

THIAMETHOXAM

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Explanation	566
Evaluation for acceptable daily intake	566
1. Biochemical aspects	566
1.1 Absorption, distribution and excretion	567
1.2 Metabolism	572
1.3 Comparison of excretion and metabolism in mice and rats.....	580
2. Toxicological studies	584
2.1 Acute toxicity	584
(a) Oral administration	584
(b) Dermal application	585
(c) Exposure by inhalation	585
(d) Dermal irritation	586
(e) Ocular irritation	586
(f) Dermal sensitization	586
2.2 Short-term studies of toxicity	587
(a) Oral administration	587
(b) Dermal application	601
2.3 Long-term studies of toxicity and carcinogenicity.	602
2.4 Genotoxicity	610
2.5 Reproductive toxicity.....	611
(a) Multigeneration studies	611
(b) Developmental toxicity	623
2.6 Special studies	629
(a) Neurotoxicity	629
(b) Developmental neurotoxicity study in rats	631
(c) Other special studies	641
3. Toxicology of metabolites	656
4. Observations in humans	657
Comments	657
Toxicological evaluation	661
References.....	664
Appendix 1: Application of the IPCS conceptual framework for cancer risk assessment.....	669

Explanation

Thiamethoxam is the International Organization for Standardization (ISO)–approved name for (EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine (International Union of Pure and Applied Chemistry [IUPAC]), with Chemical Abstracts Service (CAS) No. 153719-23-4. It is a neonicotinoid insecticide active against a broad range of commercially important sucking and chewing pests. The biological effects of this chemical class in target species are mediated primarily by an interaction with nicotinic acetylcholine receptor sites.

Thiamethoxam is being reviewed for the first time by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) at the request of the Codex Committee on Pesticide Residues (CCPR). All critical studies complied with good laboratory practice (GLP). Non-GLP studies were identified as such.

Evaluation for acceptable daily intake

1. Biochemical aspects

Kinetic studies on the absorption, distribution, metabolism and excretion of thiamethoxam were conducted in both mice and rats. This unusual extension of such studies to mice was considered necessary because neoplastic responses were observed in this species and it was believed that the additional information may go some way towards explaining the differences in response between mice and rats.

Mice

The studies in mice with thiamethoxam (identified in the original studies as CGA 293343) were mainly confined to males using unlabelled material (batch No. AMS 780/101; purity 99.3%) and a single, site-specifically radiolabelled thiamethoxam preparation: [thiazol-2-¹⁴C]thiamethoxam (batch No. ILS-208.1; radiochemical purity > 99.4%) (Mewes, 1998; Briswalter, 1999) or [oxadiazin-4-¹⁴C]thiamethoxam (batch No. ILS-235.1A; radiochemical purity 98.9%) (Mewes, 2000; Briswalter 2002a,c,d). Label positions were as shown in Figure 1.

Figure 1. Structures of the radiolabelled molecules



The oral gavage administrations to primarily male mice included the following daily dosing regimens:

- [thiazol-2-¹⁴C]thiamethoxam at 118 mg/kg bw for 14 consecutive days for the study of excretion and metabolism (Mewes, 1998) or for the identification of additional metabolites (Briswalter, 1999);
- dietary exposure to unlabelled thiamethoxam at a concentration of 0, 100, 500 or 2500 parts per million (ppm) for 29 days followed by one oral gavage dose of [oxadiazin-4-¹⁴C]thiamethoxam at 10 mg/kg bw and a second oral gavage dose of the labelled compound 72 hours later for the study of absorption, metabolism and excretion (Mewes, 2000);

- a single oral gavage dose of [oxadiazin-4-¹⁴C]thiamethoxam at 105.7 mg/kg bw for the study of blood kinetics and the identification of blood metabolites (Briswalter, 2002c);
- single oral doses of [oxadiazin-4-¹⁴C]thiamethoxam at either 0.5 or 100 mg/kg bw on a single occasion for the study of metabolism (Briswalter, 2002a) and for the study of absorption, distribution and excretion (Briswalter 2002d).

Rats

The studies in rats with thiamethoxam were performed in young male and female Tif:RAIf(SPF) rats using unlabelled material (batch No. KI-4654-18, purity > 98%; and batch No. AMS 780/101, purity > 99%) and two different, site-specifically radiolabelled thiamethoxam preparations: [thiazol-2-¹⁴C]thiamethoxam (radiochemical purity > 97%) and [oxadiazin-4-¹⁴C]thiamethoxam (radiochemical purity > 96% or > 97%) (Müller & Stampf, 1996) (Figure 1).

The administrations to rats included single oral doses to males and females of either 0.5 or 100 mg/kg bw of either radiolabelled material or 14 daily oral doses of 0.5 mg/kg bw per day of the unlabelled compound followed by a single oral dose of 0.5 mg/kg bw of radiolabelled material.

Studies in rats included a single oral gavage dose of [oxadiazin-4-¹⁴C]thiamethoxam at 106.5 mg/kg bw for the study of blood kinetics (Briswalter, 2002b).

The experimental programme also included investigation of bile duct-cannulated male rats and intravenously dosed male and female rats. Metabolism studies of specific metabolites are described below (see section 1.2). Urine, faeces, bile and expired air were individually and separately collected. For the kinetic studies in blood, samples were taken from three rats of each group and each sex by amputating the tip of the tail. Radioactivity in urine, plasma and other liquid samples was measured directly by liquid scintillation counting. Radioactivity in other collected materials was determined either after combustion or after digestion and liquid scintillation counting. Expired carbon dioxide was absorbed into a mixture of ethanolamine/ethylene glycol monomethyl ether 1:2 (volume per volume).

1.1 Absorption, distribution and excretion

Mice

Male mice received daily oral doses of [thiazol-2-¹⁴C]thiamethoxam at a dose level of 118 mg/kg bw for 14 consecutive days (Mewes, 1998) (note that this dosing regimen is different from that used on mice in the [oxadiazin-4-¹⁴C]thiamethoxam study and rats in both the [thiazol-2-¹⁴C]thiamethoxam and the [oxadiazin-4-¹⁴C]thiamethoxam studies, in which there were 14 days of dietary dosing with unlabelled thiamethoxam before gavage dosing with the radiolabelled compounds). Urine and faeces were collected daily until 72 hours after the last dose and were pooled at selected time intervals for analysis. Expired carbon dioxide was trapped during the first 3 days of the treatment.

Oral doses of thiamethoxam were apparently rapidly absorbed in mice, as administration was followed by its ready and rapid excretion, approximately 73% of the first dose appearing in urine and faeces within 24 hours. Only minor amounts of radioactivity were found in the excreta on days 2 and 3 after the last administration (time range of 360–384 hours). The administered radioactivity was predominantly excreted via the kidneys. A total of 71.8% of the administered dose was recovered in the urine, and 18.8% in the faeces. Low amounts of radioactivity were found in expired air (< 0.3% of the daily administered dose). A steady state of excretion was reached within 3 days after the first administration. The rates of excretion as percentages of the daily and cumulative doses are summarized in Table 1.

The quantitative analysis of excreted radioactivity in the urine and faeces of mice dosed with [thiazol-2-¹⁴C]thiamethoxam (Mewes, 1998) indicated that 30–60% of the daily administered

Table 1. The rate of excretion of radioactivity as percentages of the daily and cumulative doses of [thiazol-2-¹⁴C]thiamethoxam administered to male mice

Time point (h)	Percentage of daily dose		Percentage of cumulative dose	
	Mean	SD	Mean	SD
Urine				
0–24	66.03	14.69	5.29	1.18
24–48	58.83	9.75	4.65	0.27
48–72	69.01	3.13	5.49	0.63
72–96	80.32	3.51	5.92	0.87
96–120	56.85	3.46	4.18	0.64
120–144	56.51	9.28	4.18	1.14
144–168	66.91	8.57	4.95	1.16
168–192	71.75	5.69	4.71	0.15
192–216	64.14	12.20	4.25	0.57
216–240	84.13	3.96	5.59	0.36
240–264	81.17	13.12	5.37	0.65
264–288	88.02	8.38	5.91	0.56
288–312	77.77	8.59	5.19	0.19
312–336	78.71	19.18	5.19	1.12
336–360 ^a	9.60	2.08	0.63	0.10
360–384 ^a	3.76	0.40	0.25	0.04
<i>Subtotal</i>	—	—	71.75	3.50
Faeces				
0–24	6.99	1.35	0.56	0.10
24–48	34.98	11.36	2.86	1.17
48–72	14.41	3.65	1.13	0.23
72–96	9.93	1.42	0.73	0.14
96–120	18.73	3.83	1.37	0.29
120–144	21.26	11.10	1.57	0.83
144–168	16.54	7.86	1.21	0.56
168–192	14.89	2.63	0.99	0.22
192–216	19.32	4.27	1.30	0.38
216–240	27.44	8.65	1.84	0.64
240–264	18.55	4.08	1.25	0.37
264–288	14.09	3.46	0.96	0.31
288–312	17.60	7.57	1.20	0.56
312–336	17.29	10.21	1.17	0.77
336–360 ^a	6.80	6.03	0.47	0.44
360–384 ^a	3.07	3.25	0.21	0.24
<i>Subtotal</i>	—	—	18.84	3.46
Expired air				
0–24	0.17	0.01	0.01	< 0.01
24–48	0.25	0.06	0.02	< 0.01
48–72	0.26	0.03	0.02	< 0.01
<i>Subtotal</i>	—	—	0.06	< 0.01
Cage wash	—	—	3.81	0.31
Total recovery	—	—	94.46	1.27

From Mewes (1998)

^a % of daily dose calculations are based on administered radioactivity at 312 h.

Table 2. Excretion of [oxadiazin-4-¹⁴C]thiamethoxam administered to male mice after 29 days of dietary exposure to unlabelled thiamethoxam

Time point (h)	% of cumulative radioactive dose			
	Dietary concentration (ppm)			
	0	100	500	2500
Urine				
0–24	33.57	32.99	27.67	35.14
24–48	0.99	1.14	0.76	1.16
48–72	0.83	0.45	0.34	1.24
72–78	12.15	16.63	7.83	13.47
<i>Subtotal</i>	47.54	51.22	36.60	51.01
Faeces				
0–24	10.34	9.02	14.78	10.67
24–48	1.26	1.91	1.94	0.80
48–72	2.52	0.87	1.08	1.42
72–78	5.39	4.42	8.27	6.63
<i>Subtotal</i>	19.52	16.22	26.08	19.52
Cage wash				
78	10.74	7.23	8.14	8.02
Total excretion	77.79	74.67	70.82	78.56
Liver	0.43	0.73	0.72	0.53
Blood	0.08	0.11	0.11	0.09
Carcass	9.19	13.05	14.44	14.81
<i>Subtotal</i>	9.70	13.89	15.28	15.43
Total recovery	87.49	88.55	86.10	93.99

From Mewes (2000)

thiamethoxam was excreted as metabolites. As will be seen below (see [section 1.2](#)), this metabolism is much more extensive than in rats.

The effect of dietary administration of unlabelled thiamethoxam on the absorption, metabolism and excretion of [oxadiazin-4-¹⁴C]thiamethoxam was studied in male and female mice (Mewes, 2000). The mice received the unlabelled compound at a dietary concentration of 0, 100, 500 or 2500 ppm for 29 days before receiving two oral doses of [oxadiazin-4-¹⁴C]thiamethoxam, 10 mg/kg bw, 72 hours apart. Mice were killed 6 hours after the second radioactive dose. Excretion of radioactivity was very rapid at all dietary concentrations and was predominantly via the urine, and the route and rate of excretion were independent of the dietary dose level. Excretion was virtually complete by 72 hours after oral administration of a single oral dose of [oxadiazin-4-¹⁴C]thiamethoxam, with approximately 70% of the dose being recovered in urine and a further 25% of the dose being recovered in faeces (Table 2). This result was similar to that found with the [thiazol-2-¹⁴C]thiamethoxam label.

Considerable amounts of radioactivity were recovered in the liver, blood and carcass at termination (approximately 0.6%, 0.1% and 13% of the cumulative radioactive dose, respectively), as the mice were killed only 6 hours after administration of the second oral dose of [oxadiazin-4-¹⁴C]-thiamethoxam. Very little radioactivity was recovered in the bile. Residues in tissues were similar at all dietary dose levels, with residues in blood and plasma amounting to about 1 µg-equivalents/g, and residues in liver amounting to 1.7–3.3 µg-equivalents/g.

Table 3. Absorption of radioactivity by rats after a single oral administration of [thiazol-2-¹⁴C]-thiamethoxam or [oxadiazin-4-¹⁴C]thiamethoxam

	% of dose											
	[Thiazol-2- ¹⁴ C]thiamethoxam							[Oxadiazin-4- ¹⁴ C]thiamethoxam				
	Low dose × 1 ^a		Low dose × 14 ^a		High dose × 1 ^a		Low dose × 1 bile ^b	Low dose × 1 ^a		High dose × 1 ^a		Low dose × 1 bile ^b
	Male	Female	Male	Female	Male	Female	Male	Male	Female	Male	Female	Male
	Dose (mg/kg bw)											
	0.54	0.56	0.42	0.44	91.2	98.9	0.49	0.44	0.46	100.9	104.2	0.48
Urine	91.3	93.0	96.2	94.7	95.5	96.5	81.4	92.9	95.7	96.9	99.2	86.8
Bile	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.9	n.a.	n.a.	n.a.	n.a.	4.5
Tissues	0.4	0.3	0.4	0.5	0.3	0.3	1.5	0.3	0.2	0.3	0.7	1.6
Sum	91.7	93.3	96.6	95.2	95.8	96.8	86.8	93.2	95.9	97.2	99.9	92.9

From Müller & Stampf (1996)

n.a., not applicable (not collected)

^a 168 h time interval.

^b Bile duct-cannulated male rats, 48 h time interval.

Rats

Orally administered thiamethoxam was rapidly absorbed, and, as almost 100% of the oral doses were eliminated with the urine in all treatment groups, it can be assumed that thiamethoxam was virtually completely absorbed from the gastrointestinal tract irrespective of the dose level, pretreatment with non-radiolabelled thiamethoxam, the site of the label or the sex of the rats. The experiment with bile duct cannulation of male rats demonstrated that only a very small portion of the absorbed dose (approximately 4%) was excreted with the bile fluid into the duodenum (Table 3).

The routes of elimination were independent of the route of administration (oral and intravenous), the dose level, pretreatment with non-radiolabelled thiamethoxam, the site of the label and the sex of the rats. The absorbed amount was very rapidly excreted almost exclusively via the kidneys: within 24 hours, 84–95% and 3–6% of the dose were excreted in the urine and faeces, respectively. The bile duct-cannulated male rats excreted about 4%, 84% and 4% in bile, urine and faeces, respectively, within 48 hours, independent of the site of the radiolabel. Hence, these results obtained with bile duct-cannulated rats and with intravenously administered rats clearly show that the small amount eliminated with the faeces is derived from bile, providing strong evidence for the complete absorption of thiamethoxam from the gastrointestinal tract (Table 4).

After intravenous dosing, maximal blood concentrations averaged around 0.6 ppm thiamethoxam equivalents at the low dose. After oral dosing, maximal radioactivity in the blood was reached between 1 and 4 hours after administration in male and female rats independent of the dose level and the label; maximal concentrations averaged 0.18 and about 37 ppm thiamethoxam equivalents at the low and high doses, respectively. The radioactivity in the blood decreased rapidly, reaching half the maximal values 2–3 hours after intravenous administration and about 7 hours after oral administration in both sexes.

After oral administration, the areas under the concentration in blood–time curve ($AUC_{0-24\text{ h}}$) were in the same range for both labels and both sexes, accounting for an average of 1.4 and 318 $\mu\text{g}\cdot\text{h/g}$ at the low and high dose levels, respectively. Comparisons of AUCs at the low and high doses indicate a linear relationship of the bioavailability with dose in the range 0.5–100 mg/kg bw. Independent of the dose and the labels used, the bioavailability determined by the $AUC_{0-24\text{ h}}$ ratio after

Table 4. Excretion of radioactivity following a single intravenous or oral administration of thiamethoxam

Time point (h)	% of dose	[Thiazol-2- ¹⁴ C]thiamethoxam										[Oxadiazin-4- ¹⁴ C]thiamethoxam					
		Low dose × 1 iv		Low dose × 1 po		Low dose × 14 po		High dose × 1 po		Low dose × 1 po bile		Low dose × 1 po		High dose × 1 po		Low dose × 1 po	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Dose (mg/kg bw)																	
Urine																	
0-24	83.8	89.1		87.8		88.2		94.0		92.3		92.9		93.0		78.6	
24-48	1.7	2.1		1.8		2.2		1.3		1.3		1.5		2.1		2.9	
48-168	1.3	1.6		1.7		2.6		0.8		1.0		1.1		1.4		n.a.	
<i>Subtotal</i>	86.8	92.7		91.3		93.0		96.2		94.7		95.5		96.5		81.4	
Bile																	
0-24	n.a.	n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		3.8	
24-48	n.a.	n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		0.1	
<i>Subtotal</i>	n.a.	n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		n.a.		3.9	
Faeces																	
0-24	4.4	2.5		4.4		2.6		6.2		3.9		3.4		3.4		3.8	
24-48	0.8	0.5		0.6		0.5		0.5		0.4		1.4		0.7		1.0	
48-168	0.3	0.3		0.2		0.3		0.2		0.1		0.3		0.3		n.a.	
<i>Subtotal</i>	5.5	3.2		5.2		3.4		6.8		4.4		5.1		4.4		4.8	
Expired air																	
0-48	n.a.	n.a.		n.a.		n.a.		n.a.		n.a.		0.2		0.1		n.a.	
Cage wash	0.8	1.1		4.8		3.9		0.3		0.5		0.2		0.5		4.4	
Total excretion	93.1	97.0		101.3		100.2		103.3		99.6		101.0		101.5		94.5	

From Müller & Stampf (1996)
iv, intravenous; n.a., not applicable; po, by mouth (per os)

Table 5. Blood kinetics following single intravenous or oral administrations to rats of [thiazol-2-¹⁴C]thiamethoxam or [oxadiazin-4-¹⁴C]thiamethoxam

	[Thiazol-2- ¹⁴ C]thiamethoxam						[Oxadiazin-4- ¹⁴ C]thiamethoxam			
	Low dose × 1 iv		Low dose × 1 po		High dose × 1 po		Low dose × 1 po		High dose × 1 po	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	Dose (mg/kg bw)									
	0.51	0.54	0.55	0.55	91.8	100.7	0.44	0.46	101.8	104.0
$C_{\max}$ (ppm)	—	—	0.174	0.168	43.22	34.45	0.201	0.186	35.74	32.95
$T_{\max}$ (h)	0.25	0.25	4	2	2	1	2	1	4	1
$T_{\max}/2$ (h)	3	2	8	8	7	7	6	5	9	8
AUC _{0–24 h} (μg·h/g)	2.5	1.7	1.6	1.6	344.9	263.8	1.3	1.1	367.1	296.6
Bioavailability (%)	100	100	60	90	80	80	60	70	80	90

From Müller & Stampf (1996)

$C_{\max}$, maximum concentration in plasma; iv, intravenous; po, by mouth (per os); $T_{\max}$, time to reach the maximum concentration in plasma

oral and intravenous administration was about 0.7 and 0.8 for male and female rats, respectively. The major results are summarized in the Table 5.

