20)** [50.0 (90.9)]
HC: Supraoccipital hole(s)	8.0–59.3 (33.3–100.0)			
Incomplete ossification of skull: Unchanged cartilage	3 (3)	5 (2)	5 (4) [4.8 (18.2)]	7 (5) [7.1 (22.7)]
HC: Incomplete ossification of skull	0–10.8 (0.0–30.4)			
Bassioccipital hole(s)	1 (1)	0 (0)	1 (1)	5 (4) [5.1 (18.2)]
HC: Bassioccipital hole(s)	0–4.0 (0.0–12.5)			
Incomplete ossification of hyoid: Cartilage present	0 (0)	0 (0)	0 (0)	2 (2) [2.0 (9.1)]
HC: Incomplete ossification of hyoid	0–3.6 (0–8.3)			
Incomplete ossification of nasal bone: Unchanged cartilage	0 (0)	0 (0)	4 (4)*	15 (9)**
HC: Incomplete ossification of nasal bone	No historical findings			
Incomplete ossification of thoracic centrum: Unchanged cartilage	2 (2)	1 (1)	5 (3) [4.7 (13.6)]	12 (9)** [12.2 (40.9)]

Table 29 (continued)

Observations[a]	Dose (mg/kg bw per day)			
	0	5	20	60
HC: Incomplete ossification of the thoracic centrum	0.0–1.0 (0.0–4.6)			
Dumbbell ossification of lumbar centrum: Unchanged cartilage	3 (3)	4 (4)	1 (1)	6 (6) [6.1 (27.3)]
HC: Dumbbell ossification of lumbar centrum	0.0–2.0 (0.0–8.7)			
Incomplete ossification of sternebra: Unchanged cartilage	56 (21)	64 (21)	73 (20) [68.9 (90.9)]	76 (21)* [77.6 (95.5)]
HC: Incomplete ossification of sternebra	38.5–74.5 (70–100.0)			
Misshapen sternebra: Unchanged cartilage	39 (20)	37 (20)	40 (19)	64 (20)* [65.3 (90.9)]
HC: Misshapen sternebra	7.7–50.0 (37.5–96)			
Bipartite ossification of sternebra: Unchanged cartilage	0 (0)	0 (0)	0 (0)	2 (2) [2.0 (9.1)]
HC: Bipartite ossification of sternebra	0–4.9 (0.0–13.0)			
Wavy rib	17 (9)	11 (7)	34 (16)* [33.3 (72.7)]	51 (17)** [52.0 (77.3)]
HC: Wavy rib	1.0–9.3 (4.8–26.1)			
Incomplete ossification of pubis: Cartilage present	0 (0)	0 (0)	1 (1)	2 (1)
Incomplete ossification of ischium: Cartilage present	0 (0)	0 (0)	1 (1)	1 (1)

From Schneider et al. (2007 b). Data extracted from pp. 100–123 of the study report; historical control (HC) data from pp. 372–377 of the study report.

* $P < 0.05$; ** $P < 0.01$; **bolded** values are considered to be treatment related

[a] Some observations may be grouped together. [ ] percentage of fetuses and litters with finding in that treatment group.

[b] Fetal (litter) incidence.

The animals were checked for mortality or clinical signs at least daily. Body weight data were recorded on days 0, 2, 4, 6, 9, 11, 14, 16, 19, 21, 23, 25, 28 and 29 post-insemination. The consumption of feed was determined daily during days 1–29 post-insemination. Dams were sacrificed on day 29 post-insemination. Blood was taken from all females prior to sacrifice for haematological and clinical chemistry measurements. Gross pathology was conducted on all females. Liver samples were taken from five pregnant rabbits and their fetuses to determine liver porphyrins. Livers and spleens were weighed and prepared for histopathological examination. At necropsy, each fetus was weighed, sexed and examined macroscopically for any external findings. The viability of the fetuses and the condition of the placenta, umbilical cords, fetal membranes and fluids were examined. Individual placental weights were recorded. After the fetuses had been sacrificed, the abdomen and thorax were opened in order to be able to examine the organs in situ before they were removed. The heart and the kidneys were sectioned in order to assess the internal structure. The sex of the fetuses was determined by internal examination of the gonads. The heads of approximately one half of the fetuses per dam were fixed in Bouin's solution and processed and assessed according to Wilson's method. After skinning, all fetuses (including those without heads) were fixed in ethyl alcohol for skeletal examinations.

The dosing preparation was stable for 96 hours at ambient temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (greater than 90% and less than 110% of the nominal concentrations). Clinical signs were limited to the 600 mg/kg bw per day dose group, with lateral positioning, poor

general state, abortion, blood in bedding, discoloured and no urination and reduced or no defecation. Two high-dose dams were sacrificed in moribund condition on days 17 and 24, respectively. One dam from the 200 mg/kg bw per day dose group was found dead on gestation day 23, and one dam from the 600 mg/kg bw per day dose group was found dead on gestation day 27. One 50 mg/kg bw per day dam was sacrificed following abortion on gestation day 23. Two 600 mg/kg bw per day dams were sacrificed following abortion on gestation day 24, another on gestation day 25 and a final dam following abortion on gestation day 29 just prior to scheduled sacrifice. The increased mortality rate at 600 mg/kg bw per day, with four abortions, two moribund sacrifices and one premature death, is considered to be treatment related. There were no statistically significant or biologically relevant differences between the controls and the substance-treated dams in terms of body weights and body weight gain during the study. Feed consumption was decreased in the 600 mg/kg bw per day dose group from gestation day 6 to gestation day 18. Overall feed consumption was decreased in the 600 mg/kg bw per day dose group. There were no treatment-related, adverse organ weight changes. At doses at or above 200 mg/kg bw per day, there were stomach ulcerations, lack of faeces and assorted findings on implantations in dams sacrificed moribund. At 600 mg/kg bw per day, there was an increase in pale livers and kidneys, empty stomachs, enlarged bladders and assorted findings on implantations in dams that aborted. There were no treatment-related changes in the haematological parameters measured. Only slight but statistically significantly increased white blood cells and decreased platelets (high-dose does only) were observed. The haemoglobin, haematocrit, MCV, MCH and MCHC measures were unaffected by treatment at any dose level. There were no treatment-related changes in the clinical chemistry parameters. On day 29 post-insemination, total porphyrin concentrations in the liver of treated dams were statistically significantly increased compared with the animals of the control group. The liver porphyrin changes were not considered to be treatment related or adverse, as there were no other clinical, haematology or clinical chemistry changes at doses of or below 200 mg/kg bw per day. There was a treatment-related increase in maternal wastage at 600 mg/kg bw per day, with increases in dams that were found dead or sacrificed moribund and an increase in abortions. At 600 mg/kg bw per day, there was also a decrease in total litters, total live fetuses and live fetuses per dam. There were no effects on resorptions, fetal body weights, sex ratios or post-implantation loss. There were decreases in the total number of corpora lutea and corpora lutea per dam, total implantations and implantations per dam at 600 mg/kg bw per day, but as treatment did not commence until after implantation, the change is not treatment related. The findings are summarized in Table 30.

Liver porphyrins were increased at doses of 200 mg/kg bw per day and above in male and female fetuses. The nature of the changes in the total liver porphyrins was unclear due to a lack of haematological parameters measured in fetuses in the study. There were no treatment-related changes observed in the external examinations. There were no treatment-related changes to visceral malformations. There was a slight increase in the number of litters with skeletal malformations at doses at and above 200 mg/kg bw per day, especially when considering the reduced litters at 600 mg/kg bw per day. At 200 mg/kg bw per day, there was one fetus with sternebrae fused into a bony plate and one fetus with broken sternebra, ribs, humerus, tibia and fibula and knobby ulna and rib. At 600 mg/kg bw per day, there was one fetus with small interparietal and supraoccipital bones and another fetus with lumbar hemivertebra. However, as these were single incidences and there were no patterns of malformation or changes to the number of variations or unclassified changes, these malformations were not considered to be treatment related.