Depletion from the broad range of tissues sampled is assumed to follow first-order kinetics. The half-lives in all tissues were in the range of 2–6 hours, independent of the dose level, the site of the label and the sex of the rats. Tissue residues were determined in male and female rats at four different time points after oral administration of [thiazol-2-¹⁴C]thiamethoxam at both dose levels and [oxadiazin-4-¹⁴C]thiamethoxam at the low dose level. As a consequence of the rapid depletion, the tissue residues were very low 7 days after oral administration of the low dose (0.5 mg/kg bw) when the tissue residues did not exceed 0.0033 ppm thiamethoxam equivalents (found in liver). The other tissue residues were close to or even below the limit of quantification. In some tissues (e.g. bone), the residues were below the limit of detection. At the low dose level, the limits of quantification were 0.0004–0.008 ppm for blood, brain, fat, heart, kidneys, liver, muscle, plasma, spleen and testes, 0.006–0.0011 ppm for bone, lungs and uterus and 0.0024–0.0031 ppm for ovaries. Treatment for 14 consecutive days with oral administrations of non-radiolabelled thiamethoxam (0.5 mg/kg bw) followed by a single oral dose of [thiazol-2-¹⁴C]thiamethoxam did not influence the pattern of tissue residues.

After oral administration of the high dose (100 mg/kg bw), the concentrations in the tissues were proportionally (i.e. about 200 times) higher. The highest concentrations in tissues were again found in the liver. The liver residues in male rats were slightly higher after administration of the [oxadiazin-4-¹⁴C] label (i.e. 0.56 ppm thiamethoxam equivalents) than after dosing of the [thiazol-2-¹⁴C] label (0.37 ppm thiamethoxam equivalents), whereas female rats showed similar concentrations after receiving either label; however, as the residue levels in all the other tissues at the high dose and in all tissues at the low dose were essentially the same for both labels, a significant label difference cannot be reliably identified. Following liver, the tissues with the next highest concentration tended to be either kidney or blood.

1.2 Metabolism

Mice

No qualitative or quantitative differences were seen in the urinary metabolite profiles among the different dietary dose levels (Mewes, 2000). Unchanged thiamethoxam and the metabolites

CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] and CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] represented the majority of the radioactivity in urine, accounting for 36–44%, 10–15% and 8–11% of the dose, respectively. The minor metabolite CGA 353968 [1-(2-chlorothiazol-5-ylmethyl)-3-methylurea] accounted for 0.7–1.3% of the dose. Four other unidentified fractions (R1–R4) each accounted for 0.5–2% of the dose.

Minor metabolites that were detected in mouse urine are NOA 402988 [2-methylsulfanyltiazole-5-carboxylic acid], NOA 407475 [3-(2-chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylideneamine], NOA 404617 [1-(2-chlorothiazol-5-ylmethyl)-3-nitrourea], 4U [(2-chlorothiazol-5-ylmethyl)-urea], 6U [2-acetylmino-3-(2-chlorothiazol-5-ylmethylsulfanyl)-propionic acid], NOA 421275 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methylguanidine] and NOA 421276 [*N*-(2-chlorothiazol-5-ylmethyl)-guanidine]. In addition to these metabolites, two unknown non-polar metabolites (R14 and R15) were detected in mouse urine.

Approximately 90% of the radioactivity in the faeces was extractable, and the metabolites in the faecal extracts were the same as in the urine. No qualitative or quantitative differences were seen in the metabolite profiles of the faecal extracts among the different dietary dose levels. Unchanged thiamethoxam and the metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] and CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] represented the majority of the radioactivity in urine, accounting for 8–14%, 4–6% and 3–7% of the dose, respectively. The minor metabolite CGA 353968 [1-(2-chlorothiazol-5-ylmethyl)-3-methylurea] accounted for 0.3–0.4% of the dose. Four other unidentified fractions (R1–R4) each accounted for 0.5–2.5% of the dose.

Two-dimensional thin-layer chromatographic analysis of the faecal extracts revealed the presence of unchanged thiamethoxam and the metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine], CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine], NOA 407475 [3-(2-chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylideneamine], NOA 402988 [2-methylsulfanyltiazole-5-carboxylic acid], NOA 421275 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methylguanidine] and NOA 421276 [*N*-(2-chlorothiazol-5-ylmethyl)-guanidine]. Metabolite R6, accounting for 1.9–2.6% of the daily dose in mouse faeces, was isolated, with a structure proposed as 2-acetylmino-3-[5-(5-methyl-4-nitroimino-[1,3,5]oxadiazinan-3-ylmethyl)-thiazol-2-ylsulfanyl]-propionic acid, based on results from mass spectrometry. Quantitative aspects of these results are presented in [section 1.3](#), in comparison with the findings from rats.

Bile metabolite profiles showed no qualitative or quantitative differences among the different dietary dose levels. The majority of the radioactivity in bile consisted of very polar metabolites, the most important of which was metabolite M04, representing 14–22% of the dose. Unchanged CGA 293343 and the metabolites CGA 322704 and CGA 265307 each accounted for 3–6% of the dose.

Plasma metabolite profiles also showed no qualitative or quantitative differences among the different dietary dose levels. Unchanged thiamethoxam and the metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] and CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] represented the majority of the radioactivity in plasma, accounting for 17–26%, 19–26% and 43–50% of the dose, respectively.

Radioactive residues in the liver accounted for 0.4–0.7% of the total dose, and approximately 90% of this radioactivity was extractable. No qualitative or quantitative differences were seen in the metabolite profiles of the liver extracts among the different dietary dose levels. Unchanged thiamethoxam and the metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] and CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] each accounted for 0.3–2.2% of the dose. Although most of the radioactivity in liver consisted of very polar metabolites, even the more prevalent of these amounted to less than 0.25%, individually, of the total radioactive dose.

The studies of Mewes (1998, 2000) and Briswalter (1999) demonstrated that the major reaction involved in the biotransformation of thiamethoxam in mice is the cleavage of the oxadiazine ring to result in the corresponding nitroguanidine compound, which is followed by its demethylation. In this way, the two major metabolites, CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] and CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine], are formed. Another important pathway is the substitution of the chlorine of the thiazole ring in thiamethoxam by glutathione, followed by its subsequent and complex degradation. Minor pathways (< 1% of the daily dose for each metabolite) include the cleavage of the nitroguanidine group from the oxadiazine ring of thiamethoxam to result in the corresponding guanidine. The guanidine metabolite can then be demethylated and hydrolysed, the latter to form a urea derivative. As these compounds retain the chlorine, this can react with glutathione to add more metabolites and increase complexity.

Substitution of the chlorine of the thiazole ring in thiamethoxam by glutathione and subsequent reactions on the glutathione moiety resulted in metabolite R6 found in mouse faeces. Minor pathways (< 1% of the daily dose for each metabolite) include the cleavage of the nitroguanidine group of thiamethoxam to result in the corresponding guanidine, yielding metabolite NOA 407475 [3-(2-chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylideneamine]. Cleavage of the nitroguanidine group and the oxadiazine ring yielded metabolite NOA 421275 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methylguanidine]. Demethylation of NOA 421275 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methylguanidine] yielded metabolite NOA 421276 [*N*-(2-chlorothiazol-5-ylmethyl)-guanidine]. Hydrolysis of the guanidine group of CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] yielded the respective urea derivative, NOA 404617 [1-(2-chlorothiazol-5-ylmethyl)-3-nitrourea]. Substitution of the chlorine of the thiazole ring by glutathione and subsequent degradation resulted in the formation of metabolite NOA 402988 [2-methylsulfanyltiazole-5-carboxylic acid]. Metabolite 4U [(2-chlorothiazol-5-ylmethyl)-urea] is formed by hydrolysis of NOA 421276 [*N*-(2-chlorothiazol-5-ylmethyl)-guanidine]. More reactions of NOA 421276 with glutathione and subsequent metabolic transformations led to the formation of metabolite 6U [2-acetylamino-3-(2-chlorothiazol-5-ylmethylsulfanyl)-propionic acid], an *N*-acetylcysteiny derivative of the thiazole moiety of thiamethoxam.

Minor pathways include the cleavage of the nitroguanidine group to result in the corresponding guanidine, yielding metabolite NOA 407475. Demethylation of NOA 407475 yielded metabolite N1. Cleavage of the nitroguanidine group and the oxadiazine ring of metabolite NOA 407475 yielded metabolite NOA 421275. Demethylation of NOA 421275 yielded metabolite NOA 421276. Hydrolysis of the guanidine group of CGA 265307 yielded the respective urea derivative, NOA 404617. Metabolite 4U is formed by hydrolysis of NOA 421276. Cleavage between the thiazole and oxadiazine moieties occurs to a very small extent, as shown by the presence of minor metabolites 6U, MO1, MO2, CGA 359683 and NOA 402988.

Based on the similarities in the metabolites identified, it appears that the metabolism of thiamethoxam in the mouse proceeds by the same major pathways as in the rat (see below). The new minor metabolites found were R14 [3-(2-chlorothiazol-5-ylmethyl)-[1,3,5]oxadiazinan-4-ylideneamine], R15: CGA 359683 [2-chlorothiazole-5-carboxylic acid], MO1 and MO2, which are glucuronic acid and acetylcysteine conjugates, respectively, of CGA 349208 and MO3, the *N*-acetyl cysteine conjugate of CGA 265307.

A proposed metabolic pathway for thiamethoxam in mice and other vertebrate species is shown in [Figure 2](#) below.

Rats

The metabolic pathways of thiamethoxam were studied in male and female rats (Rumbëli, 1998; Thanei & Rumbëli, 1998) using the samples collected in the kinetic investigations described

above (Müller & Stampf, 1996) and stored at -18°C . The urinary pattern of metabolites determined quantitatively by two-dimensional thin-layer chromatography was complex and essentially independent of sex, dose, preliminary treatment with non-radiolabelled thiamethoxam and route of administration. Very minor label-specific fractions were observed. The faecal pattern of metabolites was similar to, although less complex than, that found for urine, with some quantitative variations, but generally was independent of sex, dose, preliminary treatment with non-radiolabelled thiamethoxam and route of administration. The faecal pattern was only slightly dependent on the position of the label. The bile pattern was even less complex and independent of the position of the label, with some quantitative variations. All the patterns of metabolites in excreta were dominated by two major fractions that together accounted for approximately 60–80% of the dose.

Ultimately, 12 metabolites were isolated from the urine of the high-dose male and female rats and identified by spectroscopy. In addition, 13 metabolites were identified by co-chromatography with authentic reference substances.

The major fraction in urine, accounting for about 70–80% of the dose, was unchanged thiamethoxam. Based on the structures of the identified metabolites, the metabolic pathways of thiamethoxam in rats were derived and proposed to consist of the following:

- cleavage of the oxadiazine ring to the corresponding nitroguanidine compound (major pathway);
- reduction of the nitroguanidine group to a hydrazine followed by either acetylation or pyruvate conjugation (minor pathway);
- cleavage of the nitroguanidine group, yielding a guanidine derivative (minor pathway);
- hydrolysis of the guanidine group to the corresponding urea (minor pathway);
- demethylation of the guanidine group (minor pathway);
- substitution of the chlorine of the thiazole ring by glutathione (minor pathway);
- cleavage between the thiazole and oxadiazine rings (minor pathway).

The proposed metabolic pathway of thiamethoxam in all of the mammalian and avian species studied (rat, mouse and—as reported in the 2010 JMPR meeting report ([Annex 1](#), reference 119)—goat and fowl) is presented in [Figure 2](#). The majority of metabolites were the result of more than one of the above-mentioned transformations. Identified metabolites are listed in [Table 6](#).

Cleavage between the thiazole and oxadiazine rings occurs to a very small extent. It is initiated either by attack of glutathione on the bridging methylene group as long as the nitroguanidine moiety is intact, rendering a good leaving group, or by oxidative dealkylation. The former reaction gives rise to a thiazole-5-ylmethyl-glutathione derivative and a nitroguanidine compound, whereas the latter reaction ultimately produces the respective carboxylic acid derivative of thiazole and the corresponding cleavage counterpart.

Three hydrazine derivative metabolites of thiamethoxam have been found in rats. Two of these derivatives were detectable in rat excreta in small amounts. All of the hydrazine derivatives detected were acylated (pyruvate or acetate) and incorporated in an enamine structure (formed by reaction of a secondary amine with a carbonyl), which suggests a reduced reactivity of the hydrazine moiety.

The glutathione derivatives are prone to further degradation of the glutathione moiety, resulting in various sulfur-containing metabolites (e.g. mercapturates, sulfides and sulfoxides). These various sulfur-containing metabolites contribute significantly to the complex pattern of metabolites found in urine.

Figure 2. Proposed integrated metabolic pathways of thiamethoxam in mice, rats, goats and fowl

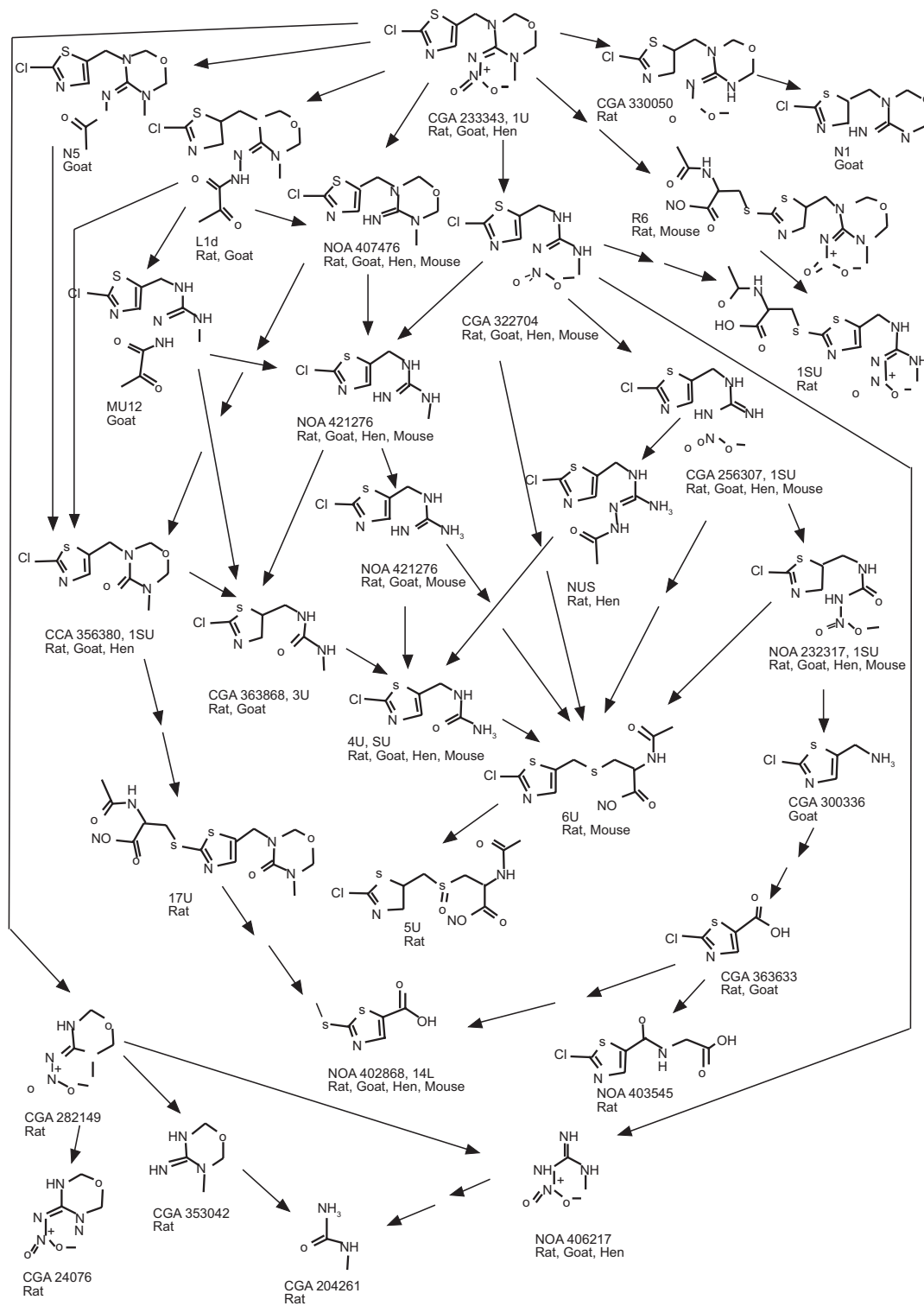


Table 6. Thiamethoxam and its metabolites as found in rats, mice, goats and fowl (hen)