The maternal toxicity LOAEL was 600 mg/kg bw per day, based on increased mortality, clinical signs (lateral positioning, poor general state, abortion, blood in bedding, discoloured or no urination and reduced or no defecation) and increased necropsy findings (stomach ulcerations, lack of faeces, increase in pale livers and kidneys, empty stomachs, enlarged bladders and assorted findings on implantations in dams that aborted or were moribund). The NOAEL for maternal toxicity was 200 mg/kg bw per day. The developmental toxicity LOAEL was 600 mg/kg bw per day, based on a decrease in total litters and total live fetuses and live fetuses per dam. The developmental toxicity NOAEL was 200 mg/kg bw per day (Schneider et al., 2006).

Table 30. Caesarean section observations in a rabbit developmental toxicity study with saflufenacil

Observation	Dose (mg/kg bw per day)			
	0	50	200	600
No. of animals assigned (mated)	25	25	25	25
No. of animals pregnant	25	24	24	24
- pregnancy rate (%)	100	96	96	96
No. non-pregnant	0	1	1	1
Maternal wastage				
- no. died[a]	0	1	1	**7****
- no. died pregnant	0	1	1	**7**
- no. died non-pregnant	0	0	0	0
- no. aborted	0	1	0	**4**
- no. premature delivery	0	0	0	0
Total no. of corpora lutea	204	186 (↓8.8)	185 (↓9.3)	126 (↓38.2)
- no. of corpora lutea/dam	8.2 ± 1.60	8.1 ± 1.35 (↓1.2)	8.0 ± 1.55 (↓2.4)	7.4 ± 1.37 (↓9.8)
Total no. of implantations	169	173 (↑2.4)	158 (↓6.5)	104 (↓38.9)
- no. of implantations/dam	6.8 ± 2.44	7.5 ± 1.08 (↑10.3)	6.9 ± 2.07 (↑1.5)	6.1 ± 1.41 (↓10.3)
Total no. of litters	25	23 (↓8)	23 (↓8)	**17** (↓32)**
Total no. of live fetuses	157	162 (↑3.2)	150 (↓4.5)	**96 (↓38.9)**
- no. of live fetuses/dam	6.3 ± 2.19	7.0 ± 1.02 (↑11.1)	6.5 ± 2.04 (↑3.2)	**5.6 ± 1.11 (↓11.1)**
Total no. of dead fetuses	2	0	0	0
Total no. of resorptions	10	11	8	8
- early	7	7	6	8
- late	3	4	2	0
Resorptions/dam	0.4 ± 0.58	0.5 ± 0.67	0.3 ± 0.49	0.5 ± 0.80
- early	0.3 ± 0.46	0.3 ± 0.56	0.3 ± 0.45	0.5 ± 0.80
- late	0.1 ± 0.33	0.2 ± 0.39	0.1 ± 0.29	0.0 ± 0.00
No. of litters with total resorptions	0	0	0	0
Mean fetal weight (g)	39.6 ± 4.43	37.1 ± 2.93 (↓6.3)	38.1 ± 6.65 (↓3.8)	39.7 ± 3.09 (↑0.3)
- males	39.8 ± 4.32	37.0 ± 3.57 (↓7.0)	37.9 ± 7.01 (↓4.8)	39.5 ± 2.72 (↓0.8)
- females	39.0 ± 4.71	36.9 ± 3.19 (↓5.4)	37.9 ± 5.71 (↓2.8)	39.3 ± 3.61 (↑0.8)
Sex ratio (% male)	50.3	42.0 (↓16.5)	51.3 (↑2.0)	46.9 (↓6.8)
Pre-implantation loss (%)	17.9 ± 24.23	6.4 ± 7.85 (↓64.2)	16.0 ± 18.57 (↓10.6)	16.9 ± 14.49 (↓5.6)
Post-implantation loss (%)	5.8 ± 8.11	6.0 ± 8.26 (↑3.4)	5.0 ± 7.40 (↓13.8)	6.4 ± 9.92 (↑10.3)

From Schneider et al. (2006). Data extracted from pp. 80–82 and 92 of the study report.
** $P < 0.01$; **bolded** values are considered to be treatment related
[a] Includes animals found dead, sacrificed moribund and sacrificed following abortion.

2.6 Special studies

(a) Acute neurotoxicity

In an acute neurotoxicity study, saflufenacil (purity 93.8%) was administered to Wistar rats, 10 of each sex per group, by oral gavage at 0, 125, 500 or 2000 mg/kg bw. The test substance was suspended in drinking-water containing 0.5% carboxymethylcellulose. The treated rats were observed for 2 weeks. Neurobehavioural assessment (FOB and motor activity testing) was performed on days

−7, 0, 7 and 14. Dosing solutions were analysed for stability, homogeneity and concentration. All animals were observed for mortality and clinical signs twice daily on working days and once during the weekends and holidays. Feed consumption was determined weekly. Body weights were recorded on days −7, 0, 7 and 14. At study termination, five rats of each sex per group were euthanized and perfusion fixed for neuropathological examination.

The test substance was stable in the vehicle for 96 hours at room temperature. The test substance was homogeneous (91.1% with standard deviation of 1%). Mean concentrations were within 10% of the nominal concentrations. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable. All rats survived the duration of the study period. There were no treatment-related effects on clinical signs of toxicity, feed consumption and feed efficiency, body weights and body weight gain, or FOB. Males at 125 mg/kg bw showed reduced hindlimb grip strength on day 0. Males at 500 mg/kg bw showed increased hindlimb grip strength on day 7. Both findings were inconsistent single occurrences without any dose–response relationship and therefore were assessed as incidental and not related to treatment. The only noted finding was a moderately decreased motor activity in high-dose males. However, the decreased motor activity was statistically significant only when evaluated over the entire summary interval period and was noted at only the day 0 time point. The finding was not accompanied by any other neuropathological changes and was considered to be a reflection of a mild and transient general systemic toxicity and not a substance-specific neurotoxic effect. At terminal sacrifice, brain weight was not affected. Gross and histopathological examination of the brain or other nervous tissues revealed no treatment-related changes.

Based on the absence of adverse neurotoxic effects, the NOAEL for neurotoxicity was 2000 mg/kg bw in male and female rats. For systemic toxicity, the LOAEL was 2000 mg/kg bw for the males, based on decreased motor activity, representing mild and transient systemic toxicity. A LOAEL for the female was not observed. The systemic toxicity NOAELs for the male and female rats were 500 and 2000 mg/kg bw, respectively (Kaspers, Kaufmann & van Ravenzwaay, 2007).

(b) Short-term study of neurotoxicity

Rats

In a 90-day oral neurotoxicity study, Wistar rats (10 of each sex per group) were administered saflufenacil (purity 93.8%) daily in the diet at 0, 50, 250, 1000 (males) or 1350 (females) ppm (equal to 0, 3.3, 16.6 and 66.2 mg/kg bw per day for males and 0, 3.9, 19.4 and 101.0 mg/kg bw per day for females, respectively). Neurobehavioural assessment (FOB and motor activity testing) was performed on days −7, 1, 22, 50 and 85. At study termination, five rats of each sex per group were euthanized and perfusion fixed. The brain and other nervous tissues were processed for histopathological examination. Diets were prepared every 4 weeks. The stability, homogeneity and concentrations were measured analytically. The rats were examined for signs of toxicity and mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays. Body weights were recorded prior to the treatment and weekly thereafter. Feed consumption was recorded weekly. Haematological parameters were evaluated at termination.