Code number	IUPAC name	Found in	Structure
CGA 293343 1U	3-(2-Chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene- <i>N</i> -nitroamine	Rat, goat, hen	
CGA 322704 2U	<i>N</i> -(2-Chlorothiazol-5-ylmethyl)- <i>N'</i> -methyl- <i>N''</i> -nitroguanidine	Rat, goat, hen	
CGA 204261	<i>N</i> -Methylurea	Rat	
CGA 265307 18U	<i>N</i> -(2-Chlorothiazol-5-ylmethyl)- <i>N'</i> -nitroguanidine	Rat, goat, hen	
MO3 CGA 282149	<i>N</i> -Acetylcysteine conjugate of CGA 265307 3,6-Dihydro-3-methyl- <i>N</i> -nitro-2H-1,3,5-oxadiazin-4-amine	Mouse Rat	
CGA 309335	(2-Chlorothiazol-5-yl)-methylamine	Goat	
CGA 330050	3-(2-Chlorothiazol-5-ylmethyl)-[1,3,5]-oxadiazinan-4-ylidene- <i>N</i> -nitroamine	Mouse, rat	
CGA 340575	3,6-Dihydro- <i>N</i> -nitro-2H-1,3,5-oxadiazin-4-amine	Rat	
CGA 349208	2-Chloro-5-thiazolemethanol	Mouse	
MO1 MO2 CGA 353042	Glucuronic acid conjugate of CGA 349208 <i>N</i> -Acetylcysteine conjugate of CGA 349208 3,6-Dihydro-3-methyl-2H-1,3,5-oxadiazin-4-amine	Mouse Mouse Rat	

Table 6 (continued)

Code number	IUPAC name	Found in	Structure
CGA 353968 3U	1-(2-Chlorothiazol-5-ylmethyl)-3-methylurea	Rat, goat	
CGA 355190 19U	3-(2-Chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-one	Rat, goat, hen	
CGA 359683	2-Chlorothiazole-5-carboxylic acid	Mouse, rat, goat	
NOA 402988 14U	2-Methylsulfanyltiazole-5-carboxylic acid	Rat, goat, hen	
NOA 404617 15U	1-(2-Chlorothiazol-5-ylmethyl)-3-nitrourea	Rat, goat, hen	
NOA 405217	<i>N</i> -Nitro- <i>N'</i> -methylguanidine	Rat, goat, hen	
NOA 407475	3-(2-Chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylideneamine	Rat, goat, hen	
NOA 408445	[(2-Chlorothiazole-5-carbonyl)-amino]-acetic acid	Rat	
NOA 421275	<i>N</i> -(2-Chlorothiazol-5-ylmethyl)- <i>N'</i> -methylguanidine	Rat, goat, hen	
NOA 421276	<i>N</i> -(2-Chlorothiazol-5-ylmethyl)-guanidine	Rat, goat	
4U 8U 13U	(2-Chlorothiazol-5-ylmethyl)-urea	Rat, goat, hen	

Table 6 (continued)

Code number	IUPAC name	Found in	Structure
5U	2-Acetylamino-3-(2-chlorothiazol-5-ylmethanesulfinyl)-propionic acid	Rat	
6U	2-Acetylamino-3-(2-chlorothiazol-5-ylmethylsulfanyl)-propionic acid	Rat	
16U	2-Acetylamino-3-[5-(N'-methyl-N''-nitroguanidinomethyl)-thiazol-2-ylsulfanyl]-propionic acid	Rat	
17U	2-Acetylamino-3-[5-(5-methyl-4-oxo-[1,3,5]-oxadiazinan-3-ylmethyl)-thiazol-2-ylsulfanyl]-propionic acid	Rat	
L14	2-Oxopropionic acid [3-(2-chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene]-hydrazide	Rat, goat	
MU3	Amino-([(2-chlorothiazol-5-ylmethyl)-amino]-methylene)-hydrazide	Rat, hen	
MU12	2-Oxopropionic acid ([3-(2-chlorothiazol-5-ylmethyl)-amino]-methylamino-methylene)-hydrazide	Goat	
N1, mouse R14	3-(2-Chlorothiazol-5-ylmethyl)-[1,3,5]-oxadiazinan-4-ylideneamine	Mouse, goat	

Table 6 (continued)

Code number	IUPAC name	Found in	Structure
N5	Acetic acid [3-(2-chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene]-hydrazide	Goat	
R6	2-Acetylamino-3-[5-(5-methyl-4-nitroimino-[1,3,5]oxadiazinan-3-ylmethyl)-thiazol-2-ylsulfanyl]-propionic acid	Mouse, rat	

From Briswalter (1999, 2002a,b,c); Mewes (2000)

Both the thiazole and oxadiazine moieties are susceptible to oxidative attack. These minor pathways proceed rapidly to small molecules, which may, at least partially, enter the general metabolism. As described previously (Müller & Stampf, 1996), a small but measurable amount of radioactivity was exhaled after administration of either label, most probably as carbon dioxide. The small metabolites resulting from thiazole and oxadiazine ring degradation also contribute to the metabolite complexity found in urine.

The degradation resulted in metabolites that, together with unchanged thiamethoxam, were eliminated very rapidly. The administered dose was almost completely absorbed and was degraded partially and eliminated almost completely via urine. Excretion via bile and ultimately via faeces was of very minor importance. Enterohepatic circulation was negligible.

1.3 Comparison of excretion and metabolism in mice and rats

In toxicity studies, described below, in which thiamethoxam was administered to rats and mice, a clear species difference was observed with respect to hepatotoxicity and the incidence of liver tumours. The latter was observed only in mice. Consequently, in an effort to understand this species difference, several metabolism studies were conducted in mice in order to compare the metabolic rate and fate of thiamethoxam in mice with those in rats.

There was about a 4-fold increased faecal excretion of radioactivity in mice (Mewes, 1998) compared with rats in the kinetic study (Müller & Stampf, 1996), revealing a species difference in the routes of excretion. About 19% of the total administered radioactivity was found in the mouse faeces, whereas only 5.1% of the dose was recovered in the rat faeces. The data are summarized in Table 7.

As the relative amounts excreted in mouse urine and faeces did not change markedly over the treatment period, it is likely that the different excretion patterns can be attributed to differences in the species rather than in the dose regimens. Of the daily administered thiamethoxam dose, 30–60% was metabolized and excreted in mice, whereas only 20–30% of the dosed thiamethoxam was metabolized and excreted in rats.

Identified metabolites were quantified in urine and faecal samples collected over 0–24 hours from rats receiving 91 mg/kg bw [thiazol-2-¹⁴C]thiamethoxam on a single occasion and compared with samples collected over 0–24, 72–96, 144–168, 216–240, 288–312 and 360–384 hours from mice receiving 118 mg/kg bw [thiazol-2-¹⁴C]thiamethoxam per day for 14 days. The 360- to 384-hour collection represented the collection from the last dose, which was administered at 312 hours.

Table 7. Species differences in metabolism of thiamethoxam in rats and mice

	Excretion (% of total dose)	
	Rat ^a	Mouse ^b
	Single oral, 91.2 mg/kg bw	Multiple oral, 14 × 118.2 mg/kg bw
Urine	95.49	71.75
Faeces	5.14	18.84
Expired air	0.18	0.06 ^c
Cage wash	0.18	3.81
Tissue residues	0.29	Not determined
Total recovery	101.28	94.46

From Müller & Stampf (1996); Mewes (1998)

^a Data are taken from Müller & Stampf (1996).

^b Data are from Mewes (1998).

^c Collection period 0–72 h after the first administration.

In urine, unchanged thiamethoxam represented 73% of the dose to rats, whereas it represented 33–41% of the dose to mice at the five collection times covering 0–312 hours. The metabolite CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] represented 12% of the dose to rats and about the same, 8–12%, of the dose to mice at the five successive collection times. Only for the metabolite CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] was there a marked difference in urinary representation, this being 1.9% of the dose to rats and 9.4–17.9% in mice. All other identified metabolites found in urine represented less than 0.9% of the dose in both rats and mice. There was, however, unresolved polar material that represented 0.3% of the dose to rats and between 4.7% and 8.1% of the dose to mice.

In faeces, unchanged thiamethoxam represented 1.2% of the dose to rats, whereas in mice, at the successive collection times, it represented 0.9%, 1%, 3.5%, 7.8% and 4.5% of the dose to mice. As there was but a single collection time from rats, it is not known whether the higher proportion of the administered dose found in mice at the three later collection times represents a true difference from rats or not. Similarly, the metabolite CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] represented just 0.2% of the dose to rats and 0.3% of the dose to mice in faeces collected over 0–24 hours, whereas it tended to increase as a proportion of the administered dose at the successively later times to 0.5%, 1.7%, 3.2% and 1.8%. However, there did appear to be a species difference in the proportion of CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine], which represented just 0.04% of the dose in rats, but 0.59% in mice, over the 0- to 24-hour sampling time. At the successively later sampling times for mice, it represented 1.9%, 3.9%, 6.3% and 3.3% of the dose. The only other metabolite for which there was a substantial species difference, even when comparing the 0- to 24-hour sampling times, was R6 [2-acetylamino-3-[5-(5-methyl-4-nitroimino-[1,3,5]oxadiazinan-3-ylmethyl)-thiazol-2-ylsulfanyl]-propionic acid], which was not detected in rats but represented 2.15% of the dose to mice. This proportion in mice was maintained over the later sampling times, when it varied between 1.9% and 2.6%. However, eight other metabolites were also not detected in rat faeces but occurred in measurable amounts in mouse faeces, accounting for up to 0.8% of the administered dose (although more usually at much lower levels).

Comparisons of rats and mice are also possible from the results of three later studies (Briswalter, 2002a,b,c). First, aspects of these studies will be summarized.

Briswalter (2002a) studied the metabolism of [oxadiazin-4-¹⁴C]thiamethoxam in mice after oral administration at a single oral dose of 0.5 or 100 mg/kg bw with a special emphasis on metabolite identification. The excretion and tissue distribution of thiamethoxam were investigated in a

Table 8. Summary of kinetic data following administration of a single oral dose of [oxadiazin-4-¹⁴C]thiamethoxam at 106.5 mg/kg bw to male rats

Parameter	Total ¹⁴ C residues	Thiamethoxam	CGA 322704 [<i>N</i> -(2-chlorothiazol-5-ylmethyl)- <i>N'</i> -methyl- <i>N''</i> -nitroguanidine]	CGA 265307 [<i>N</i> -(2-chlorothiazol-5-ylmethyl)- <i>N'</i> -nitroguanidine]
<i>C</i> _{max} (ppm)	50	41	8	0.6
<i>T</i> _{max} (h)	6	6	6	6
<i>t</i> _½ (h)	3	2	4	8
AUC _{0–24 h} (µg·h/g)	581	467	92	8

From Briswalter (2002b)

*C*_{max}, maximum concentration in plasma; *t*_½, half-life of elimination from plasma; *T*_{max}, time to reach the maximum concentration in plasma

previous, oral, multiple-dose study (Briswalter, 2002d) from which samples were used during this study. Approximately 72–73% of the administered dose was excreted in urine and a further 14–20% was excreted in faeces at the low dose level, and 82–90% and 11–15% were excreted in the urine and faeces, respectively, at the high dose level.

In urine, unchanged thiamethoxam and the metabolites CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] and CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] were identified and represented the majority of the radioactivity in urine, accounting for 25–41%, 10–16% and 13–19% of the dose, respectively. Two minor metabolites—M010 (*N*-formyl-*N'*-hydroxyurea) and M011 (*N*-formyl-*N'*-(hydroxymethyl)-urea)—accounted for 5–8% and 1–3% of the dose, respectively. Metabolites M012 [*N'*-formylureidoacetic acid] and CGA 330050 [3-(2-chlorothiazol-5-ylmethyl)-[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine] were detected in trace amounts.

The faecal extracts contained the same metabolites as urine. Unchanged thiamethoxam accounted for 2–4% of the dose, and metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine], CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine], M010 (*N*-formyl-*N'*-hydroxyurea) and M011 [*N*-formyl-*N'*-(hydroxymethyl)-urea] accounted for 1–3%, 2–6%, 1–2% and 0.1–0.6% of the dose, respectively.

Briswalter (2002b) studied the blood kinetics of thiamethoxam and its metabolites in male rats after a single oral administration of [oxadiazin-4-¹⁴C]thiamethoxam at a dose of 107 mg/kg bw. Total residue levels in blood reached a maximum 6 hours after dosing of 50 µg of thiamethoxam equivalents per gram and declined rapidly thereafter. The half-life of elimination of total radioactivity in blood was 3 hours.

The radioactivity in the blood of rats was almost quantitatively extractable with acetonitrile. Thiamethoxam was the major component detected in blood, followed by CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine]. Only trace amounts of CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] were found. As for the total radioactive residues, the maximum levels of thiamethoxam and metabolites in blood were reached 6 hours after dosing and declined rapidly thereafter. The maximum concentration of thiamethoxam in blood was 41 µg/g, with a half-life of elimination of 2 hours. The maximum concentrations of the metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine] and CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] in blood were 8 µg/g and 0.6 µg/g, respectively, with half-lives of 4 hours and 8 hours, respectively (Table 8).

Following administration of a single oral dose of [oxadiazin-4-¹⁴C]thiamethoxam at a mean dose of 105.7 mg/kg bw to male mice (Briswalter, 2002c), radioactivity was rapidly absorbed into the systemic circulation. Total residue levels in blood reached a maximum 0.5 hour after dosing of 41 µg

Table 9. Summary of toxicokinetic data following administration of a single oral dose of [oxadiazin-4-¹⁴C]thiamethoxam at 105.7 mg/kg bw to male mice

Parameter	Total ¹⁴ C residues	Thiamethoxam	CGA 322704 [<i>N</i> -(2-chlorothiazol-5-ylmethyl)- <i>N'</i> -methyl- <i>N''</i> -nitroguanidine]	CGA 265307 [<i>N</i> -(2-chlorothiazol-5-ylmethyl)- <i>N'</i> -nitroguanidine]	CGA 330050 [3-(2-chlorothiazol-5-ylmethyl)-[1,3,5]oxadiazinan-4-ylidene- <i>N</i> -nitroamine]
$C_{\max}$ (ppm)	41	32	6	6	4
$T_{\max}$ (h)	0.5	0.5	2	2	2
$t_{1/2}$ (h)	4	3	3	3	3
AUC _{0-24 h} (µg·h/g)	277	122	39	66	28

From Briswalter (2002c)

$C_{\max}$, maximum concentration in plasma; $t_{1/2}$, half-life of elimination from plasma; $T_{\max}$, time to reach the maximum concentration in plasma

thiamethoxam equivalents per gram and declined rapidly thereafter. The half-life of elimination of total radioactivity in blood was 4 hours (Table 9).

The radioactivity in the blood of mice was almost quantitatively extractable, with the exception of a subgroup sampled 24 hours after dose administration. Thiamethoxam was the major component detected in blood. Lower amounts of the metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine], CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] and CGA 330050 [3-(2-chlorothiazol-5-ylmethyl)-[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine] were also detected in blood.

Of the radioactive residues identified, the maximum level of thiamethoxam in blood was reached 0.5 hour after dosing and declined rapidly thereafter. The maximum concentration of thiamethoxam in blood was 32 µg/g, with a half-life of elimination of 3 hours. The metabolites CGA 322704 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-methyl-*N''*-nitroguanidine], CGA 265307 [*N*-(2-chlorothiazol-5-ylmethyl)-*N'*-nitroguanidine] and CGA 330050 [3-(2-chlorothiazol-5-ylmethyl)-[1,3,5]oxadiazinan-4-ylidene-*N*-nitroamine] reached maximum concentrations in blood of 4–6 µg/g after 2 hours and had half-lives of elimination of 3 hours.

This study was followed by one in which [oxadiazin-4-¹⁴C]thiamethoxam was administered to male and female mice by gavage at a nominal dose of 0.5 or 100 mg/kg bw. Urine and faeces were collected over a number of periods spanning 0–72 hours after dosing, and tissues were collected 72 hours after dosing.

Absorption of [oxadiazin-4-¹⁴C]thiamethoxam from the gastrointestinal tract into the systemic circulation amounted to approximately 76% at the low dose level and about 88% at the high dose level, based on the recovery of radioactivity in urine, cage wash, tissues and carcass. The metabolite profiles were similar in urine and faeces (Briswalter, 2002a), and it was concluded that the portion of the dose excreted in faeces represents absorbed thiamethoxam excreted in the bile.

The routes and rates of elimination were independent of dose level and sex. Following oral administration of [oxadiazin-4-¹⁴C]thiamethoxam at both the high and low dose levels, excretion was rapid and almost complete within 24 hours, with most of the radioactivity excreted via urine. At the low dose level, means of 72% (males) and 73% (females) of the dose were excreted in urine, and 20% (males) and 14% (females) of the dose were excreted via faeces. The routes and rates of excretion were similar at the high dose level, with urinary excretion accounting for 82% (males) and 90% (females) of the dose and faecal excretion accounting for 11% (males) and 15% (females) of the dose.

At both the low and high dose levels, radioactive residues remaining in the tissues and carcass 72 hours after dosing were low, accounting for approximately 1% of the dose in total. The distribution

of residues in the tissues and organs was essentially independent of dose level and sex, although tissue residues were slightly higher in female mice than in male mice.