The test substance was homogeneously distributed and was stable for 49 days at room temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable. There were no deaths observed in the study. Urine-smeared anogenital region was observed in one female at 1350 ppm and in one male at 1000 ppm. This finding was not considered to be toxicologically significant. The body weights of high-dose males and females were lower than those of the concurrent controls, attaining statistical significance on several time intervals. The overall body weight gains for the high-dose male and female rats were 14.0% and 15.9% lower than the control values at the end of the study period (Table 31). Feed consumption was decreased in high-dose males, occasionally attaining

Table 31. Mean body weights and body weight gains during 90 days of dietary administration of saflufenacil to rats

	Mean values ± standard deviation							
	Males (n = 10 per group)				Females (n = 10 per group)			
	Dietary concentration (ppm)							
	0	50	250	1000	0	50	250	1350
Body weight (g)								
Day 0	195.8 ± 10.0	199.1 ± 13.0	190.2 ± 11.1	196.8 ± 9.4	152.5 ± 7.0	150.4 ± 8.7	150.7 ± 9.0	149.3 ± 10.2
Day 91	404.4 ± 25.0	417.4 ± 25.3	384.3 ± 34.8	376.3 ± 35.5 (−6.9%)	250.5 ± 14.7	240.6 ± 16.2	246.9 ± 24.6	231.7 ± 17.2 (−7.8%)
Body weight change (g)								
Days 0–91	208.6 ± 23.4	218.3 ± 20.0	194.1 ± 32.0	**179.5 ± 34.2 (−14.0%)**	98.0 ± 16.4	90.3 ± 16.9	96.2 ± 19.0	**82.5 ± 17.9 (−15.9%)**

From Kaspers et al. (2007g). Data taken from table 1A, pp. 71–80 of the study report.
Bolded values are considered to be treatment related.

Table 32. Selected haematological data after 90-day dietary administration of saflufenacil to rats

	Mean values ± standard deviation							
	Males (n = 10 per group)				Females (n = 10 per group)			
	Dietary concentration (ppm)							
	0	50	250	1000	0	50	250	1350
Haemoglobin (mmol/l)	9.1 ± 0.3	9.1 ± 0.2	9.2 ± 0.4	**7.9 ± 0.4****	9.1 ± 0.3	8.8 ± 0.2	8.8 ± 0.3	**8.2 ± 0.4***
Haematocrit (%)	40.6 ± 1.2	40.6 ± 1.6	41.7 ± 1.6	**36.8 ± 1.9****	40.9 ± 1.5	40.2 ± 1.3	39.6 ± 1.1	37.8 ± 1.7
MCV (fl)	49.2 ± 1.2	51.2 ± 1.1*	47.4 ± 1.6	**44.5 ± 2.6****	53.3 ± 0.9	52.7 ± 0.9	53.3 ± 1.1	**47.4 ± 2.8***
MCH (fmol)	1.11 ± 0.04	1.15 ± 0.06	1.04 ± 0.04*	**0.96 ± 0.06****	1.18 ± 0.03	1.16 ± 0.02	1.19 ± 0.02	**1.03 ± 0.07****
MCHC (mmol/l)	22.5 ± 0.29	22.4 ± 0.77	21.9 ± 0.31	21.6 ± 0.24**	22.2 ± 0.33	22.0 ± 0.26	22.3 ± 0.19	**21.7 ± 0.54***

From Kaspers et al. (2007g). Data taken from table 1B, pp. 189–190 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered to be treatment related

statistical significance. No difference in feed consumption was noted between the high-dose males and controls towards the end of the study. Feed consumption was statistically significantly decreased in the high-dose females throughout the study. There were no treatment-related ophthalmoscopic findings (part of FOB measurements). Treatment-related decreases in haemoglobin, haematocrit (males only), MCV, MCH and MCHC were observed in high-dose animals at the end of the study (Table 32). These changes were regarded to be associated with moderate to severe anaemia. Other changes were not considered toxicologically significant because of the small magnitude of changes, lack of a dose–response relationship and/or inconsistency of the findings.

There were no treatment-related effects on the absolute or relative brain weights. There were no gross lesions that were attributable to the treatment. There were no treatment-related histopathological findings. A single (grade 1) "axonal degeneration" was recorded in the peripheral nerves of one high-dose female. This single finding was regarded as incidental or spontaneous in nature and not related to treatment, as single nerve fibre degenerations have been reported to occur spontaneously in central and peripheral nerve fibres in short-term neurotoxicity studies in rats. Neurotoxicity assessment, including an extensive FOB, motor activity, brain weights, as well as gross pathology and histopathology of the brain and other nervous tissues, did not reveal any adverse findings.

The NOAELs for neurotoxicity for the males and females were 1000 and 1350 ppm, respectively (equal to 66.2 and 101.0 mg/kg bw per day for males and females, respectively). A LOAEL for neurotoxicity was not established. The LOAELs for general systemic toxicity for the males and females were 1000 and 1350 ppm, respectively (equal to 66.2 and 101.0 mg/kg bw per day in males and females, respectively), based on treatment-related decreases in haemoglobin, haematocrit, MCV, MCH and MCHC. The NOAEL for systemic toxicity was 250 ppm (equal to 16.6 and 19.4 mg/kg bw per day in males and females, respectively) (Kaspers et al., 2007g).

(c) Immunotoxicity

Saflufenacil (purity 93.8%) was administered in the diet to groups of eight male C57BL/6J Rj mice at a dose level of 0, 50, 125 or 250 ppm (equal to 0, 10, 27 and 52 mg/kg bw per day, respectively) for a 4-week period. In addition, a concurrent positive control group of eight male C57BL/6J Rj mice received cyclophosphamide monohydrate in water at a dose level of 10 mg/kg bw per day by gavage for 4 weeks. All animals were immunized with a 0.5 ml intraperitoneal injection of sheep red blood cells on day 23. On day 29, blood was collected for primary T cell–dependent antibody response (anti-sheep red blood cell immunoglobulin M [IgM] enzyme-linked immunosorbent assay evaluation) and necropsied. Selected tissues (liver, spleen and thymus) were removed, weighed and prepared for histopathological examination.

No treatment-related effects on clinical signs, mortality, body weight, body weight gain or feed consumption were observed in saflufenacil treatment groups. Decreased red blood cell counts, haemoglobin and haematocrit values were observed in the 125 and 250 ppm dose groups. In mice of the positive control group, in addition to the decreased red blood cell, haemoglobin and haematocrit values, MCV and MCH were increased. ALT activity was increased in the 250 ppm dose group. Alkaline phosphatase activity was reduced in the 125 and 250 ppm dose groups and also in the positive control group. There were no treatment-related effects on the absolute or relative spleen and thymus weights in any saflufenacil-treated groups. No histopathological findings in the spleen and thymus were noted in the saflufenacil-treated mice. As expected, there were treatment-related increases in the spleen and thymus weights in the positive control group. Increased liver weight was observed at 250 ppm. Histopathological investigation of the liver revealed a slight to moderate centrilobular fatty change in almost all animals (seven out of eight) at 250 ppm. Additionally, two animals showed minimal lymphoid infiltration, and a single animal showed minimal extramedullary haematopoiesis in the liver. Six days after immunization, no changes in the sheep red blood cell IgM titres were found in male mice dosed with the test substance, whereas the sheep red blood cell titres were significantly lower in mice of the positive control group.

Under the study conditions utilized, no signs of immunotoxicity were observed following administration of saflufenacil to male C57BL/6J Rj mice. The oral administration of the positive control substance led to findings indicative of immunotoxicity, demonstrating the sensitivity of the assay (Buesen, 2010; Buesen et al., 2010).

(d) Mechanistic studies of effects on porphyrins

In a repeated-dose mechanistic toxicity study, saflufenacil (purity 94.2%) was administered in the diet to groups of Wistar rats (10 of each sex per group) at 0, 1, 5 or 25 ppm (equal to 0, 0.1, 0.4 and 2.0 mg/kg bw per day in males and 0, 0.1, 0.5 and 2.3 mg/kg bw per day in females, respectively) for an 8-week period. The diets were analysed for stability, homogeneity and concentration. The rats were examined for signs of toxicity and mortality twice a day during weekdays and once a day during weekends and holidays. Body weights and feed consumption were determined once a week. Blood and faeces from all rats were sampled after 1, 2, 4 and 8 weeks of saflufenacil treatment. Haematological examinations were performed, and total porphyrin concentrations in faeces were measured. At study termination, all rats were sacrificed under carbon dioxide anaesthesia and assessed for gross pathological changes.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable (104–114% of the nominal). There were no adverse effects of treatment on mortality, clinical observation, body weight, feed consumption or haematological parameters. Dietary administration of saflufenacil at 25 ppm caused an increase in porphyrin in faeces of male and female rats (Table 33), whereas saflufenacil at 5 ppm caused an increase in faecal porphyrin only in males. At 1 ppm, there were no effects on porphyrin excretion in the faeces.

The LOAEL was established based upon effects on porphyrin metabolism seen in males at 5 ppm (equal to 0.4 mg/kg bw per day) and in females at 25 ppm (equal to 2.3 mg/kg bw per day). The NOAEL was 1 ppm for males (equal to 0.1 mg/kg bw per day) and 5 ppm for females (equal to 0.5 mg/kg bw per day) (Cunha et al., 2005a).

In a repeated-dose mechanistic toxicity study, saflufenacil (purity 94.2%) was administered in the diet to groups of Wistar rats (10 of each sex per group) at 0, 10, 50 or 1000 ppm (equal to 0, 0.8, 4.1 and 80.6 mg/kg bw per day in males and 0, 0.9, 4.6 and 89.5 mg/kg bw per day in females, respectively) for an 8-week period. A 2-week recovery period was maintained for five animals of each sex per group. The effects of saflufenacil administration on porphyrin levels in plasma, urine, faeces and liver were monitored. The rats were examined for signs of toxicity and mortality twice a day during weekdays and once a day during weekends and holidays. Body weights and feed consumption were determined once a week. All surviving rats were sacrificed by carbon dioxide after a fasting period (withdrawal of feed and water) of about 16–20 hours. The liver was processed for porphyrin concentration examinations. No other examinations were carried out.

No analyses of the test substance preparations were carried out for this study. No treatment-related clinical signs or mortality occurred during the study. No treatment-related changes in body weight were observed in any of the treated animals. High-dose males had lower body weights in the first 2 weeks of the treatment; however, the overall weight gain of high-dose males was similar to that of control males. Feed consumption was found to be significantly reduced only in males treated with the high concentration (1000 ppm) on study days 7 (−9%) and 21 (−10%). At the end of the administration period, significantly decreased haemoglobin, haematocrit, MCV and MCH values were found in the peripheral blood of the male and female animals receiving 1000 ppm of the test compound. In addition, MCHC was significantly reduced in the males of the 1000 ppm group. No treatment-related effects on any of the haematological parameters were noted in either sex of the 10 or 50 ppm groups. At the end of the recovery period (week 11), high-dose rats still

Table 33. Total porphyrin values in faeces

	Mean total porphyrin values in faeces ± standard deviation (µmol/l)							
	Males (*n* = 10 per group)				Females (*n* = 10 per group)			
	Dietary concentration (ppm)							
	0	1	5	25	0	1	5	25
Day 7	76.1 ± 29.4	97.6 ± 43.5	**109 ± 40.4***	**283 ± 60.5****	103 ± 30.5	88.5 ± 40.6	109 ± 49.8	**175 ± 60.9****
Day 14	48.8 ± 31.5	46.3 ± 20.5	**75.9 ± 36.9***	**207 ± 74.0****	76.9 ± 54.7	70.9 ± 35.0	95.6 ± 38.7	**128 ± 44.1***
Day 28	57.2 ± 26.6	58.8 ± 26.8	**116 ± 56.8***	**204 ± 70.7****	71.2 ± 24.3	70.6 ± 32.9	87.5 ± 34.0	**145 ± 39.6****
Day 57	48.4 ± 19.6	58.2 ± 15.8	**110 ± 61.1***	**163 ± 57.0****	86.7 ± 23.4	109 ± 54.6	102 ± 40.3	140 ± 63.0

From Cunha et al. (2005a). Data taken from table IB, pp. 88–103 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered to be treatment related

showed statistically significantly decreased MCHC (males), MCV (females) and MCH (females) values when compared with the controls, but the differences were only marginal, indicating that the effects of saflufenacil on the blood parameters were reversible after a 2-week recovery period. Distinct increases in total porphyrin concentrations were measured in the plasma of the 1000 ppm groups of males (approximately 3- to 10-fold higher than the controls) and females (approximately 3-fold higher than the controls) after 3, 5 and 9 weeks of test substance administration (Table 34). In addition, porphyrin levels were slightly increased in the males of the 50 ppm group (approximately 2-fold higher than the controls) at weeks 3 and 5. Increases in total porphyrins were observed in the urine samples of the 1000 ppm groups of the male (approximately 4-fold higher than the controls) and female (approximately 5- to 8-fold higher than the controls) animals at the 3-, 5- and 9-week intervals. Slight, but statistically significant, increases in urinary total porphyrin concentrations were also found in the 50 ppm groups of both sexes (approximately 2-fold higher than the controls) after 3 and 9 weeks of test substance administration. No treatment-related effects on total porphyrin levels in urine of either sex were seen at 10 ppm. Throughout the administration period, statistically significantly increased total porphyrin concentrations were found in the faeces of the 50 ppm (males: approximately 3- to 7-fold higher; females: approximately 2- to 3-fold higher than the controls) and 1000 ppm (males: approximately 8- to 11-fold higher than the controls; females: approximately 7- to 11-fold higher than the controls) groups of both sexes. In the males, significantly increased porphyrin levels were also noted in the animals of the 10 ppm group (approximately 2-fold higher than the controls) at the 5- and 9-week intervals. No changes were seen in the females given 10 ppm of the test compound. After cessation of treatment, all test compound–related findings were reversible within a recovery period of 2 weeks. Statistically significant increases in porphyrin levels in liver homogenates were observed in rats at 10 ppm (females 1.5-fold higher than controls; statistically non-significant), 50 ppm (males approximately 5-fold and females approximately 3-fold higher than controls) and 1000 ppm (males approximately 27-fold and females approximately 24-fold higher than controls).