At the low dose level, the highest tissue residue concentrations were found in the liver, amounting to 0.0139 ppm in males and 0.0271 ppm in females. Residues in all other tissues and organs were below 0.0032 ppm. Residues were correspondingly higher at the high dose level, with residues in liver amounting to 2.675 ppm in males and 5.114 ppm in females. Residues did not exceed 0.53 ppm in any other tissues.

Based on the metabolites identified in urine and faeces, the metabolism of thiamethoxam in mice proceeds by the same major pathway as in rats. There are, however, quantitative interspecies differences that deserve mention. Taking into consideration all of the absorption, distribution, metabolism and elimination studies summarized in this document, it can be concluded that about 20–30% of the dose was eliminated as metabolites of thiamethoxam in rats, whereas metabolism was more extensive in mice, with 30–60% of the dose appearing as metabolites of thiamethoxam. The predominant metabolites in rats were CGA 322704 and CGA 265307, which accounted for 10% and 1% of the dose, respectively, whereas the remaining 20 metabolites were below 1% of the dose. These same two metabolites, CGA 322704 and CGA 265307, were dominant in mice, accounting for about 20% and 15% of the dose, respectively. The $AUC_{0-24\text{ h}}$ for CGA 265307 was significantly greater in mice than in rats (66 versus 8 $\mu\text{g}\cdot\text{h/g}$ blood). These species differences will be of particular interest in considering rat–mouse differences in neoplastic developments.

2. Toxicological studies

2.1 Acute toxicity

(a) Oral administration

In an acute oral toxicity study conducted according to Organisation for Economic Co-operation and Development (OECD) guideline 401 (1987), groups of five male and five female fasted Sprague-Dawley (Crj:CD SD) rats were each administered thiamethoxam (batch No. P.506006; purity 98.6% weight per weight) in 0.5% weight per volume (w/v) aqueous methylcellulose at a dose volume of 20 ml/kg bw by gavage as a single oral dose of 0, 900, 1500, 2300, 3800 or 6000 mg/kg bw. Mortality, clinical signs and body weight changes were recorded. Autopsies were performed on rats at termination on day 14.

Deaths occurred in both sexes at dose levels of 1500 mg/kg bw and above between 2 and 6 hours after dosing. In most instances, tonic convulsions preceded death. All rats died in the two highest dose groups, four of five of each sex at 2300 mg/kg bw and three of five of each sex at 1500 mg/kg bw. Ptosis occurred in all treated groups 1 hour after dosing, and survivors of both sexes treated at 1500 mg/kg bw and above showed reduced locomotor activity on the day of treatment. Clinical observations on the surviving rats returned to normal on the following day. Body weight loss or retarded weight gain was recorded for 2 days after dosing, after which body weight gain was normal. Autopsy and postmortem examination did not reveal any treatment-related abnormalities in either the rats that died or the survivors.

The acute oral median lethal dose (LD_{50}) of thiamethoxam in male and female rats was 1563 mg/kg bw (Oda, 1996a).

In an acute oral toxicity study conducted according to OECD guideline 401 (1987), groups of five male and five female fasted SPF (Crj:CD1(ICR) strain) mice were each administered thiamethoxam (batch No. P.506006; purity 98.6%) in 0.5% w/v aqueous methylcellulose at a dose volume of 20 ml/kg bw by gavage as a single oral dose of 0, 500, 700, 1000, 1400 or 2000 mg/kg bw. Mortality,

clinical signs and body weight changes were recorded. Autopsies were performed on mice at termination on day 14.

Deaths occurred in both sexes at dose levels of 700 mg/kg bw and above between 15 minutes and 24 hours after dosing. All mice died in the highest dose group, all males and four of five females at 1400 mg/kg bw, four of five males and three of five females at 1000 mg/kg bw and two of five males and one of five females at 700 mg/kg bw. Reduced locomotor activity or prostration occurred in all thiamethoxam-treated mice within 5–15 minutes of treatment, and clonic convulsions occurred 15 minutes to 4 hours after treatment. Clinical observations on the surviving mice returned to normal on the following day. The body weight development of all male treated groups was unaffected throughout the observation period, but the body weight gain of all female treated groups was slightly reduced on the day following dosing. Thereafter, body weight development of female groups returned to normal. Autopsy and postmortem examination did not reveal any treatment-related abnormalities in either the mice that died or the survivors.

The acute oral LD₅₀ of thiamethoxam in mice was 783 mg/kg bw (95% confidence interval 619–1000 mg/kg bw) in males, 964 mg/kg bw (729–1271 mg/kg bw) in females and 871 mg/kg bw (735–1028 mg/kg bw) in both sexes combined (Oda, 1996b).

(b) *Dermal application*

In an acute dermal toxicity study conducted according to OECD guideline 402 (1987), five male and five female Sprague-Dawley (Crj:CD strain) rats were administered thiamethoxam (batch No. P.506006; purity 98.6%) applied to the closely clipped dorsum at a dose level of 0 or 2000 mg/kg bw. The applied thiamethoxam was covered by an occlusive dressing for 24 hours. Local or systemic signs of reaction to treatment were recorded during a subsequent 14-day period of observation. All rats were killed on day 15 and subjected to gross postmortem examination.

There were no deaths or clinical signs of reaction to treatment in any rat throughout the study. There was no evidence of a dermal response to treatment throughout the study. Body weight development was unaffected by treatment; a slight reduction in body weight was observed on the day following application in both control and treated groups. Autopsies performed on day 14 did not reveal any gross abnormalities.

The acute lethal dermal dose (LD₅₀) of thiamethoxam in rats was greater than 2000 mg/kg bw (Oda, 1996c).

(c) *Exposure by inhalation*

In an acute inhalation study conducted according to OECD guideline 403 (1981), groups of five male and five female Sprague-Dawley (CrI:CD strain) rats were exposed (nose only) for 4 hours to a dust aerosol of thiamethoxam (batch No. P.506006; purity 98.6%) at a nominal concentration of 10.9 or 56.6 g/m³. The rats were observed for 14 days after exposure, then killed and autopsied. Parameters monitored included mortality, clinical signs and weekly body weights.

The characteristics of the achieved atmosphere were, respectively, mean achieved analytical atmospheric concentrations of 1.02 ± 0.05 and 3.72 ± 0.73 g/m³; and mean mass aerodynamic diameters (MMAD) of 5.1 ± 0.3 and 5.6 ± 0.1 µm. The percentages of particles less than 7.07 µm were 67.5% and 66.9%, respectively. Higher atmospheric concentrations were not technically achievable.

There was no mortality and there were no treatment-related clinical signs of toxicity during the exposure and observation periods. Soiled fur in the nasorostral region was observed to a slight degree in all rats of both groups on the day of exposure, but this was considered to be related to the procedure rather than to thiamethoxam. Rats recovered to appear normal on the first day (low dose) and third day (high dose) after exposure. Normal body weight gain was noted during the study in the low-dose rats and in all high-dose rats except for two females. These showed slight decreases in

body weight on day 7, but had recovered by day 14. Autopsies performed on day 14 did not reveal any gross abnormalities.

The 4-hour acute inhalation median lethal concentration (LC_{50}) of thiamethoxam in rats was greater than 3.72 g/m^3 , the highest technically achievable concentration (Shutoh, 1996).

(d) *Dermal irritation*

In a dermal irritancy study conducted according to Japanese Ministry of Agriculture, Forestry and Fisheries guideline 4200 and OECD guideline 404 (1992), six female Japanese White rabbits were exposed to 0.5 g of thiamethoxam (batch No. P.506006; purity 98.6%) under a semioclusive dressing on the closely clipped dorsal skin for 4 hours. Dermal reactions were assessed at 1, 24, 48 and 72 hours after removal of the dressing. Dermal irritation was graded according to the Draize scoring method.

No dermal irritation was observed during the study. The mean irritation score over 24–72 hours was 0 for erythema and 0 for oedema. Thiamethoxam was not irritating to rabbit skin (Shibata, 1996a).

(e) *Ocular irritation*

In an ocular irritation study conducted according to OECD guideline 405 (1992), nine female Japanese White rabbits were exposed to 0.1 g of thiamethoxam (batch No. P.506006; purity 98.6%) instilled into the conjunctival sac of the left eye on day 1. Ocular reactions were assessed at 1, 24, 48 and 72 hours and 7 days after treatment. The eyes of three rabbits were rinsed 2–3 minutes after instillation. Twenty-four hours after application, a drop of 2% fluorescein (sodium salt) solution was instilled into the eyes and immediately rinsed with water in order to assist in the recognition of corneal damage. Ocular irritation was graded according to the Draize scoring method.

All rabbits survived, and there were no clinical signs of toxicity. Minimal (grade 1) erythema and oedema of the conjunctivae occurred at 1 hour in the unwashed eye of all rabbits and in the washed eye of two of three rabbits. The other rabbit in the washed group developed minimal conjunctival oedema only. Transient eye closure and discharge were observed in all rabbits immediately after application. No signs of eye irritation were present at 24, 48 and 72 hours in unwashed or washed eyes.

Thiamethoxam was transiently minimally irritating to the rabbit eye (Shibata, 1996b).

(f) *Dermal sensitization*

In a skin sensitization study conducted according to Japanese Ministry of Agriculture, Forestry and Fisheries guideline 4200 and OECD guideline 406 (1992), skin hypersensitivity in guinea-pigs exposed to thiamethoxam (batch No. P.506006; purity 98.6%) was assessed by the Magnusson & Kligman maximization method. Based on the findings of a preliminary study, the closely clipped dorsa of groups of 10 male and 10 female Pirbright White guinea-pigs were given, on day 1, 0.1 ml intradermal injections of 1% thiamethoxam in peanut oil, 1% thiamethoxam in Freund's complete adjuvant/saline or adjuvant/saline alone (test groups); 0.5% mercaptobenzothiazole in peanut oil alone or 0.5% mercaptobenzothiazole in adjuvant/saline alone (positive control groups); or peanut oil alone, adjuvant/saline mixture or adjuvant/saline mixture with peanut oil (negative control groups). One week later, the same areas of skin were treated by topical application of 30% w/v thiamethoxam in petroleum jelly, 50% mercaptobenzothiazole in petroleum jelly or petroleum jelly alone, respectively, and covered by an occlusive dressing for 48 hours. Two weeks after the topical induction, all guinea-pigs were challenged by occluded application of 10% thiamethoxam in petroleum jelly (test and negative control groups) or 10% mercaptobenzothiazole in petroleum jelly. The occlusive dressings were removed on the following day, and the condition of the test sites was assessed approximately 24 and 48 hours later and scored according to the Draize scale.

A skin reaction (erythema reaction with Draize score 1) was observed in one male (corresponding to a reaction rate of 5%) from the test group guinea-pigs after 48 hours only. No irritant skin reactions occurred in the negative control group. In contrast, the positive control group showed positive reactions (Draize scores 1–3) in 17 of 20 animals after 24 and 48 hours, corresponding to a sensitization rate of 85%. No effect on body weights occurred during the experimental period.

Thiamethoxam produced a skin reaction incidence of 5% and on this basis is judged not to be a skin sensitizer in this guinea-pig Magnusson & Kligman test (Winkler, 1995).

2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

In a 90-day dietary toxicity study in mice performed according to OECD guideline 408 (1987), groups of 10 male and 10 female mice (Tif:MAGf, SPF strain) were administered thiamethoxam (batch No. KI-4654/18; purity 98.4%) in the diet at a nominal concentration of 0, 10, 100, 1250, 3500 or 7000 ppm, providing measured intakes equal to 0, 1.41, 14.3, 176, 543 and 1335 mg/kg bw per day for males and 0, 2.01, 19.2, 231, 626 and 1163 mg/kg bw per day for females. Mortality and clinical signs were checked daily, and feed/water consumption and body weight were recorded weekly. Haematology was performed on all animals at the end of the treatment period. All mice were killed at termination and subjected to postmortem examination, selected organs were weighed and a range of tissues was taken, fixed and examined microscopically.

Preliminary diet analyses demonstrated the stability at room temperature of thiamethoxam in diet. Analysis of representative test diets demonstrated achieved concentrations to be in the range 94.2–101% of nominal concentrations.

No treatment-related deaths occurred during the study, but one male at 7000 ppm died during blood sampling. Clinical signs of an adverse effect of treatment were restricted to transient respiratory sounds, without dyspnoea, at 3500 and 7000 ppm. Body weight gain was markedly reduced in males and to a lesser extent in females at 7000 ppm and slightly reduced in males at 3500 ppm. In females, feed consumption was reduced at 7000 ppm and to a lesser extent at 3500 ppm. It was not possible to evaluate any effect on feed consumption in the males due to spillage.

Trend tests on the haematological data indicated anaemia associated with a tendency to hyperchromasia and macrocytosis manifest as significant reductions in erythrocyte count, haemoglobin concentration and haematocrit in males and females of the 7000 ppm group. In addition, in females of the 1250, 3500 and 7000 ppm groups, there were minimal, but statistically significant, elevations in platelet numbers.

No treatment-related changes were observed at autopsy. Statistically significant increases were recorded in absolute and relative liver weights of female mice at 3500 and 7000 ppm and in the relative liver weight of male mice at 7000 ppm (Table 10). Ovary and spleen weights of females were significantly reduced at 3500 and 7000 ppm. Marked depressions of carcass weight at 7000 ppm in both males (32.5%) and females (14.9%) were associated with changes in the absolute and/or relative weights of the heart, liver, kidneys, adrenal glands, thymus (males only), gonads, spleen and thyroid gland, but in most cases there were no histological correlates for these changes. Histological correlates were identified for the liver and ovarian weight changes. These consisted of hepatocellular hypertrophy, single-cell necrosis, pigment accumulation in Kupffer cells and an increase in lymphocytic infiltration of the liver. Ovarian weight loss was associated with atrophy.

Table 10. Organ weight changes in mice treated with thiamethoxam in the diet for 90 days

	Organ weight											
	Male						Female					
	Dietary concentration (ppm)											
	0	10	100	1250	3500	7000	0	10	100	1250	3500	7000
Carcass (g)	47.48	47.76	46.62	48.61	45.30	32.01* ⁻	30.10	30.84	30.58	31.89	30.26	25.63*
Liver (g)												
- Absolute	2.774	2.636	2.502	2.641	2.738	2.138	1.787	1.698	1.732	2.005	2.108 ⁺	1.822 ⁺
- Relative ^a	58.13	55.13	53.40	54.26	60.32	66.85 ⁺	59.36	55.15	56.70	62.82	69.57*	71.12*
Ovary (mg)												
- Absolute	—	—	—	—	—	—	48.10	50.07	41.26	43.21	38.71	31.64* ⁻
- Relative ^a	—	—	—	—	—	—	1.598	1.632	1.354	1.354	1.288 ⁻	1.235 ⁻
Spleen (g)												
- Absolute	0.093	0.090	0.087	0.092	0.88	0.097	0.097	0.098	0.104	0.092	0.087	0.085 ⁻
- Relative ^a	1.961	1.886	1.868	1.896	1.936	3.105	3.216	3.180	3.398	2.904	2.874	3.406

From Bachmann (1996a)

* $P < 0.01$ (Lepage); -/+ negative/positive trend (Jonckheere)^a Relative to body weight.**Table 11. Significant microscopic changes in mice treated with thiamethoxam in the diet for 90 days**

	Incidence of finding											
	Male						Female					
	Dietary concentration (ppm)											
	0	10	100	1250	3500	7000	0	10	100	1250	3500	7000
<i>No. examined</i>	10	10	10	10	10	10	10	10	10	10	10	10
Liver												
Lymphocytic infiltration	0	0	1	1	4	5	1	1	0	0	5	3
Fatty change	9	8	4	9	10	5	9	9	8	10	9	10
Kupffer cell pigment	0	0	0	0	1	10	0	0	0	0	2	9
Hepatocyte: necrosis	0	0	0	0	0	8	0	0	0	0	3	10
Hepatocyte: hypertrophy	0	0	8	9	10	10	0	0	0	2	10	10
Ovaries												
Atrophy	—	—	—	—	—	—	0	0	1	1	5	10

From Bachmann (1996a)

Minimal to marked hypertrophy of centrilobular hepatocytes, minimal pigmentation of Kupffer cells and an increased incidence of minimal lymphocytic infiltration of the parenchyma were present in the livers of males and females at 3500 and 7000 ppm (Table 11). At 7000 ppm, minimal to moderate necrosis of single hepatocytes was also present. Both sexes at 1250 ppm also showed minimal centrilobular hypertrophy. Males at 100 ppm showed minimal hypertrophy as an isolated change in the liver, without the occurrence of other progressive hepatic alterations noted at higher dose levels. The ovaries of females at 3500 and 7000 ppm showed minimal to moderate atrophy in the form

of reduced numbers of corpora lutea. These effects on the ovary are most likely due to the growth retardation seen at these dose levels, rather than a direct effect of thiamethoxam. There were no other treatment-related morphological findings (Bachmann, 1996a).

The no-observed-adverse-effect level (NOAEL) in the 90-day dietary study in mice with thiamethoxam was 100 ppm, equal to a dose level of 14.3 mg/kg bw per day (males) and 19.2 mg/kg bw per day (females), based on the finding of liver hypertrophy at 1250 ppm, equal to 176 mg/kg bw per day in males, and liver hypertrophy and raised platelet counts at 1250 ppm, equal to 231 mg/kg bw per day in females. A minimal hepatocyte hypertrophy was observed in males only at 100 ppm, but in the absence of any other hepatic changes, this was considered an adaptive response.

Rats

In an oral study of toxicity, groups of five male and five female Tif:RAIf (SPF) rats were administered thiamethoxam (batch No. KGL4654/12; purity 95.0%) in the diet at a dose level of 0, 100, 1000, 2500 or 10 000 ppm, equal to 0, 8.0, 81.7, 198.6 and 710.6 mg/kg bw per day in males and 0, 8.7, 89.3, 210.6 and 762.6 mg/kg bw per day in females, for 28 consecutive days. Mortality and clinical signs were checked daily, and feed/water consumption and body weight were recorded weekly. Haematological, blood chemistry and urinalysis investigations were performed on all rats towards the end of the treatment period. All rats were killed at termination and subjected to postmortem examination, selected organs were weighed and a range of tissues was taken, fixed and examined microscopically. In a separate investigation, measurement of replicating deoxyribonucleic acid (DNA) synthesis was performed on liver samples from the male animals. The results of this separate investigation are presented below (see [Table 54](#)).

Preliminary analysis of diets demonstrated the stability and homogeneity of the distribution of thiamethoxam. Analysis of test diets demonstrated achieved concentrations to be in the range 98–104% of nominal concentrations.

No deaths occurred and no clinical signs of a reaction to treatment were evident during the administration period. Body weight gain was reduced by 29% in males at 10 000 ppm, but females at this level were not affected. Minimal retardation of body weight gain occurred in males at 2500 ppm. Feed consumption was markedly reduced at 10 000 ppm in males throughout the treatment period, but in females, it was only slightly reduced during the first week.

There were no treatment-related effects on haematological profiles at dose levels up to and including 10 000 ppm. Isolated statistically significant differences from the controls, having no relationship to dose, are considered incidental to treatment. Platelet counts at 10 000 ppm were higher in both males (23%) and females (18%), but remained within the historical control range and were considered to be of no toxicological significance. Plasma cholesterol concentrations were significantly higher in males of the 1000 ppm (22%), 2500 ppm (26%) and 10 000 ppm (43%) groups and in females of the 10 000 ppm (70%) group. Other treatment-related effects were confined to this 10 000 ppm group and are considered to be minor consequences of renal and hepatic morphological changes. The statistically significant plasma changes at the highest dose consisted of a 35% increase in aspartate aminotransferase (AST) activity in males but not in females, a 6% reduction in albumin concentration in males but not in females, a 20% increase in urea level in females (and a non-significant 19% increase in males) and very small, yet significant, reductions in sodium concentrations of 1.7% in males and 0.5% in females. There were no effects on any urinary physiological parameters or on cellular and chemical constituents.

Treatment-related effects on organ weights occurred in both males and females at 1000 ppm and above ([Table 12](#)). At 10 000 ppm, absolute liver weights were increased in females and relative (to body weight) liver weights were increased in males and females. Relative kidney weights were increased at 2500 ppm in males, whereas in females, both absolute and relative kidney weights were increased at 1000 ppm, but not at 2500 ppm, and only relative kidney weights were increased at

Table 12. Organ weights in rats treated with thiamethoxam in the diet for 28 days

Parameter	Dietary concentration (ppm)				
	0	100	1000	2500	10 000
Males					
Body weight (g)	314.2	314.7	316.4	303.6	250.1* ⁻
Liver					
- Absolute (g)	14.95	14.92	14.87	14.89	16.07
- Relative (% body weight × 10)	47.54	47.47	47.00	49.02	64.28* ⁺
Kidneys					
- Absolute (g)	2.260	2.359	2.470	2.524	1.918
- Relative (% body weight × 10)	7.209	7.464	7.812	8.306* ⁺	7.670
Adrenals					
- Absolute (mg)	81.46	73.86	72.02	78.18	68.38*
- Relative (% body weight × 10)	0.261	0.236	0.227	0.256	0.274
Thymus					
- Absolute (mg)	712.0	643.8	675.9	598.5	541.1*
- Relative (% body weight × 10)	2.263	2.027	2.133	1.974	2.166
Females					
Body weight (g)	199.2	211.7	215.9	211.5	184.4
Liver					
- Absolute (g)	8.476	9.285	9.695	8.969	10.41*
- Relative (% body weight × 10)	42.42	43.77	44.87	42.39	56.37*
Kidneys					
- Absolute (g)	1.668	1.810	1.984* ⁺	1.826	1.757
- Relative (% body weight × 10)	8.390	8.536	9.204* ⁺	8.658	9.575*
Adrenals					
- Absolute (mg)	85.46	88.72	93.60	90.96	93.22
- Relative (% body weight × 10)	0.428	0.420	0.435	0.433	0.506
Thymus					
- Absolute (mg)	448.2	484.1	469.5	508.0	426.6
- Relative (% body weight × 10)	2.255	2.291	2.178	2.397	2.300

From Bachmann (1995)

* $P < 0.05$ (Wilcoxon); -/+ negative/positive trend (Jonckheere)

10 000 ppm. Other statistically significant organ weight changes were reductions in absolute adrenal and thymus weights of male but not female rats at 10 000 ppm.

At autopsy, dilatation of the renal pelvis was observed in two male rats of the 10 000 ppm group, but no other treatment-related gross lesions were recorded. Treatment-related microscopic changes were observed in males treated at 1000 ppm and above and in females at 2500 ppm and above (Table 13). The organs affected were liver, kidneys, adrenal glands and thyroid glands. Minimal to marked hypertrophy of centrilobular hepatocytes occurred particularly in males, but also in females, at 2500 and 10 000 ppm. Glycogen depletion was observed in males at 10 000 ppm, but it is not clear whether other hepatic lesions recorded were the result of treatment (i.e. glycogen depletion in females and cholangiofibrosis in males and females). In the kidneys, minimal to moderate hyaline change to the tubule epithelium occurred in males at 1000 ppm (3/5) and 2500 ppm (5/5), but not at

Table 13. Selected histopathology from rats treated with thiamethoxam for 28 days

Organ/finding	Incidence of finding									
	Males					Females				
	Dietary concentration (ppm)									
	0	100	1000	2500	10 000	0	100	1000	2500	10 000
<i>Number of animals</i>	5	5	5	5	5	5	5	5	5	5
Liver										
Intrahepatic bile duct: cholangiofibrosis	2	0	1	1	2	0	0	0	0	2
Hepatocytes: glycogen deposition	4	3	2	3	0	2	2	0	1	0
Hepatocytes: hypertrophy	0	0	0	4	5	0	0	0	1	5
Kidneys										
Cyst	0	0	0	1	0	0	0	0	0	0
Lymphohistiocytic infiltration	0	0	0	1	0	0	0	0	0	0
Acute tubular lesion	0	0	0	2	0	0	0	0	0	0
Renal cortex: calcification	0	0	1	0	0	0	0	0	0	0
Renal tubule: hyaline change	0	0	3	5	0	0	0	0	0	0
Tubular basophilic proliferation	1	4	5	5	2	3	3	3	4	4
Renal pelvis: dilatation	0	0	1	0	3	0	0	0	0	0
Pelvic epithelium hyperplasia	0	0	1	0	1	0	0	0	0	1
Adrenal gland										
Cortical fatty change	0	0	0	0	3	0	0	0	0	2
Thyroid gland										
Follicular epithelial hypertrophy	1	1	1	2	3	0	0	0	0	2

From Bachmann (1995)

any other dose level, including 10 000 ppm, and hyperplasia of the pelvic epithelium occurred in one rat of each sex at 10 000 ppm. However, this latter lesion was also recorded for one male rat of the 1000 ppm group, so its relation to treatment is unclear. The hyaline (eosinophilic) change was later demonstrated to be the outcome of α_2 -urinary microglobulin (α_{2u} -globulin) deposition (see Weber, 2000a,b,c,d). Many of the affected male rats also showed one or more additional kidney lesions (focal calcification, pelvic dilatation, renal cyst, basophilic proliferation, lymphohistiocytic infiltration or an acute tubular lesion), but with little or no evidence of a treatment relationship. A common lesion recorded in both males and females, tubular basophilic proliferation, may be the beginnings of chronic progressive nephropathy development, which is not a toxic response but a naturally occurring pathology specific to rats and with no human counterpart. There was no indication of a dose-response relationship for basophilic proliferation in females, of which the incidences in the 0, 100, 1000, 2500 and 10 000 ppm groups, respectively, were 3/5, 3/5, 3/5, 4/5 and 4/5. In males, the respective incidences were 1/5, 4/5, 5/5, 5/5 and 2/5. In the adrenal cortex, minimal to moderate fatty change mainly of the zona fasciculata was observed in both males and females of the 10 000 ppm group, but in no

other group. In the thyroid gland, the incidence of follicular cell hypertrophy was increased in males, combined with a moderately increased severity, at 10 000 ppm. In females, this observation was also made in two females, with minimal severity. No other treatment-related microscopic lesions were observed (Bachmann, 1995). There was no effect on replicating DNA synthesis in hepatocytes of male rats at dose levels up to 10 000 ppm (Persohn, 1995).

The NOAEL in the 28-day dietary study in rats was 100 ppm, equal to 8.0 mg/kg bw per day, based on increased kidney weights in both sexes (although these were not clearly dose related) and hyaline changes in the renal tubule epithelium of males at a dose level of 1000 ppm, equal to 81.7 mg/kg bw per day. As the latter, male rat-specific kidney effects have no human relevance, they are not considered as contributing to the identification of a relevant NOAEL. The NOAEL of human relevance in the 28-day dietary study in rats was 100 ppm, equal to 8.0 mg/kg bw per day, based on increased plasma cholesterol concentrations at a dose level of 1000 ppm, equal to 81.7 mg/kg bw per day (Bachmann, 1995).

In a 90-day dietary toxicity study, groups of 10 male and 10 female Tif:RAIf strain Sprague-Dawley-derived rats were administered thiamethoxam (batch No. KI-4654/18; purity 98.4%) in the diet at a concentration of 0, 25, 250, 1250, 2500 or 5000 ppm, equal to 0, 1.74, 17.6, 84.9, 168 and 329 mg/kg bw per day in males and 0, 1.88, 19.2, 92.5, 182 and 359 mg/kg bw per day in females, for 13 weeks. Rats were observed daily for clinical signs, and body weight and consumption of feed and water were measured weekly. Eye examinations were conducted before the start and at week 13 of treatment. Haematology, blood chemistry and urinalysis were performed towards the end of the study. At autopsy, the weights of selected organs were recorded for all rats. All major organs and tissues were examined microscopically.

Preliminary diet analyses demonstrated the stability at room temperature of thiamethoxam in diet. Representative analyses of test diets demonstrated achieved concentrations to be in the 94.2–104.6% range of nominal concentrations.

There were no treatment-related deaths or clinical signs of an adverse reaction to treatment, although one female at 1250 ppm died on day 57 with no histopathological treatment-related findings. Ophthalmoscopic examinations revealed no evidence of ocular toxicity. In the groups of male rats, body weight gains were statistically significantly retarded at 1250, 2500 and 5000 ppm by 22%, 19% and 27%, respectively, and the feed consumption in these groups was reduced (non-significantly) relative to the controls by 11%, 7% and 14%, respectively. Weight gains and feed consumption of all female groups and the male groups at dietary concentrations up to 250 ppm were unaffected by treatment. Water consumption in both sexes at 5000 ppm was slightly increased.

The only haematological effect was a 12% increase in the number of circulating platelets in males at 5000 ppm. Other, minimal differences between control and high-dose haematological values that were statistically significant or showed a positive trend were considered to reflect physiological variation, as individual values were within reference ranges. Minor treatment-related effects on blood chemistry occurred in males at 1250 ppm and above and in females at 2500 ppm and above. The effects included higher plasma urea concentrations in both sexes that were increased statistically significantly (24%) in males of the 5000 ppm group and increased plasma creatinine concentrations in males only at 1250 ppm and above, reaching 22% at 5000 ppm. In addition, plasma glucose concentrations were reduced in male rats at 1250 ppm and above, whereas plasma cholesterol concentrations were raised in both males (22%) and females (24%), but only at 5000 ppm. Minimal changes in the electrolyte balance and phosphate levels of both sexes are considered to be of no toxicological consequence. There were no treatment-related effects on urinary physiological parameters or cellular and chemical constituents of urine.

Gross examination at autopsy revealed no treatment-related effects on any tissue or organ examined. There appeared to be no statistically significant changes in the absolute weights of any

organs; however, the relative weights of the adrenals (44%), liver (21%), kidneys (22%), heart (15%) and spleen (27%) were increased in males at 5000 ppm. In male rats at 2500 ppm, there were also marginally increased relative liver (6%) and kidney (11%) weights. There was no histopathological correlation to the increased heart weight of males at 5000 ppm. Treatment-related effects in females were confined to a 16% reduction in thymus weights at 5000 ppm.

On the basis of histopathology, the liver, kidney, spleen and adrenal glands were identified as target organs for thiamethoxam (Table 14). Changes in liver morphology at 2500 and 5000 ppm were minimal to moderate centrilobular hypertrophy and an increased incidence of lymphohistiocytic infiltration of the parenchyma and, in the males only, an increased incidence of cholangiofibrosis of bile ducts. Females at 5000 ppm also showed minimal pigmentation of Kupffer cells. Changes in kidney morphology occurred at 250 ppm and above in males and 2500 ppm and above in females. Lesions observed in males were minimal to marked hyaline change in the tubular epithelium, acute and chronic tubular lesions and an increased incidence of tubular basophilic proliferation at 1250 ppm and above. Increased incidences of pelvic dilatation and epithelial hyperplasia as well as tubular cast formation were also evident. Renal lesions in females were confined to an increased incidence of chronic tubular lesions at 250 ppm and above and an increase in the severity of nephrocalcinosis (which occurred in all females of all groups). The pattern of effects in male rat kidneys at 250 ppm and above is consistent with α_{2u} -globulin nephropathy and chronic progressive nephropathy. Immunohistochemical studies have demonstrated the occurrence of α_{2u} -globulin nephropathy following thiamethoxam treatment (Weber, 2000b; see Table 54 below). α_{2u} -Globulin nephropathy is generally accepted to be unique to male rats, and male rats also have a tendency to develop chronic progressive nephropathy more than female rats. Neither process has any relevance in human risk assessment. There was an increased incidence of fatty change in the adrenal cortex in males at 1250 ppm and above and in females at 2500 ppm and above. In the spleen, there were increases in the incidence and severity of extramedullary haematopoiesis and haemosiderosis in males at 5000 ppm and an increase in the severity of haemosiderosis in females at 2500 and 5000 ppm.

Rat-specific pathology (α_{2u} -globulin nephropathy) was observed in males, but as this has no human relevance, it was not expected to form the basis for a NOAEL. There is no equivalent explanation available for the dose-related increase in chronic tubular lesions in female rats in the 3-month study, in which there was an increased incidence of chronic tubular lesions at 250 ppm, equal to 19.2 mg/kg bw per day; however, while this description presents a strict account of the observations within this particular study, it is significant that there was no similar increased incidence of kidney lesions in female rats after 12 months or 24 months (see below). It is concluded that these observations of renal tubule pathology in female rats represent an incidental finding that was not replicated in studies of longer-term duration.

The NOAEL of human relevance in the 90-day dietary study in rats was 250 ppm, equal to 17.6 mg/kg bw per day, based on reduced body weight gain and increased incidence of fatty change in the adrenals in males at 1250 ppm, equal to 84.9 mg/kg bw per day (Bachmann, 1996b).

Dogs

In a 90-day oral toxicity study in dogs, groups of four male and four female purebred Beagle dogs were administered thiamethoxam (batch No. P.506006; purity 98.6%) in the diet at a concentration of 0, 50, 250, 1000 or 2500/2000 ppm, equal to 0, 1.58, 8.23, 32.0 and 54.8 mg/kg bw per day in males and 0, 1.80, 9.27, 33.9 and 50.5 mg/kg bw per day in females, for 13 weeks. Dogs were observed daily for clinical signs of toxicity; feed consumption was measured daily, and body weight was recorded weekly. Eye examinations were conducted before the start and at week 13 of treatment. Haematology, blood chemistry and urinalysis were performed before exposure and at weeks 7 and 13. At autopsy, the weights of selected organs were recorded for all dogs. All major organs and tissues were examined microscopically.