After 2 and 4 weeks of dietary exposure to saflufenacil, δ-aminolevulinic acid concentrations in urine samples of high-dose males were slightly, but statistically significantly, increased (approximately 3-fold higher than controls). After 2 and 4 weeks of dietary exposure to saflufenacil, increases of porphobilinogen concentrations were observed in high-dose males (approximately 8-fold higher than controls) and females (approximately 2-fold higher than controls at week 4). After 1, 2 and 4 weeks of dietary exposure to saflufenacil, dose-dependent increases in coproporphyrin III concentrations were observed in urine of treated rats. The concentration of coproporphyrin III was generally higher in male than in female animals. Significant increases of coproporphyrin III concentration in faeces were observed in all test rats at 1000 ppm (males, approximately 17-, 12- and 10-fold higher than controls on study days 7, 14 and 28, respectively; females, approximately 22-, 25- and 14-fold higher

Table 34. Porphyrin values in plasma, urine, faeces and liver

	Porphyrin values (mean ± standard deviation)							
	Males (*n* = 5 per group)				Females (*n* = 5 per group)			
	Dietary concentration (ppm)							
	0	10	50	1000	0	10	50	1000
Total porphyrin in plasma, nmol/l (*n* = 10 per group at week 9)								
Week 3	27.8 ± 9.3	44.6 ± 24.6	**69.1 ± 38****	**190 ± 119****	32.9 ± 8.6	24.5 ± 9.0	33.8 ± 11.7	**106 ± 16****
Week 5	21.8 ± 5.4	26.6 ± 7.5	**37.4 ± 9.1***	**219 ± 113****	23.8 ± 7.8	15.8 ± 3.5	24.7 ± 4.4	**76.1 ± 10****
Week 9	43.6 ± 20.5	53.2 ± 27.4	53.0 ± 17.1	**133.2 ± 88****	38.8 ± 23.6	27.8 ± 16.3	35.0 ± 14.3	**109 ± 138****
δ-Aminolevulinic acid in urine, µmol/l								
Week 3	48.6 ± 18.3	57.4 ± 9.1	54.7 ± 27.5	**119.8 ± 59***	62.1 ± 24.6	65.4 ± 34.1	49.9 ± 12.8	68.0 ± 27.4
Week 5	41.1 ± 20.4	45.2 ± 5.2	39.6 ± 8.4	**97.8 ± 26***	50.3 ± 17.9	67.5 ± 31.7	44.6 ± 7.6	56.7 ± 7.7
Porphobilinogen in urine in urine, µmol/l								
Week 3	4.8 ± 0.5	5.0 ± 1.5	6.1 ± 1.3	**35.9 ± 22****	5.1 ± 1.1	6.1 ± 2.7	4.3 ± 0.9	**9.8 ± 2.22****
Week 5	5.6 ± 0.6	6.2 ± 1.4	5.6 ± 0.6	**39.3 ± 20****	5.0 ± 0.9	5.8 ± 0.8	4.9 ± 0.9	7.3 ± 2.1
Total porphyrin in urine, µmol/l (*n* = 10 per group at week 9)								
Week 3	3.4 ± 1.9	5.3 ± 2.7	**8.6 ± 1.9****	**15.4 ± 2.1****	1.3 ± 0.3	1.2 ± 0.3	2.8 ± 1.2*	**10.3 ± 3.6****
Week 5	3.3 ± 2.1	3.8 ± 1.9	5.9 ± 0.7	**14.0 ± 1.3****	1.6 ± 0.8	1.1 ± 0.3	2.1 ± 0.5	**7.5 ± 3.0****
Week 9	3.8 ± 2.3	3.7 ± 1.8	**6.7 ± 1.9***	**17.2 ± 4.6****	1.1 ± 0.4	1.1 ± 0.3	2.4 ± 1.0**	**10.4 ± 5.3****
Total porphyrin in faeces, µmol/l (*n* = 10 per group at week 9)								
Week 3	74.0 ± 39.5	150.7 ± 68	**447 ± 127****	**615 ± 247****	67.0 ± 10.4	126.9 ± 104	**220 ± 139****	**615 ± 247****
Week 5	47.0 ± 2.9	**123.6 ± 61***	**328 ± 174****	**539 ± 203****	97.4 ± 40.3	83.9 ± 29.1	**183 ± 50***	**539 ± 203****
Week 9	63.8 ± 33.5	**106.4 ± 46***	**217 ± 102****	**618 ± 155****	89.8 ± 50.9	112.9 ± 53	**176 ± 75****	**635 ± 209****
Week 11	90.3 ± 63.3	40.0 ± 15	53.3 ± 39.9	54.1 ± 19.3	81.0 ± 63.9	74.6 ± 39.7	53.3 ± 39.9	64.3 ± 25.9
Total porphyrin in liver, pmol/l								
Week 9	96.6 ± 39.7	175.6 ± 45	**504 ± 79****	**2572 ± 489****	115 ± 28.2	196 ± 42	**389 ± 80****	**2753 ± 856****

From Cunha et al. (2006). Data taken from table IB, pp. 88–103 of the study report.
* $P \leq 0.05$; ** $P \leq 0.01$; **bolded** values are considered to be treatment related

than controls on study days 7, 14 and 29, respectively), 50 ppm (males, approximately 6-, 6- and 4-fold higher than controls on study days 7, 14 and 28, respectively; females, approximately 3-fold higher than controls on study days 7, 14 and 29) and 10 ppm (males, approximately 2-fold higher than controls on study days 7 and 14; females, approximately 1.5-fold higher than controls on study days 7 and 29). There were no pronounced increases of protoporphyrin IX in faeces of both male and female rats. Significant increases in the concentration of mesoporphyrin in faeces were found at 1000 ppm and 50 ppm in males and females and also at 10 ppm in males only. Comparison of the specific (HPLC) and nonspecific (spectrofluorometric) methods of determination of porphyrin excretion in urine and faeces indicated that the total values obtained from both methods were similar. There were no treatment-related gross pathological findings.

In summary, dietary administration of saflufenacil at 1000 ppm caused moderate MHA (sideroblastic anaemia) due to inhibition of haem biosynthesis. The inhibition of PPO resulted in increased accumulation and excretion of large amounts of porphyrins and their precursors, resulting in marked porphyria. Animals treated with lower concentrations of saflufenacil showed no signs of anaemia. At 1000 ppm, anaemia was observed in both sexes. However, at 50 ppm, saflufenacil still caused significant porphyria in male and female rats. Small, but statistically significant, increases in porphyrin levels in urine of males and in faeces of both sexes at 10 ppm were still noted. In general,

the total porphyrin concentrations in the plasma, urine, faeces and liver were statistically significantly increased in male and female rats at 50 and 1000 ppm. Following a 2-week recovery period, total porphyrins in the faeces of both sexes and most haematological parameters returned to normal or showed trends of reversibility. Therefore, it was concluded that the inhibition of PPO by saflufenacil was rapidly and completely reversible after cessation of treatment. Both individual porphyrin (HPLC) and total porphyrin (spectrofluorometric) measurements were made to assess the correlation of the two methods. The results indicated a strong correlation, and both types of measurements provided reliable dose–response information regarding PPO inhibition. Total porphyrins in faeces and liver provided the most reliable and sensitive data. Statistically significant effects on porphyrin metabolism could be detected at exposure concentrations well below those associated with adverse haematological effects.

Effects on porphyrins were seen down to the lowest dose tested, giving a lowest-observed-effect level (LOEL) of 10 ppm (equal to 0.8 mg/kg bw per day) and a corresponding no-observed-effect level (NOEL) of less than 10 ppm (equal to 4.1 mg/kg bw per day) (Cunha et al., 2006).