Table 14. Histopathology of rats treated with thiamethoxam in the diet for 90 days

	Incidence of finding					
	Dietary concentration (ppm)					
	0	25	250	1250	2500	5000
<i>Number of animals</i>	<i>10</i>	<i>10</i>	<i>10</i>	<i>10</i>	<i>10</i>	<i>10</i>
Males						
Liver						
- Hepatocellular hypertrophy	0	0	0	0	6	10
- Lymphohistiocytic infiltration	3	4	6	5	4	9
- Cholangiofibrosis	2	4	2	4	5	6
- Pigmentation of Kupffer cells	0	0	0	0	0	0
Kidneys						
- Hyaline change	1	0	4	8	10	10
<i>weighted grade</i>	<i>1.0</i>	<i>0</i>	<i>1.3</i>	<i>1.5</i>	<i>1.7</i>	<i>2.3</i>
- Chronic tubular lesion	0	1	3	6	10	9
- Acute tubular lesion	0	0	0	3	8	9
- Lymphohistiocytic infiltration	2	2	1	3	6	3
- Basophilic proliferation	2	1	2	4	6	10
- Dilatation of renal pelvis	1	0	2	1	5	1
- Hyperplasia of pelvic epithelium	0	0	0	1	3	0
- Cast formation	1	3	2	3	3	5
- Calcification of renal cortex	0	0	0	0	0	1
- Nephrocalcinosis	0	0	0	0	0	0
Spleen						
- Haemosiderosis incidence	7	8	9	7	9	10
<i>weighted grade</i>	<i>1.0</i>	<i>1.9</i>	<i>1.4</i>	<i>1.6</i>	<i>1.7</i>	<i>2.0</i>
- Extramedullary haematopoiesis	3	2	3	5	2	5
<i>weighted grade</i>	<i>1.0</i>	<i>1.0</i>	<i>1.0</i>	<i>1.0</i>	<i>1.0</i>	<i>1.8</i>
Adrenal gland						
- Fatty change	4	5	5	7	7	8
Females						
Liver						
- Hepatocellular hypertrophy	0	0	0	0	0	8
- Lymphohistiocytic infiltration	4	5	6	7	9	10
- Cholangiofibrosis	2	1	2	1	3	1
- Pigmentation of Kupffer cells	0	0	0	0	0	6
Kidneys						
- Hyaline change	0	0	0	0	0	0
- Chronic tubular lesion	4	5	7	7	9	10
- Acute tubular lesion	0	0	0	0	0	0
- Lymphohistiocytic infiltration	0	1	0	0	0	0
- Basophilic proliferation	2	1	1	2	0	1
- Dilatation of renal pelvis	2	0	1	1	0	0
- Hyperplasia of pelvic epithelium	0	0	0	0	0	0

Table 14 (continued)

	Incidence of finding					
	Dietary concentration (ppm)					
	0	25	250	1250	2500	5000
- Cast formation	0	1	0	0	2	0
- Calcification of renal cortex	0	0	0	0	0	1
- Nephrocalcinosis	10	10	10	10	10	10
<i>weighted grade</i>	<i>1.6</i>	<i>1.8</i>	<i>1.9</i>	<i>1.7</i>	<i>2.4</i>	<i>2.5</i>
Spleen						
- Haemosiderosis incidence	9	10	10	10	10	10
<i>weighted grade</i>	<i>1.7</i>	<i>2.3</i>	<i>2.1</i>	<i>1.9</i>	<i>2.6</i>	<i>2.7</i>
- Extramedullary haematopoiesis	4	6	6	7	2	3
Adrenal gland						
- Fatty change	0	0	2	1	4	6

From Bachmann (1996b)

Preliminary diet analyses demonstrated the stability of thiamethoxam in diet at room temperature. Representative analyses of test diets demonstrated achieved concentrations to be in the 91.4–97.8% range of nominal concentrations.

All dogs survived the scheduled treatment period, and there were no clinical signs of an adverse effect of treatment. Seven dogs in the highest dose group (receiving 2500 ppm) lost weight during the first 2 weeks, necessitating a dose reduction to 2000 ppm. Three of these dogs continued to show markedly depressed weight gain, whereas weight gain of the remaining animals was unaffected by treatment. In female dogs of this 2000 ppm group, there were group mean body weight losses at weeks 4 and 8 and a modest cumulative weight gain at week 13. Weight gains of the dogs receiving diets containing up to 1000 ppm thiamethoxam were unaffected at any time. Feed consumption was depressed at 2500 ppm and to a lesser extent when the dose level was reduced to 2000 ppm. Feed consumption was also slightly depressed in females at 1000 ppm. There was no evidence of ocular toxicity at any dose level.

Treatment-related haematology and blood chemistry effects occurred at 1000 and 2000 ppm (Table 15). Slight anaemia, associated with a tendency to hypochromasia, anisochromasia and microcytosis, had developed by week 13 in females at 2000 ppm. Reduced white cell counts (total, neutrophils, monocytes and lymphocytes) also occurred in females. Males at 2000 ppm showed reduced monocyte counts and a tendency to hypochromasia and anisochromasia of red blood cells. Eosinophilia occurred in some dogs at this dose level. Some dogs in the 1000 and 2000 ppm groups had prolonged thromboplastin (prothrombin) times. Consideration of the development of thromboplastin times within the individual dogs showed that, when compared with their own control (i.e. pretreatment) values, there were effects in males, with a NOAEL of 250 ppm, but no effect in females.

Lower plasma calcium concentration and alanine aminotransferase (ALT) activity were observed in both sexes at 1000 and 2000 ppm (Table 16). The latter was not considered to be an adverse effect, as no accompanying hepatocellular changes were observed microscopically. Furthermore, consideration of the development of plasma calcium concentrations within the individual dogs showed that, when compared with their own control (i.e. pretreatment) values, there were no effects in males at any dose level, but there was an effect in females, with a NOAEL of 1000 ppm.

Minimally reduced plasma albumin levels in both sexes at 1000 and 2000 ppm and minimally lower cholesterol and phospholipid levels in males at 2000 ppm are considered to be physiological adaptive responses rather than primary effects of thiamethoxam. There were no treatment-related effects on urinary physiological parameters or cellular and chemical constituents of urine.

Table 15. Selected haematology of dogs treated with thiamethoxam in the diet for 90 days

Parameter (unit)	Week	Dietary concentration (ppm)				
		0	50	250	1000	2000
Males						
White blood cells (10 ⁹ /l)	7	10.77	10.91	13.20	9.352	7.985
	13	9.233	10.41	10.65	9.548	10.24
Neutrophils (10 ⁹ /l)	7	5.940	6.095	8.334	4.950	3.418
	13	5.035	6.150	6.675*	5.998	6.138
Basophils (10 ⁹ /l)	7	0.055	0.045	0.046	0.043	0.039
	13	0.068	0.043	0.045*	0.043	0.035*
Monocytes (10 ⁹ /l)	7	0.550	0.385	0.561	0.364	0.126*
	13	0.328	0.368	0.335	0.340	0.058*
Red blood cells (10 ¹² /l)	7	6.470	6.300	5.885	6.357	6.415
	13	6.625	6.163*	6.218	6.550	6.446
Haemoglobin (mmol/l)	7	8.575	8.425	8.038	8.400	8.525
	13	8.975	8.400	8.575	8.650	8.450
Haematocrit (l)	7	0.421	0.420	0.390*	0.413	0.416
	13	0.444	0.409	0.417	0.420	0.416
Thromboplastin (prothrombin) time (s)	7	33.64	33.68	32.11	39.43*	37.24
	13	36.18	35.82	36.63	41.61	41.16 ⁺
Females						
White blood cells (10 ⁹ /l)	7	9.155	9.895	10.99	11.24*	4.581*
	13	8.223	8.590	10.08*	9.905	4.156*
Neutrophils (10 ⁹ /l)	7	5.138	5.413	6.040	6.125	1.701*
	13	4.958	4.808	6.235	5.745	1.631*
Monocytes (10 ⁹ /l)	7	0.375	0.408	0.445	0.445	0.084*
	13	0.183	0.198	0.225	0.303	0.093
Red blood cells (10 ¹² /l)	7	6.330	6.083	6.618	6.078	6.036
	13	6.688	6.348	6.573	6.703	5.971*
Haemoglobin (mmol/l)	7	8.875	8.200	8.825	8.100	7.963
	13	9.400	8.550	8.850	8.850	7.813* ⁻
Haematocrit (l)	7	0.433	0.393*	0.428	0.390	0.377*
	13	0.456	0.415*	0.434	0.431	0.381* ⁻
MCV (fl)	7	86.33	64.53*	64.55*	64.10*	62.61* ⁻
	13	68.28	65.43	65.98	64.40*	63.86
MCH (fmol)	7	1.403	1.345	1.333	1.333*	1.323*
	13	1.408	1.348	1.345	1.323*	1.305*
Thromboplastin (prothrombin) time (s)	7	31.26	35.95*	34.66	39.33*	36.59
	13	35.37	37.88	38.29	44.26* ⁺	41.92 ⁺

From Altmann (1996)

MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; * $P \leq 0.05$ by Dunnett's t -test (two-tailed); -/+ negative/positive trend (Jonckheere)

Table 16. Selected blood chemistry parameters of dogs treated with thiamethoxam in the diet for 90 days

Parameter (unit)	Week	Dietary concentration (ppm)				
		0	50	250	1000	2000
Males						
ALT (U/l)	7	35.50	41.13	29.85	25.00*	18.13*-
	13	81.03	49.00	35.08	25.40*-	13.10*-
Albumin (g/l)	7	31.89	32.13	31.88	30.65	30.02*
	13	33.10	32.85	33.69	31.18	30.31
Ca ²⁺ (mmol/l)	7	2.803	2.758	2.788	2.678	2.710*
	13	2.743	2.755	2.810	2.698	2.698
Phospholipid (mmol/l)	7	4.465	4.160	4.285	4.208	3.703*
	13	4.455	4.235	4.108	4.068	3.698*
Cholesterol (mmol/l)	7	3.933	3.698	3.730	3.663	3.308
	13	3.930	3.853	3.633	3.753	3.368
Females						
ALT (U/l)	7	50.20	43.35	37.70*	26.63*-	17.41*-
	13	53.83	42.50	40.73	25.23*-	13.10*-
Albumin (g/l)	7	33.65	32.95	33.28	30.83	30.66-
	13	34.76	33.81	33.90	31.96*	30.15*-
Ca ²⁺ (mmol/l)	7	2.798	2.723	2.840	2.689	2.640*
	13	2.783	2.735	2.815	2.695	2.663*
Cholesterol (mmol/l)	7	3.698	3.523	3.225	3.150	3.650
	13	3.733	3.665	3.170	3.175	3.488
Phospholipid (mmol/l)	7	4.223	4.193	3.875	3.775	4.028
	13	4.210	4.265	3.720*	3.678	3.815

From Altmann (1996)

U, units; * $P \leq 0.05$ by Dunnett's *t*-test (two-tailed); -/+ negative/positive trend (Jonckheere)

No treatment-related macroscopic changes were evident at necropsy. Testis and ovary weights were reduced at 2000 ppm by 43% and 35%, respectively, and histological correlates were identified. Slightly reduced heart, liver and kidney weights in females at 2000 ppm and increased thyroid weights in males at 50, 1000 and 2000 ppm were not associated with histological changes and are considered incidental to treatment with thiamethoxam. Minimal to marked reductions in spermatogenesis and increased incidences of spermatid giant cells occurred in the testes of all males at 2000 ppm (Table 17). One male also showed moderate tubular atrophy. These changes represent an immature histological appearance of the testis. Immature ovaries, consistent with retarded maturation, were observed in three females at 2000 ppm. The three dogs affected included two that failed to gain weight throughout the 13-week period and one that gained weight only modestly. The fourth female, which was least affected by general body weight effects, also did not demonstrate retarded ovarian development. The maturity of the uterus in two of these dogs reflected the immature stage of ovarian development. These delays in sex organ development are likely to have been a consequence of the general delay in growth and development resulting from thiamethoxam treatment and not a specific effect of thiamethoxam on the ovary. It is noted that the ages of these dogs at the beginning of treatment ranged from 23 to 27 weeks for males and from 22 to 30 weeks for females.

Table 17. Histopathology of selected sex organs of dogs treated with thiamethoxam in the diet for 90 days

Organ and finding	Males					Females				
	Dietary concentration (ppm)									
	0	50	250	1000	2000	0	50	250	1000	2000
<i>Number of animals</i>	4	4	4	4	4	4	4	4	4	4
Testes										
Tubular atrophy	0	0	0	0	1	—	—	—	—	—
Spermatogenesis reduced	0	1	0	0	4	—	—	—	—	—
Spermatic giant cells	1	1	0	1	4	—	—	—	—	—
Uterus										
Immature	—	—	—	—	—	0	0	0	0	2
Ovary										
Immature	—	—	—	—	—	0	0	0	0	3

From Altmann (1996)

The NOAEL in the 90-day oral toxicity study in dogs was 250 ppm, equal to 8.23 mg/kg bw per day, based on prolonged thromboplastin times at 1000 ppm, equal to 32.0 mg/kg bw per day (Altmann, 1996).

In a 52-week oral toxicity study, groups of four male and four female purebred Beagle dogs were administered thiamethoxam (batch No. P.506006; purity 98.6%) in the diet at a concentration of 0, 25, 150, 750 or 1500 ppm, equal to 0, 0.70, 4.05, 21.0 and 42.0 mg/kg bw per day in males and 0, 0.79, 4.49, 24.6 and 45.1 mg/kg bw per day in females. Dogs were observed twice daily for mortality and clinical signs of toxicity; feed consumption was measured daily, and body weight was recorded weekly. Eye examinations were conducted before the start and at the end of treatment. Haematology, blood chemistry and urinalysis were performed before exposure and at weeks 13, 26 and 52. At autopsy, the weights of selected organs were recorded for all dogs. All major organs and tissues were examined microscopically.

Analysis of diet samples was performed on the 13 diet mixes prepared throughout the study. The first mix was also analysed for homogeneity and stability. Analysis demonstrated thiamethoxam to be stable in the diet for at least 7 weeks at room temperature and to be homogeneously mixed. Achieved concentrations ranged from 86.3% to 107.4% of nominal concentrations. The overall mean achieved concentrations were 23.8, 140.6, 702 and 1433 ppm, in order of increasing nominal concentration.

No deaths or treatment-related clinical signs occurred during the study. The overall body weight gain of males at 1500 ppm was reduced by 26% during the study. Body weight gain was transiently lower among male dogs of the 25, 150 and 750 ppm groups at week 13 by 36%, 50% and 50%, respectively, but had recovered by week 26, so that cumulative weight gains were similar to those of the controls in all groups of males up to and including the 750 ppm group. Transient body weight loss occurred in females at 1500 ppm at the study start, but body weight subsequently increased and overall weight gain was comparable to that of the controls by week 26. Mean weight gain in 750 ppm females was depressed due to two animals that lost weight during the treatment period in spite of normal feed intake. As weight gain was not substantially affected at 1500 ppm, this finding is considered incidental to treatment. Mean feed consumption and conversion ratios were unaffected by treatment in male groups but were slightly reduced in females at 750 and 1500 ppm at the start of treatment. No ocular toxicity was evident at week 52.

Table 18. Selected blood chemistry parameters of dogs treated with thiamethoxam in the diet for up to 52 weeks

	Week	Dietary concentration (ppm)				
		0	25	150	750	1500
Males						
Urea (mmol/l)	−1	3.23	3.99	4.16	4.20	4.43
	13	3.09	3.75*	4.08	4.13*	5.53*+
	26	3.85	4.50	4.94	4.89*	5.30
	52	3.79	4.78	4.65	4.83	4.97*
Creatinine (μmol/l)	−1	60.48	61.70	62.65	62.60	64.35
	13	61.95	65.44	65.73	68.93	85.23*
	26	60.63	65.34	66.05	71.15*	81.58*+
	52	66.53	75.10	72.88	77.73*	85.95*+
ALT (U/l)	−1	52.60	52.23	65.90	51.88	64.28
	13	49.10	53.28	49.73	33.38	22.05*−
	26	49.40	45.80	49.90	32.60	23.65*−
	52	54.10	45.40	53.05	33.95	30.53*−
Females						
Urea (mmol/l)	−1	4.21	3.86	5.09	3.96	4.22
	13	3.82	3.92	4.23	5.35+	5.08
	26	3.67	4.07	4.40	5.06*+	4.21
	52	4.99	4.60	4.40	5.65	4.68
Creatinine (μmol/l)	−1	64.05	60.48	67.20	64.78	65.28
	13	62.63	65.53	71.38	79.15*	81.04*+
	26	64.48	62.66	70.63	77.14	82.25*+
	52	71.98	72.35	78.95	86.29	86.45
Albumin (g/l)	−1	35.32	35.15	33.42*	35.04	33.80
	13	35.48	35.60	34.14	33.55	31.90
	26	35.35	34.94	33.36	33.32	32.30*−
	52	35.00	36.03	32.13	32.34	30.61
Albumin/globulin ratio	−1	1.50	1.51	1.59	1.57	1.44
	13	1.43	1.45	1.27	1.26	1.11 [−]
	26	1.38	1.36	1.27	1.31	1.19*
	52	1.27	1.35	1.25	1.18	1.10

From Altmann (1998)

U, units; * $P \leq 0.04$, Wilcoxon; –/+ negative/positive trend ($P \leq 0.01$, Jonckheere)

There were no treatment-related changes in haematological profiles at any dose level, except for prothrombin times, which were lower in both sexes at 1500 ppm throughout treatment compared with the controls.

There was a dose-related, minimal to slight increase in plasma creatinine and a tendency to higher plasma urea levels throughout the treatment period in both sexes at 750 and 1500 ppm (Table 18). A slight to moderate decrease in ALT activity, relative to pretest values, occurred in males at 750 and 1500 ppm during treatment, but could not be correlated with histological changes in the liver. Therefore, a reduction in the activity of this enzyme is considered not to represent an adverse

Table 19. Weights of selected organs in dogs treated with thiamethoxam in the diet for 52 weeks

	Males					Females				
	Dietary concentration (ppm)									
	0	25	150	750	1500	0	25	150	750	1500
Carcass										
- Absolute (kg)	11.46	11.43	11.80	10.95	11.17	10.56	10.27	10.38	9.61	10.46
Heart										
- Absolute (g)	112.7	112.8	114.0	112.4	123.1	102.8	101.2	88.9	98.9	109.1
- Relative (% body weight × 10)	9.85	9.87	9.65	10.25	11.03	9.78	9.86	8.58	10.52	10.48
Testes/ovaries										
- Absolute (g)	19.07	20.48	19.83	20.65	16.06	1.51	1.10	1.66	1.06	1.03
- Relative (% body weight × 10)	1.66	1.79	1.69	1.89	1.44	0.14	0.11	0.15	0.12	0.10
Spleen										
- Absolute (g)	59.59	47.05	37.17	29.92 ⁻	47.40	73.58	66.69	50.89	46.52	47.37
- Relative (% body weight × 10)	5.24	4.12	3.15	2.72 ⁻	4.22	6.91	6.38	4.82	5.26	4.49
Thyroid gland										
- Absolute (g)	1.17	1.08	0.99	1.24	1.18	1.08	1.06	1.14	2.38	1.22
- Relative (% body weight × 10)	0.10	0.09	0.08	0.11	0.11	0.10	0.10	0.11	0.21	0.12

From Altmann (1998)

– negative trend ($P \leq 0.01$, Jonckheere)

effect. Females at 1500 ppm had minimally lower albumin levels, associated with lower albumin to globulin ratios. No other treatment-related effects on blood chemistry occurred. There were no treatment-related quantitative or qualitative effects on urinary composition at any dose level.