(e) Comparative study of hydrate and anhydrate forms of saflufenacil

Saflufenacil hydrate (purity 93.9%) or saflufenacil anhydrate (purity 99.0%) was administered to groups of 10 male Wistar rats at a dietary concentration of 0 or 1350 ppm (equal to 126.0–129.1 mg/kg bw per day) over a period of 4 weeks in order to determine if there are toxicologically significant differences between the two different crystalline forms of saflufenacil. After dosing, the rats were examined for signs of toxicity or mortality twice a day. Body weights were determined on day 0, then weekly thereafter. Feed consumption was recorded weekly. Faeces and blood from all rats were sampled on days 14 and 28 for haematological and porphyrin determinations. At study termination, all rats were sacrificed under carbon dioxide anaesthesia and assessed for gross pathological changes. The weights of the spleen and liver were recorded. The livers were also analysed for porphyrin concentrations.

The results indicated very similar effects following dietary exposure to the hydrated or anhydrate form of saflufenacil. The effects included impairment of feed intake; significant decreases in erythrocyte counts, haemoglobin, haematocrit, MCV, MCH, MCHC; increased reticulocyte and platelet counts; increases in porphyrin levels in the liver and faeces; and increases in absolute and relative spleen weights. Based on the similarity of the findings, it was concluded that the bioavailability and toxicity potential of the hydrated and anhydrated form of saflufenacil were similar (Cunha et al., 2005b).

(f) Dermal absorption

Rats

The absorption, distribution and excretion of radiolabelled saflufenacil were studied in male Wistar rats following a single dermal application of [^{14}C]saflufenacil (radiochemical purity 95%) suspended in the formulation concentrate (BAS 800 02 H) and 1/10 and 1/100 aqueous dilutions thereof. The nominal dose levels were 1.1723 mg/cm^2, 0.1172 mg/cm^2 and 0.0117 mg/cm^2, which correspond to about 11.723, 1.172 and 0.117 mg/animal at the high, middle and low doses, respectively. The high, middle and low doses selected in this study correspond to approximately 36.9, 4.0 and 0.4 mg/kg bw. Hairs were clipped from the dorsal region 24 hours before treatment, and the region was then cleaned with acetone. All animals were exposed for 10 hours. Application sites (all dose groups) were washed at 10 hours with a mild soap solution. Four animals were killed at 10, 24, 72 and 120 hours post-application. Radioactivity in the carcass, urine, faeces, blood, treated skin (after washing), surrounding skin, cage wash, gauge and bandages was analysed.

Mean recoveries of radioactivity from all dose groups were in the range of 92.00–115.20% of the total radioactivity administered. The largest proportion of radioactivity was recovered from the carcass and the faecal samples from the high-dose group and in the skin washes of the mid-dose (about 82–93% of the applied dose) and low-dose groups (about 72–86% of the applied dose). At the

high dose (i.e. the formulation concentrate), a systemic absorption of about 66.41% was observed after a 10-hour period of exposure to saflufenacil. At sacrifice after 120 hours, the absorption was 81.01% of the dose. In the high-dose group, about two thirds of the radioactivity remaining in the skin after the end of exposure penetrated through the skin during the 5-day post-observation period. At the middle dose (i.e. an 1/10 aqueous dilution of the formulation concentrate), a systemic absorption of about 2.78% was observed immediately after a 10-hour exposure period to saflufenacil. At sacrifice after 24, 72 and 120 hours, the absorptions were 3.96%, 8.57% and 3.36%, respectively. At the low dose (i.e. a 1/100 aqueous dilution of the formula concentrate), a systemic absorption of about 3.39% was observed immediately after a 10-hour exposure period for saflufenacil. At sacrifice after 24, 72 and 120 hours, the absorptions were 4.80%, 4.01% and 5.94% of the radioactivity applied, respectively. In most dermal absorption studies, the highest per cent absorption is usually observed at the low doses, whereas in this study, the highest dermal absorption was at the highest dose. There was evidence of skin irritation/corrosion at the application site in the high dose, which may enhance the dermal absorption. About 21–30% of the radioactivity was bound to the protective device in the high-dose group compared with 1.2–8.26% at the mid- and low-dose groups, suggesting the potential for even higher absorption potential at the high dose if the protective device–bound material was available for absorption (Fabian & Landsiedel, 2007b).

To estimate the dermal absorption of saflufenacil simulating the user-specific exposure scenario, the absorption, distribution and excretion of radiolabelled saflufenacil were studied in male Wistar rats following a single dermal application of saflufenacil in the formulation concentrate (BAS 800 04H) and 1/10 and 1/100 aqueous dilutions thereof. The target dose levels were 3.42 mg/cm^2, 0.342 mg/cm^2 and 0.0342 mg/cm^2 (corresponding to about 34.2, 3.42 and 0.342 mg/animal and about 105.8, 11.2 and 1.06 mg/kg bw) at the high, middle and low doses, respectively. Skin wash was performed at 8 hours after exposure and also before sacrifice at each time point. Four treated rats per dose were sacrificed at 24, 72 and 168 hours post-exposure. Excreta (urine and faeces) were collected throughout the exposure period, and blood cells, plasma, treated skin, skin surrounding the treated area and the carcass were collected at sacrifice. In addition, the cage and skin washes as well as the protective cover were retained for the determination of radioactivity.

There was no skin irritation observed at the skin application site. Mean recoveries of radioactivity from all dose groups ranged from 95.27% to 107.6% of the applied dose. The radioactivity in skin wash ranged from about 78% to 99% of the applied dose. Mean dermal absorption for saflufenacil in the BAS 800 04 H formulation concentrate (3.42 mg/cm^2), 1/10 dilution of the concentrate (0.342 mg/cm^2) and 1/100 dilution of the concentrate (0.0342 mg/cm^2) was 0.32%, 0.56% and 1.63%, respectively, following an exposure period of 8 hours and sacrifice after 168 hours. This study (Fabian & Landsiedel, 2008) gave drastically reduced dermal absorption values compared with the previous study (Fabian & Landsiedel, 2007b), probably due to differences in the vehicle used in these two studies.

(g) Protoporphyrinogen IX oxidase in vitro

A non-guideline study was conducted to investigate the inhibitory effects of saflufenacil (purity 93.8%) on PPO in liver mitochondrial preparations from female Wistar rats, female C57BL/6NCrl mice, female Himalayan rabbits and female human donors. The porphyrin pathway is an essential metabolic pathway in haem and chlorophyll biosynthesis. PPO catalyses the oxygen-dependent oxidation of protoporphyrinogen IX to protoporphyrin IX. In plants, the porphyrin pathway is a target for herbicides and growth-regulating chemicals. In mammals, inhibition of PPO may induce toxic effects. PPO is localized in two different compartments: in plants, PPO is localized in chloroplasts and in mitochondria; in mammals, PPO is localized in mitochondria. Mitochondrial fractions of female Wistar rats, C57BL/6N Crl mice, Himalayan rabbits and human donors were prepared and characterized

according to the activity of marker enzymes. Measurements of PPO activities of liver mitochondrial preparations were based on the spectrofluorometric detection of protoporphyrin formation over time. Butafenacil and oxyfluorfen (known PPO inhibitors) were used as positive controls.

PPO activities were linear to the amount of mitochondrial homogenate and varied across species, with human mitochondrial fractions showing lower PPO activity per milligram of protein, followed by the rabbit, mouse and rat. The rate of inhibition was determined using similar amounts of protein per assay, following the addition of various concentrations of saflufenacil. Butafenacil and oxyfluorfen, known PPO inhibitors, were similarly used as positive controls for PPO activity inhibition. PPO inhibition values were used for the determination of the 50% inhibitory concentrations (IC_{50}s) for interspecies comparison of inhibitory potency. The results demonstrated interspecies differences in saflufenacil inhibition of PPO activities in rat, mouse, rabbit and human liver mitochondrial preparations.