Analysis of absolute and relative organ weights indicated that there was a minimal decrease in absolute and relative testis weights in 1500 ppm dogs, due mainly to the low testis weights of two animals (Table 19). Higher heart and thyroid weights in 1500 ppm males and lower ovary and spleen weights in females at 750 and 1500 ppm did not have histopathological correlates and are therefore considered incidental.

At autopsy, pulmonary nodules or mottled lungs were observed in several control and treated animals of both sexes. Microscopically, these findings represented various inflammatory or post-inflammatory changes that commonly occur in control animals from this source and are considered incidental to treatment. Microscopic examination of tissues/organs revealed a higher incidence of tubular atrophy in the testes at 750 and 1500 ppm; as this observation was also made in control animals, it is not clearly associated with treatment (Table 20). At 1500 ppm, the observation correlated with reduced testis weights in two animals. Although not clearly treatment related, this increase in the recorded incidence of testicular atrophy is consistent with the observation of an immature testis. Together with the lower testis weights in two animals, this may represent delayed testicular development, most likely as a consequence of the general growth and developmental delay resulting from treatment at the higher dose levels. Other microscopic alterations observed, particularly nonspecific inflammatory changes, thymic atrophy and fatty change of adrenal cortical cells, occur commonly in this colony of dogs, and the morphology and severity did not indicate an effect of treatment. Significantly, in comparison with the 90-day study in dogs, there was no evidence for developmental delay in female sex organs in this longer-term study.

Table 20. Histopathology of selected organs of dogs treated with thiamethoxam in the diet for 52 weeks

	Males					Females				
	Dietary concentration (ppm)									
	0	25	150	750	1500	0	25	150	750	1500
<i>Number of animals examined</i>	4	4	4	4	4	4	4	4	4	4
Adrenal glands										
Cortical fatty change	0	0	0	1	0	1	3	3	3	3
Lung										
Fibrosis	1	0	1	0	1	3	2	1	1	2
Bronchial-alveolar hyperplasia	0	0	1	0	0	1	1	0	0	0
Chronic inflammation	3	2	2	3	4	4	4	4	4	2
Osseous metaplasia	0	0	0	0	0	0	0	0	1	0
Testes										
Tubular atrophy	1	1	1	2	2	—	—	—	—	—
Spermatid giant cells	2	1	1	0	1	—	—	—	—	—
Inflammatory cell infiltration	0	0	1	0	0	—	—	—	—	—
Thymus										
Atrophy	3	4	3	3	4	3	2	2	4	3
Developmental cyst	2	3	1	2	1	—	3	2	3	1
Granuloma	0	0	0	0	0	0	0	0	1	0
Phagocytic cells	0	0	0	0	0	1	0	0	0	0

From Altmann (1998)

The NOAEL in the 52-week oral toxicity study in dogs was 750 ppm, equal to 21.0 mg/kg bw per day, based on prolonged thromboplastin times and reduced testis weights at 1500 ppm, equal to 42.0 mg/kg bw per day (Altmann, 1998).

(b) Dermal application

In a 28-day dermal toxicity study, groups of five male and five female Sprague-Dawley-derived (Tif:RAIf, SPF strain) rats were administered thiamethoxam (batch No. P.506006; purity 98.6%) at a dose level of 0, 20, 60, 250 or 1000 mg/kg bw per day applied under a semioclusive dressing to intact shaven dorsal skin for 6 hours per day, 5 days per week, for 4 weeks. Thiamethoxam was formulated as a suspension in aqueous 1% carboxymethylcellulose/0.1% Tween 80 at nominal concentrations of 0, 10, 30, 125 and 500 mg/ml in order of increasing dose level. Mortality and clinical signs were checked daily, and individual body weights and feed and water consumption were recorded weekly. Haematological and blood chemistry investigations were performed on all rats towards the end of the treatment period. All animals were subjected to autopsy and postmortem examination, major organs were weighed and selected tissues were examined microscopically.

There were no deaths or clinical signs of toxicity during the study. Body weight gains in males of the 1000 mg/kg bw per day group were retarded during the first 2 weeks of treatment, but not at lower dose levels or in females at any dose level. Feed consumption was not affected in any group. Haematological profiles were unaffected by treatment at all dose levels. All statistically significant values showed no dose–response relationship, or the recorded values were within historical ranges. Females at 250 and 1000 mg/kg bw per day had slightly raised plasma glucose concentrations and minimally raised alkaline phosphatase activities (Table 21). Triglyceride concentrations were also

Table 21. Selected blood chemistry parameters in rats treated with thiamethoxam by skin application for 28 days

Parameter	Males					Females				
	Dose (mg/kg bw per day)									
	0	20	60	250	1000	0	20	60	250	1000
Glucose (mmol/l)	7.528	7.786	7.886	7.592	6.622	6.390	6.126	6.159	9.592*	9.739*+
Alkaline phosphatase (U/l)	171.5	140.0	144.7	155.2	135.6	108.3	95.42	95.62	174.5*	139.8+
Triglycerides (mmol/l)	0.501	0.623	0.688	0.708	0.516	0.515	0.506	0.438	0.700	1.091*+

From Gerspach (1996)

U, units; * $P < 0.05$ (Wilcoxon); + positive trend ($P < 0.01$, Jonckheere)

slightly raised in females at 1000 mg/kg bw per day. Significantly elevated plasma potassium concentrations in all groups of male rats were considered not to have been influenced by thiamethoxam, as control values were unusually low. Other differences from the controls in blood chemistry are not considered to be treatment related because they were too small to be of toxicological relevance (e.g. inorganic phosphate levels in males at 1000 mg/kg bw per day) or their occurrence showed no relationship to dose level.

No anomalies were observed at autopsy, and all absolute organ weights and their ratios to body weight were unaffected by treatment. Morphological changes in the skin at the application site occurred at low incidence in both treated and control animals and are considered to have been induced by the treatment procedure rather than by a response to thiamethoxam. The liver, kidneys and adrenal glands were identified as target organs on the basis of histological findings (Table 22). An increased incidence of minimal inflammatory cell infiltration and necrosis of single hepatocytes occurred in females at 250 and 1000 mg/kg bw per day; the elevated incidence of this lesion at 60 mg/kg bw per day and higher was considered to fall within the limits of normal variation for this strain of rat. Effects on liver morphology were absent in the males. In the kidneys, minimal tubular hyaline change in males and minimal chronic tubular lesions in females were apparent at 1000 mg/kg bw per day. A single female at this dose level also showed minimal basophilic cell infiltration in the renal tubules. Lower dose levels were unaffected by these renal changes. In the adrenal glands, minimal inflammatory cell infiltration was evident in the cortex of females at 1000 mg/kg bw per day. No other groups were affected.

The NOAEL in the 28-day dermal toxicity study in rats was 60 mg/kg bw per day, based on the blood chemistry changes (increased glucose and alkaline phosphatase levels) in females at 250 mg/kg bw per day. In view of the low order of toxicity by the percutaneous route in this study, a 90-day dermal study is not required (Gerspach, 1996).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female albino mice (Tif:MAGf, SPF strain) were administered thiamethoxam (batch No. P.506006; purity 98.6%) orally for 78 weeks in the diet at a concentration of 0, 5, 20, 500, 1250 or 2500 ppm, equal to 0, 0.65, 2.63, 63.8, 162 and 354 mg/kg bw per day in males and 0, 0.89, 3.68, 87.6, 215 and 479 mg/kg bw per day in females. Ten mice of each sex per group were designated for clinical laboratory investigations, and 50 mice of each sex per group for the oncogenicity study. Additional groups of 10 mice of each sex in the control and high-dose groups were similarly treated with thiamethoxam for 35 weeks and then killed for interim evaluation. Clinical

Table 22. Selected histopathological findings in rats treated with thiamethoxam by skin application for 28 days

Organ/finding	Incidence of finding									
	Males					Females				
	Dose (mg/kg bw per day)									
	0	20	60	250	1000	0	20	60	250	1000
<i>Number of animals examined</i>	5	5	5	5	5	5	5	5	5	5
Adrenal glands										
Cortical fatty change	4	4	3	3	4	1	0	1	1	1
Inflammatory cell infiltration	0	0	0	0	0	0	1	0	1	3
Kidneys										
Chronic progressive nephropathy	0	0	0	0	1	0	1	0	0	0
Glomerulosclerosis	0	1	0	0	0	0	0	0	0	0
Chronic tubular lesion	1	0	1	1	1	1	2	2	1	4
Nephrocalcinosis	1	0	0	1	0	3	5	5	5	5
Renal tubular cast	0	0	1	0	1	0	1	0	1	0
Hyaline change	1	2	1	1	3	0	0	0	0	0
Calcification	0	1	0	0	0	0	0	0	0	0
Basophilic infiltration	2	2	0	2	1	0	0	0	0	1
Liver										
Inflammatory cell infiltration	3	4	4	3	2	2	2	4	5	5
Intrahepatic bile duct cholangiofibrosis	1	0	0	1	1	0	0	0	0	0
Extramedullary haematopoiesis	0	0	0	0	0	1	0	0	0	1
Kupffer cell hyperplasia	0	0	0	0	0	1	0	1	0	0
Hepatocyte necrosis	0	0	0	0	0	2	0	2	3	4

From Gerspach (1996)

observations were made daily, and body weights and feed consumption were recorded weekly for 13 weeks and monthly thereafter. Haematological investigations were performed at weeks 53 and 78 on samples from 10 mice of each sex per group. All mice were subjected to detailed autopsy and postmortem examination. Organ weights of all mice that survived to the end of their scheduled dosing period were recorded. Tissue/organ samples from all mice killed at 35 weeks, those that died or were killed during the study and all mice killed after 78 weeks were fixed and preserved. Microscopic examination of tissues was performed on all mice of the oncogenicity subgroup. The livers of the additional 10 mice of each sex in the control and high-dose groups were also examined microscopically after haematoxylin–eosin staining and appropriate special stains. In-life and organ weight data were statistically analysed by a univariate technique, using non-parametric methods where appropriate. Survival analysis was by Cox's regression model. Neoplastic lesions were analysed using Peto's mortality prevalence test, and non-neoplastic lesions by the Cochran-Armitage linear trend test.

Diet analyses demonstrated thiamethoxam to be stable in feed for at least 5 weeks at room temperature, whereas fresh diets were prepared monthly. Analysis of diet samples demonstrated a homogeneous distribution of thiamethoxam. The overall mean analytically determined concentrations were 4.9, 20.3, 502, 1251 and 2598 ppm.

Survival incidence and the incidences of single/multiple palpable masses were not affected by treatment in either sex. Clinical signs were restricted to a slightly increased incidence of abdominal distension in females at 1250 ppm and in males at 1250 and 2500 ppm. This finding correlated histologically, in most cases, with benign or malignant liver tumours. There was a slightly increased incidence of hypoactivity in females at 2500 ppm, which correlated with perimortal findings in unscheduled deaths. At 2500 ppm, body weight gain was retarded in males from week 7 and in females from week 38, resulting in an overall depression of weight gains of 18% and 14%, respectively. Feed consumption was unaffected by treatment in both sexes at all dose levels.

There were no treatment-related changes in haematological profiles at 53 and 78 weeks at any dose level. Significantly higher mean corpuscular haemoglobin occurred in males at 2500 ppm at 53 and 78 weeks, but as erythrocyte numbers, haemoglobin concentration and haematocrit values were unaffected by treatment, the differences were considered to have no toxicological significance. Occasional statistically significant differences from the controls occurred in some haematological parameters but were considered not to be toxicologically significant because there was no dose-response relationship or the changes were too small to be of biological relevance. Lymphatic leukaemia occurred in two control females, one female at 5 ppm, one female and two males at 500 ppm and one female at 1250 ppm. Females with leukaemia contributed to higher mean white blood cell counts and unstained cell counts in control and 500 ppm animals at week 78. No toxicological significance was attributed to these findings.

At week 35, the only effects on organ weights attributable to treatment were increased liver and adrenal weights in females treated at 2500 ppm. At week 79, mean carcass weight was reduced in males at 2500 ppm. Liver weights were increased dose-dependently in both sexes at 500, 1250 and 2500 ppm (Table 23). Other significant differences at 2500 ppm are considered to be incidental to treatment because of an effect on body weight (male brain weight), no histological correlate (male kidney weight) or aberrant mean value in the control group due to the presence of tumours (female spleen weight).

There were no treatment-related gross lesions at autopsy in week 35. Gross examination at week 79 revealed an increased incidence of masses and nodules in the liver at doses of 500 ppm and above, particularly in male groups. Thickening of the stomach in three males at 2500 ppm, a decreased incidence of enlarged seminal vesicles at 1250 and 2500 ppm and a decreased incidence of enlarged spleen in females at 2500 ppm are considered to be treatment related, as histological correlates were noted. A slight increase in the incidence of uterine masses at 2500 ppm was not supported by microscopic findings, and this was therefore considered incidental to treatment.

Liver histopathology performed at week 35 revealed non-neoplastic lesions only. A treatment-related increase occurred in the incidences of inflammatory cell infiltration, necrosis of single hepatocytes, Kupffer cell pigmentation and hepatocyte hypertrophy in both sexes at 2500 ppm (Table 24). The severity ranged from minimal to moderate. At week 79, treatment-related, non-neoplastic lesions in the liver occurred at 500 ppm and above, comprising increased incidences of focal cellular alteration (mainly eosinophilic), inflammatory cell infiltration, necrosis of single hepatocytes, hepatocellular hypertrophy, increased mitotic activity, deposition of pigments (lipofuscin and haemosiderin) and hyperplasia of Kupffer cells. The lesions ranged in severity from minimal to marked, and their incidences generally showed a positive dose-response relationship.

Treatment-related, non-neoplastic effects in tissues other than liver were increased incidences and severity of splenic extramedullary haematopoiesis and gastric mucosal epithelial hyperplasia in both sexes at 2500 ppm. The latter lesion was frequently accompanied by mild inflammatory cell infiltration and, in males, could be correlated with grossly observable thickening of the stomach wall. The incidences of some degenerative and inflammatory lesions were decreased at 2500 ppm and occasionally at 500 and 1250 ppm. Effects observed at reduced incidences included adrenal cortical hyperplasia, cataract formation, chronic nephropathy in males, pancreatic islet cell hyperplasia in

Table 23. Treatment-related organ weight changes in mice treated with thiamethoxam in the diet for 35 or 79 weeks

Organ	Dietary concentration (ppm)	Males				Females			
		Week 35		Week 79		Week 35		Week 79	
		Absolute weight	Relative weight ^a	Absolute weight	Relative weight ^a	Absolute weight	Relative weight ^a	Absolute weight	Relative weight ^a
Carcass (g)	0	53.93	—	53.15	—	35.84	—	40.57	—
	5	—	—	53.34	—	—	—	38.48	—
	20	—	—	52.17	—	—	—	38.83	—
	500	—	—	53.48	—	—	—	40.17	—
	1250	—	—	51.65	—	—	—	40.82	—
	2500	48.88	—	48.62* ⁻	—	35.69	—	37.62	—
Liver (g)	0	3.02	55.62	2.97	55.73	1.88	53.35	2.34	57.87
	5	—	—	3.08	58.67	—	—	2.19	57.44
	20	—	—	2.92	55.91	—	—	2.29	59.37
	500	—	—	3.20	60.02	—	—	2.56	63.79**
	1250	—	—	3.79* ⁺	73.75* ⁺	—	—	2.67 ⁺	65.55* ⁺
	2500	2.69	54.81	5.18* ⁺	106.4* ⁺	2.30	64.44*	2.97* ⁺	78.54* ⁺
Adrenals (mg)	0	9.81	0.18	10.02	0.19	20.36	0.58	19.35	0.49
	5	—	—	9.27	0.18	—	—	19.09	0.50
	20	—	—	8.80*	0.17	—	—	17.56	0.46
	500	—	—	9.11*	0.17	—	—	20.84	0.53
	1250	—	—	9.50	0.19	—	—	18.88	0.48
	2500	9.95	0.21	10.02	0.21	25.58	0.72	18.44	0.50

From Bachmann (1998b)

* $P \leq 0.01$ (LePage); -/+ negative/positive trend ($P \leq 0.01$, Jonckheere)

^a % body weight $\times 10$.