The relative inhibitory potency of saflufenacil on mice hepatic mitochondria was 0.6 times higher when compared with what was observed in rat mitochondria. The greatest differences in mitochondrial inhibition between species were observed in humans (14.1 times) and rabbits (16.2 times), when compared with inhibitory effects on rats, meaning that humans are approximately 14 times less sensitive than rats to PPO inhibition. No statistically significant differences were observed between human and rabbit mitochondria. The inhibitory activity of the other PPO inhibitors tested (butafenacil and oxyfluorfen) in different species was similar to that observed for saflufenacil; however, saflufenacil showed the lowest overall inhibition of PPO enzyme activity (Fabian, Niggeweg & Landsiedel, 2008).

3. Observations in humans

No clinical cases or poisoning incidents relating to saflufenacil have been reported.

Comments

Biochemical aspects

Absorption, distribution, excretion and metabolism of orally administered (gavage) saflufenacil were studied in male and female rats using (phenyl-U-^{14}C)- and (uracil-4-^{14}C)-labelled saflufenacil. The time to reach the maximum concentration of radioactive material in plasma was less than 1 hour. Thereafter, the plasma level of radioactivity declined rapidly, and only residual radioactivity was detected at 168 hours (0.2% of the administered dose). The AUC values indicated a sex difference, with up to 3-fold higher internal exposures for males than for females. Saflufenacil was rapidly and extensively (> 79%) absorbed from the gastrointestinal tract and rapidly excreted from the body in urine and faeces (> 97% of the administered dose) within 168 hours. The majority of excretion occurred in the first 24–48 hours, and excretion was complete by 96 hours. In 48 hours, bile duct–cannulated rats excreted approximately 67.8% and 35.5% of the administered dose in the bile in males and females, respectively. The urinary and biliary excretion data suggested that significant enterohepatic circulation of saflufenacil had occurred. Within 1 hour after oral administration of [^{14}C]saflufenacil, the highest radioactivity was found in the liver, gastrointestinal tract, kidney, lung and thyroid.

In the urine, the unchanged parent compound accounted for 10.9–48.2% and 78.1–88.9% of the administered dose for male and female rats, respectively. The predominant metabolic reactions of saflufenacil in the rat were demethylation of the uracil ring system, stepwise degradation of the *N*-methyl-*N*-isopropylsulfonamide to form an unsubstituted sulfonamide and cleavage of the uracil ring with loss of a three-carbon fragment to form an *N*-methylurea attached to the phenyl ring. The major metabolites identified in the urine of male and female rats were M800H01 (3.5–9.1% of the

dose) and M800H07 (0.6–4.6% of the dose), respectively. In faeces, the parent compound accounted for 3–16% of the dose. The main metabolite in faeces was M800H01, which amounted to 18–44% and 1–3% of the dose in male and female rats, respectively.

Toxicological data

The LD_{50} in rats treated orally and dermally with saflufenacil was greater than 2000 mg/kg bw. The LC_{50} in rats treated by inhalation (nose only) was greater than 5.3 mg/l. Saflufenacil was minimally irritating to the eyes and non-irritating to the skin of rabbits. Saflufenacil was not a skin sensitizer in guinea-pigs, as determined by the Magnusson and Kligman (maximization) test.

Short-term toxicity studies in mice, rats and dogs showed similar profiles of toxicity with respect to blood and liver. Males were more susceptible than females. The haematological effects were mostly related to the pesticidal mode of action of saflufenacil (i.e. inhibition of PPO). Effects indicative of this included increased total porphyrins in urine, faeces and liver, as well as increased total bilirubin and urinary bilinogen. Decreased haematological parameters indicative of MHA are consistent with this mode of action. Indicators of MHA included increased normoblasts, reticulocytes and polychromasia, increased microcytosis and anisocytosis, increased spleen weight, extramedullary haematopoiesis in liver and spleen (iron storage) and erythroid hyperplasia in bone marrow. At higher doses, an indication of liver toxicity, which included increased serum liver enzymes, centrilobular fatty change and lymphoid cell infiltration, was observed.

In 28-day and 90-day toxicity studies in mice, MHA, altered clinical chemistry (increased ALT, AST, urea and total bilirubin) (28-day study) and liver pathology (increased weight and centrilobular fatty change) were observed. In addition, decreased body weight and body weight gain were observed in the 90-day toxicity study. The NOAEL in the 28-day and 90-day studies of toxicity in mice was 50 ppm (equal to 12.8 mg/kg bw per day). The LOAEL in the 28-day and 90-day toxicity studies in mice was 150 ppm (equal to 36.6 mg/kg bw per day).

In a 28-day toxicity study in rats, the NOAEL was 150 ppm (equal to 13.4 mg/kg bw per day), based on MHA at 450 ppm (equal to 39.2 mg/kg bw per day). In addition to MHA, decreased total protein and decreased globulin were observed in a 90-day toxicity study in rats. The NOAEL in the 90-day toxicity study was 150 ppm (equal to 10.5 mg/kg bw per day), and the LOAEL was 450 ppm (equal to 32.3 mg/kg bw per day).

In a 28-day toxicity study in dogs, the NOAEL was 30 mg/kg bw per day, based on MHA at 100 mg/kg bw per day. The NOAEL in a 90-day toxicity study in dogs was 10 mg/kg bw per day, based on MHA in both sexes at 50 mg/kg bw per day. At the highest dose tested, more severe anaemia was seen, along with decreased body weight and body weight gain and dark brown/red brown discoloured faeces. In a 1-year toxicity study in dogs, the NOAEL was 20 mg/kg bw per day, based on discoloured faeces, lower body weight in males, decreased feed consumption, MHA, increased serum alkaline phosphatase activity and lowered total blood protein and albumin levels at 80 mg/kg bw per day. The overall NOAEL for the 90-day and 1-year toxicity studies in dogs was 20 mg/kg bw per day.

The carcinogenic potential of saflufenacil was studied in mice and rats. In mice, there was unusually high mortality in controls and all dose groups after approximately 16 months (485 days) of treatment. However, survival was adequate to assess the carcinogenic potential of saflufenacil. The early mortality was greatest in the control and low-dose male mice and was clearly unrelated to test substance treatment. There were no treatment-related effects on clinical signs of toxicity, mortality, body weight and body weight gain, feed consumption and feed efficiency, gross pathology or organ weights. The NOAEL was 25 ppm (equal to 4.6 mg/kg bw per day), based on MHA seen in the satellite group (killed at 10 months) at 75 and 150 ppm (equal to 13.8 and 38.1 mg/kg bw per day) in males and females, respectively. No treatment-related tumours were observed in mice.

In a 2-year study of toxicity and carcinogenicity in rats, the NOAEL was 100 ppm (equal to 6.2 mg/kg bw per day), on the basis of decreased body weight and body weight gains (males), anogenital region smeared with urine in females and MHA in males and females at 500 ppm (equal to 24.2 mg/kg bw per day). No treatment-related tumours were observed in rats.

The Meeting concluded that saflufenacil was not carcinogenic in mice or rats.

Saflufenacil gave a negative response in an adequate range of in vitro and in vivo genotoxicity tests, except for a positive finding that occurred with metabolic activation in an in vitro chromosomal aberration assay in mammalian cells. In contrast, no clastogenicity was observed in an in vivo mouse micronucleus assay.

The Meeting concluded that saflufenacil was unlikely to be genotoxic in vivo.

On the basis of the absence of genotoxicity in vivo and the absence of carcinogenicity in mice and rats, the Meeting concluded that saflufenacil is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, reproductive parameters were not affected at the highest dose tested (50 mg/kg bw per day). The NOAEL for parental systemic toxicity was 15 mg/kg bw per day, based on adverse effects on feed intake, body weight gain and MHA at 50 mg/kg bw per day. The NOAEL for offspring toxicity was 15 mg/kg bw per day, based on the increased number of stillborn pups, increased pup mortality during the early phase of lactation, reduced pup weight gains and indications of MHA at 50 mg/kg bw per day.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on MHA at 60 mg/kg bw per day. The developmental toxicity NOAEL was 5 mg/kg bw per day, based on decreased fetal body weights in males and females, an increased incidence of skeletal anomalies and delayed ossification at 20 mg/kg bw per day. In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 200 mg/kg bw per day, based on increased mortality, clinical signs (lateral positioning, poor general state, abortion, blood in bedding, discoloured and no urination and reduced or no defecation) and increased necropsy findings (stomach ulcerations, lack of faeces, increase in pale livers and kidneys, empty stomachs, enlarged bladders and assorted findings on implantations in dams that aborted or were moribund) at 600 mg/kg bw per day. The developmental toxicity NOAEL was 200 mg/kg bw per day, based on a decrease in total litters and total live fetuses and live fetuses per dam at 600 mg/kg bw per day.

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

In an acute neurotoxicity study in rats via gavage, no effects on FOB parameters, motor activity or neuropathology were observed at doses up to 2000 mg/kg bw. For systemic toxicity, the NOAEL was 5000 mg/kg bw for male rats, based on the decreased motor activity, representing mild and transient systemic toxicity, likely due to general malaise, at 2000 mg/kg bw, the highest dose tested.

In a 90-day dietary study of neurotoxicity in rats, no effects on FOB parameters, motor activity or neuropathology were observed in males and females at doses up to 1000 and 1350 ppm, respectively (equal to 66.2 and 101.0 mg/kg bw per day for male and female rats, respectively). The NOAEL for systemic toxicity was 250 ppm (equal to 16.6 mg/kg bw per day), based on MHA at 1000 ppm (equal to 66.2 mg/kg bw per day).

In an immunotoxicity study, no evidence of immunotoxicity was observed in male mice treated with saflufenacil in the diet for 4 weeks at doses up to 250 ppm (equal to 52 mg/kg bw per day).

Two dietary toxicity studies were conducted in rats to evaluate the effects of saflufenacil administration on porphyrin levels in plasma, urine, faeces and liver and also to evaluate the reversibility of porphyrin levels. Total porphyrin measurements showed significantly higher total porphyrin levels in the faeces of the males at 5 and 25 ppm (equivalent to 0.4 and 2.0 mg/kg bw per day, respectively) and of the females at 25 ppm (equivalent to 2.3 mg/kg bw per day). These findings are considered to be treatment related and are a consequence of increased accumulation and excretion of porphyrins due to inhibition of PPO by saflufenacil. In the recovery study, during a

treatment-free recovery period of 2 weeks, the statistically significant increases in total porphyrins in the faeces of both sexes returned to normal. Most of the haematological effects indicated their complete reversibility.

In studies in rats, bioavailability and toxicity were comparable between hydrated and anhydrate crystalline forms of saflufenacil.

An in vitro study was conducted to investigate the inhibitory effects of saflufenacil on PPO in the liver mitochondrial preparations from female rats, mice, rabbits and human donors. The results of this study indicated that rats are approximately 14 and 16 times more susceptible than humans and rabbits, respectively, to PPO inhibition. This difference in species susceptibility is consistent with the absence of haematological effects in rabbits at doses at least 14 times the NOAEL for these effects in rats.

No adverse effects due to occupational exposure to saflufenacil were reported in employees having contact with the active substance.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.05 mg/kg bw on the basis of a NOAEL of 4.6 mg/kg bw per day in the carcinogenicity study in mice, based on MHA at 13.8 mg/kg bw per day, and using a safety factor of 100. This ADI was supported by the NOAEL of 6.2 mg/kg bw per day observed in the chronic toxicity and carcinogenicity study in rats, on the basis of anogenital region smeared with urine in female rats and MHA in rats seen at 24.2 mg/kg bw per day. It is further supported by the NOAEL of 5 mg/kg bw per day observed in the developmental toxicity study in rats on the basis of increased skeletal anomalies at 20 mg/kg bw per day.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for saflufenacil in view of its low acute toxicity and the absence of developmental toxicity or any other toxicological effects that would be likely to be elicited by a single dose. MHA is not considered to be an appropriate end-point on the basis of which to establish an ARfD because it is not expected to appear after a single exposure due to the mechanism of toxicity by which it is produced.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Sixteen-month study of toxicity and carcinogenicity[a]	Toxicity	25 ppm, equal to 4.6 mg/kg bw per day	75 ppm, equal to 13.8 mg/kg bw per day
		Carcinogenicity	75 ppm, equal to 13.8 mg/kg bw per day[b]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 6.2 mg/kg bw per day	500 ppm, equal to 24.2 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 24.2 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	15 mg/kg bw per day	50 mg/kg bw per day
		Reproductive toxicity	50 mg/kg bw per day[b]	—
		Offspring toxicity	15 mg/kg bw per day	50 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	20 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	5 mg/kg bw per day	20 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Acute neurotoxicity study[c]	Systemic toxicity	500 mg/kg bw	2000 mg/kg bw
		Neurotoxicity	2000 mg/kg bw[b]	—
Rabbit	Developmental toxicity study[c]	Maternal toxicity	200 mg/kg bw per day	600 mg/kg bw per day
		Embryo and fetal toxicity	200 mg/kg bw per day	600 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[a,d]	Toxicity	20 mg/kg bw per day	50 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two or more studies combined.

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw

Estimate of acute reference dose

Not necessary

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly absorbed, complete within 168 h
Dermal absorption	No data available
Distribution	Widely distributed in tissues; highest residues in liver, gastrointestinal tract, liver, kidney, lung and thyroid
Potential for accumulation	None
Rate and extent of excretion	Rapid and extensive
Metabolism in animals	Moderately metabolized
Toxicologically significant compounds (animals, plants and environment)	Saflufenacil
Acute toxicity	
Rat, LD_{50}, oral	> 2000 mg/kg bw (female rats)
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.3 mg/l (4 h exposure, nose only)
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Minimally irritating
Guinea-pig, dermal sensitization (Magnusson and Kligman test)	Not a sensitizer
Short-term studies of toxicity	
Target/critical effect	MHA (mice, rats and dogs)
Lowest relevant oral NOAEL	10.5 mg/kg bw per day (90-day study of toxicity in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rats)
Lowest relevant inhalation NOAEC	Not available

Long-term studies of toxicity and carcinogenicity	
Target/critical effect	MHA
Lowest relevant NOAEL	4.6 mg/kg bw per day (carcinogenicity study in mice)
Carcinogenicity	Not carcinogenic in mice and rats
Genotoxicity	
	Not genotoxic in vivo
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	50 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	Developmental toxicity, including skeletal anomalies in rats
Lowest relevant developmental NOAEL	5 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity target/critical effect	Not neurotoxic (acute and 90-day studies in rats)
Lowest relevant neurotoxicity NOAEL	66.2 mg/kg bw per day, highest dose tested
Mechanistic data	
	Mechanistic studies indicating species differences in PPO inhibition and reversibility of porphyria
Medical data	
	No adverse effects reported

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Carcinogenicity study in mice supported by 2-year study of toxicity and carcinogenicity in rats and developmental toxicity study in rats	100
ARfD	None established		

References

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