Table 24. Incidences of non-neoplastic hepatic lesions in mice treated with thiamethoxam in the diet for 35 or 79 weeks

	Incidence of lesion											
	Males						Females					
	Dietary concentration (ppm)											
	0	5	20	500	1250	2500	0	5	20	500	1250	2500
Week 35												
<i>Number examined</i>	10	0	0	0	0	10	10	0	0	0	0	10
Inflammatory cell infiltration	1	—	—	—	—	8	3	—	—	—	—	8
Single-cell necrosis	0	—	—	—	—	10	0	—	—	—	—	9
Kupffer cell pigmentation	0	—	—	—	—	8	0	—	—	—	—	4
Hepatocyte hypertrophy	0	—	—	—	—	9	0	—	—	—	—	10
Week 79												
<i>Number examined</i>	50	50	50	50	50	50	50	50	50	50	50	50
Deposition of pigment	2	2	3	13	33	44	6	5	3	5	14	30

Table 24 (continued)

	Incidence of lesion											
	Males						Females					
	Dietary concentration (ppm)											
	0	5	20	500	1250	2500	0	5	20	500	1250	2500
Focus of alteration	7	4	4	11	22	32	2	2	2	2	14	37
Hepatocellular hypertrophy	8	11	6	41	40	45	3	2	3	19	39	45
Increased mitotic activity	0	0	0	1	10	8	1	1	0	4	5	4
Inflammatory cell infiltration	13	9	13	33	41	43	18	20	20	24	33	45
Kupffer cell hyperplasia	0	0	1	0	0	10	2	1	0	0	1	2
Single-cell necrosis	5	3	5	40	40	46	3	2	5	18	36	46

From Bachmann (1998b)

Table 25. Incidences of neoplastic hepatic lesions in mice treated with thiamethoxam in the diet for 79 weeks

	Malignant or benign	Incidence of lesion											
		Males						Females					
		Dietary concentration (ppm)											
		0	5	20	500	1250	2500	0	5	20	500	1250	2500
<i>Number examined</i>		<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>50</i>
Hepatocellular adenocarcinoma	m	3	3	2	4	4	16	0	0	0	0	2	3
1st hepatocellular adenoma	b	9	5	8	17	21	39	0	0	0	5	8	28
2nd hepatocellular adenoma	b	3	2	3	3	12	31	0	0	0	0	5	14
Multiple hepatocellular adenomas	b	0	1	0	0	6	14	0	0	0	0	2	7
Metastatic carcinoma	m	0	0	0	0	1	0	1	0	0	0	1	0
Haemangioma	b	0	1	1	0	0	0	2	1	0	0	0	0
1st haemangiosarcoma	m	1	0	0	0	1	0	1	0	0	0	1	0
2nd haemangiosarcoma	m	1	0	0	0	1	0	0	0	0	0	0	0
Hepatoblastoma	m	0	0	0	0	1	0	0	0	0	0	0	0
Metastatic osteosarcoma	m	0	0	0	0	0	0	0	0	1	0	0	0
Any hepatic neoplastic lesion		11	7	10	19	22	44	0	0	0	5	9	29

From Bachmann (1998b)

b, benign; m, malignant

males, lymphocytic infiltration of the salivary gland in females, chronic inflammation and dilatation of the seminal vesicles (correlated with reduced incidence of enlarged seminal vesicles at necropsy), splenic white pulp hyperplasia (correlated in females with decreased incidence of enlarged spleen at necropsy) and testicular tubular atrophy. Particularly in males, reduced incidences are considered to be associated with depressed body weight gain and are not regarded as adverse effects.

At 79 weeks, the incidence of hepatocellular adenoma was significantly greater than concurrent and historical control levels in both sexes at 500 ppm and above. The incidence of hepatocellular adenocarcinoma was also significantly greater than concurrent and historical control levels in females at 1250 ppm and in both sexes at 2500 ppm (Table 25). No increased incidences of neoplasms in other organs were observed. Decreased incidences of systemic malignant lymphomas in females were consistent with significantly lower spleen weights.

The NOAEL in the 78-week dietary study in mice was 20 ppm, equal to 2.63 mg/kg bw per day, based on increased liver weights, hepatocellular hypertrophy, pigment deposition, inflammatory cell infiltration and single-cell necrosis at 500 ppm, equal to 63.8 mg/kg bw per day. Thiamethoxam was tumorigenic in mice and induced hepatocellular adenomas in male and female mice at a dose level of 500 ppm, equal to 63.8 mg/kg bw per day in male mice and 87.6 mg/kg bw per day in female mice, and hepatocellular adenocarcinomas in male mice at 2500 ppm, equal to 354 mg/kg bw per day. A mouse-specific mode of (toxic and carcinogenic) action was proposed through which it was clear that hepatotoxicity and hepatocarcinogenicity were unlikely to occur at human levels of dietary exposure (Bachmann, 1998b). A proposed mode of carcinogenic action of thiamethoxam in mice is evaluated in [Appendix 1](#).

The NOAEL for non-hepatic effects was 1250 ppm (equal to 162 mg/kg bw per day), based on reductions in body weight and effects on spleen and stomach at 2500 ppm (equal to 354 mg/kg bw per day).

Rats

Groups of 70 male and 70 female Sprague-Dawley rats (strain Tif:RAIf, SPF) were administered thiamethoxam (batch No. P.506006; purity 98.6%) orally for 104 weeks in the diet at a concentration of 0, 10, 30, 500 or 1500 ppm, equal to 0, 0.41, 1.29, 21.0 and 63.0 mg/kg bw per day, in males and 0, 10, 30, 1000 or 3000 ppm, equal to 0, 0.48, 1.56, 50.3 and 155 mg/kg bw per day, in females. Twenty rats of each sex per group were designated for clinical laboratory investigations, and 50 rats of each sex per group for the carcinogenicity study. Additional groups of 10 rats of each sex per group were similarly treated with thiamethoxam for 52 weeks and then killed for interim evaluation. Clinical observations were made daily, body weights and feed consumption were recorded weekly for 13 weeks and monthly thereafter, and water consumption was recorded monthly. The eyes of all rats in the carcinogenicity subgroup were examined before dosing and at 104 weeks. Control and high-dose rats were also examined at weeks 26, 52 and 77. Laboratory investigations were performed at weeks 13, 27, 53 and 78 and at the end of the treatment period on 20 rats of each sex per group for haematology and on 10 rats of each sex per group for blood chemistry and urinalysis. All rats were subjected to detailed autopsy and postmortem examination. Organ weights of all rats that survived to the end of their scheduled dosing period were recorded. Tissue/organ samples from all rats killed at 52 weeks, those that died or were killed during the study and all rats killed at the end of the study were fixed and preserved. Microscopic examination of tissues was performed on all rats of all treatment and control groups, with the exception of the 20 rats of each sex per group designated for laboratory investigations. In-life and organ weight data were statistically analysed by a univariate technique, using non-parametric methods where appropriate. Survival analysis was by Cox's regression model. Neoplastic lesions were analysed using Peto's mortality prevalence test, and non-neoplastic lesions by the Cochran-Armitage linear trend test.

Diet analyses demonstrated thiamethoxam to be stable in feed for at least 7 weeks at room temperature, whereas fresh diets were prepared monthly. Analysis of diet samples 13 times demonstrated a homogeneous distribution of thiamethoxam and achieved concentrations in the range 89.3–107.6% of nominal during the 4-week use period. The overall mean analytically determined concentrations were 10, 31, 507, 1011, 1502 and 3033 ppm.

Survival incidence was not affected by treatment, there were no clinical signs of an adverse effect of treatment and the incidences of single and multiple palpable masses were unaffected by treatment at any dose level. Body weight development in all male groups was unaffected by treatment, but females at 3000 ppm had slightly depressed body weight gain from week 3 until termination, at which time cumulative weight gain was depressed by 12.6%. Feed consumption was unaffected by treatment in both sexes at all dose levels, but water consumption of males at 1500 ppm was raised by 13%. No effect on water consumption occurred in other groups. Eye examinations revealed no evidence of ocular toxicity at any time.

There were no treatment-related changes in haematology, blood chemistry or urinalysis parameters. Occasional statistically significant differences from the controls, or trends, occurred in these parameters, but were considered not to be toxicologically significant because there was no consistency between sampling intervals, there was no dose–response relationship or the changes were too small to be of biological relevance. A myeloid leukaemia was identified in one male rat at 1500 ppm in week 53. This type of neoplasm occurs spontaneously at low incidence in rats of this source and strain, and it was considered unrelated to treatment.

There were no treatment-related effects on organ weights at any dose level at either 52 or 104 weeks. At 104 weeks, mean carcass weight in high-dose males and females was reduced by 4% and 8%, respectively. There were no significant differences from control values in absolute and relative organ weights among the males, with the exception of a minor negative trend in the relative thyroid weight of males at 1500 ppm. The trend was considered not to be of toxicological significance because the mean value was similar to historical control values and no related histological changes were evident. Mean absolute adrenal weights appeared elevated in males at 10, 500 and 1500 ppm, but not at the intermediate dose of 30 ppm. The highest mean value was for the 10 ppm dose in both males and females. These are clearly erratic results and include some particularly high individual adrenal weights in the 10 and 1500 ppm group males. The variations in adrenal weights cannot be interpreted with justification as being due to thiamethoxam. Female mean relative thyroid weights were higher than control values in all treated groups, but remained within the historical control range. In the absence of corroborative clinical chemistry or histological changes, the differences were judged to be without toxicological significance.

Postmortem examination of rats sacrificed after 52 and 104 weeks and unscheduled deaths between 52 and 104 weeks revealed no treatment-related lesions. Most gross lesions occurred at comparable frequencies in the control and treated groups. Morphological correlates of gross lesions showing intergroup differences in incidence did not indicate an effect of treatment. The nature of the lesions was similar to those occurring spontaneously in this strain of rat.

No treatment-related neoplastic lesions occurred in rats that were killed at 52 weeks or that died during the first 52 weeks. Three malignant and seven benign neoplasias were identified in total. All were diagnosed as neoplasms that occur spontaneously in this strain of rat, and only pituitary adenomas occurred in more than one individual. The distribution between the control and treated groups did not indicate an effect of treatment. Non-neoplastic, treatment-related lesions at 52 weeks were increased incidences of renal tubular regenerative changes, chronic tubular lesion and tubular basophilic proliferation in males at 500 and 1500 ppm. Minimally increased incidences of renal tubular and pelvic lymphocytic infiltration also occurred at 1500 ppm, but without tubular hyaline change. These alterations are considered to represent the sequelae of $\alpha_2\mu$ -globulin-mediated nephropathy. The kidneys of females at all dose levels were unaffected. There was a minimal increase in the severity of splenic haemosiderosis in females at 3000 ppm from a mean score of 2.4 in the controls to 3.0 in the 3000 ppm group. In the liver, there were increased incidences of cholangiofibrosis in males at 10 ppm (5/10), 500 ppm (6/10) and 1500 ppm (4/10) compared with the controls (2/10) and an increased incidence of inflammatory cell infiltration in males at 10 ppm (4/10), 30 ppm (3/10) and 500 ppm (5/10) relative to controls (1/10). These findings, which were not apparent after 104 weeks of treatment, are considered incidental to treatment because of the lack of a dose–response relationship. Other non-neoplastic lesions occurred in animals that died during the first year or that were sacrificed at 52 weeks, but all commonly occur in this strain of rat, and the incidence, distribution and morphology did not indicate an effect of treatment.

In rats that were killed at 104 weeks or that died between weeks 53 and 104, treatment-related, non-neoplastic changes in the kidneys and liver were observed. In the kidneys, a slightly increased incidence of slight/moderate chronic nephropathy occurred in males at 1500 ppm (Table 26), accompanied by a minimal increase in the incidence of lymphocytic infiltration of the renal cortex at 1500

Table 26. Incidences of non-neoplastic renal hepatic lesions in rats treated with thiamethoxam in the diet for 104 weeks

	Incidence of lesion									
	Males					Females				
	Dietary concentration (ppm)									
	0	10	30	500	1500	0	10	30	1000	3000
Kidneys										
<i>No. examined</i>	50	50	49	50	50	50	50	49	50	50
Lymphocytic infiltration	10	10	7	14	17	2	3	4	2	2
<i>Average grade</i>	1.6	1.7	1.4	1.9	1.7	1.5	2.0	1.5	1.5	2.0
Chronic nephropathy	30	35	32	37	42	12	10	8	6	10
<i>Average grade</i>	2.4	2.6	2.4	2.5	2.7	2.8	2.9	2.0	2.2	2.5
Tubular hyaline change	0	1	1	0	2	2	1	0	0	0
Liver										
<i>No. examined</i>	50	50	49	50	50	50	49	49	50	50
Focus of cellular alteration	20	21	15	21	20	10	21	12	15	26
<i>Average grade</i>	2.2	2.3	1.6	2.0	2.0	1.9	1.6	1.5	1.4	2.1
Subtype:										
- Amphophilic	3	3	1	5	1	3	2	1	1	3
- Basophilic	1	2	0	2	0	4	6	5	5	6
- Clear cell	13	10	11	11	14	3	5	2	5	15
- Eosinophilic	3	5	2	3	5	0	8	4	4	2
- Mixed	0	1	1	0	0	0	0	0	0	0

From Bachmann (1998a)

ppm. Two animals also showed tubular hyaline change. In the liver, a treatment-related increase occurred in the incidence of slight/moderate focal cellular alteration, generally of the clear cell subtype, in females at 3000 ppm. All other non-neoplastic findings in the liver and kidneys were of a similar nature, severity and incidence in both treated and control groups. In other tissues, some non-neoplastic lesions occurred at slightly higher incidences in treated groups than in controls, but in all instances, these are considered unrelated to treatment with thiamethoxam, as their occurrence is not dose related, the incidence is within the historical control range or the lesions are known to occur spontaneously in aged rats of this strain.

All neoplastic findings, both malignant and benign, occurring at 104 weeks are considered incidental to treatment with thiamethoxam, as the incidences in treated and control groups are similar, the intergroup distribution shows no relationship to dose level, or the incidences are within historical control ranges and the lesions are known to occur spontaneously in aged rats.

The NOAEL in the 104-week dietary study in rats was 30 ppm, equal to 1.3 mg/kg bw per day in males, based on increased incidences of renal chronic tubular lesions and basophilic proliferation in males at 500 ppm, equal to 21.0 mg/kg bw per day. This renal lesion was considered to be α_2 -globulin mediated and therefore has no human relevance. The relevant NOAEL in rats in the 104-week dietary study was 1000 ppm, equal to 50.3 mg/kg bw per day in females, based on foci of cellular alteration in the liver and increased severity of splenic haemosiderosis at 3000 ppm, equal to 155 mg/kg bw per day (Bachmann, 1998a).

Table 27. Results of studies of genotoxicity with thiamethoxam

End-point	Test object	Concentration/dose ^a (LED/HID)	Batch No.; purity (%)	Result	Reference
In vitro					
Gene mutation	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537; <i>Escherichia coli</i> WP2uvrA (± Aroclor 1254-treated rat liver S9), standard plate test	5000 µg/plate	P.506006; 98.6	Negative	Hertner (1995a)
Gene mutation	<i>S. typhimurium</i> strains TA98, TA100, TA102, TA1535, TA1537 (± thiamethoxam-treated mouse liver S9), standard plate test	5000 µg/plate	P.506006; 98.6	Negative	Deparade (1999)
Gene mutation	Chinese hamster lung V79 cells <i>hprt</i> locus	2220 µg/ml –S9 3330 µg/ml +S9	P.506006; 98.6	Negative Negative	Ogorek (1996a)
Chromosomal aberration	Chinese hamster ovary cells	2270 µg/ml –S9 4540 µg/ml +S9	P.506006; 98.6	Negative Negative	Zeugin (1996)
Unscheduled DNA synthesis	Primary cultures of male Tif:RAIf rat hepatocytes	1665 µg/ml	P.506006; 98.6	Negative	Ogorek (1996b)
Unscheduled DNA synthesis	Primary cultures of male Tif:MAG mouse hepatocytes	235 µg/ml	P.506006; 98.6	Negative	Ogorek (2000)
In vivo					
Micronucleus formation	Male and female Tif:MAGf mice, bone marrow cells	1000 mg/kg bw (males), 1250 mg/kg bw (females) × 1 po, observations on males at 16, 24 and 48 h and on females at 16 h after dosing	OP235005; 99.4	Negative	Hertner (1995b)

HID, highest ineffective dose; LED, lowest effective dose; po, by mouth (per os); S9, 9000 × g rat or mouse liver supernatant

2.4 Genotoxicity

Thiamethoxam was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 27; all study references are listed in this table). There was no evidence of mutagenic activity in two bacterial reverse mutation assays performed over dose ranges of up to 5000 µg/plate. One of these assays was performed in the presence and absence of an exogenous metabolic activating system (S9 mix) prepared in the standard way from male rats and using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA. The other assay used *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of S9 mix prepared from mice treated with three dose levels of thiamethoxam for 14 days. Also, no mutagenic activity was observed at the *hprt* locus in a single study in the presence and absence of S9 mix with Chinese hamster lung V79 cells, and no clastogenic activity was observed in a single study in the presence and absence of S9 mix with Chinese hamster ovary cells. Unscheduled DNA synthesis activity was measured in two studies with primary cultures of hepatocytes, one from rats and one from mice. No increased DNA repair synthesis was observed in either study.

A single in vivo study was conducted for micronucleus induction in polychromatic erythrocytes from the bone marrow of male and female Tif:MAGf mice treated with thiamethoxam orally by gavage on a single occasion. No increased frequencies of micronucleated cells from thiamethoxam-treated mice were observed.

It is concluded from this battery of tests that thiamethoxam demonstrated no genotoxic or mutagenic activity and is considered unlikely to present a genotoxic hazard to humans.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Two multigeneration studies were conducted on thiamethoxam, the first to comply with regulations in Japan, the second to comply with regulations in the United States of America (USA).

In a two-generation study of reproduction in rats, groups of male and female Sprague-Dawley-derived Tif:RAIf, SPF strain rats were administered thiamethoxam (batch No. P.506006; purity 98.6%) continuously in the diet at a dose level of 0, 10, 30, 1000 or 2500 ppm throughout the two generations. These dietary concentrations were equal to 0, 0.4–1.5, 1.4–4.3, 45.6–144.0 and 117.6–362.9 mg/kg bw per day for males and 0, 0.6–2.1, 1.8–6.4, 59.3–219.6 and 14.8–541.3 mg/kg bw per day for females.

The F₀ generation comprised 30 males and 30 females in each dose group. They received the treated diet for 10 weeks before pairing and throughout mating, gestation, littering and lactation for two litters per generation. Litters were culled to four pups of each sex, where possible, on day 4 post-partum. The F₁ generation was selected from the first litters of the F₀ generation. Clinical signs, body weights, feed consumption, mating, gestation and parturition parameters, pup survival and developmental or behavioural landmarks were recorded. A gross necropsy examination was performed on all pups not selected for mating. All parental animals were necropsied after weaning of the second litters and subjected to macroscopic examination and histopathology of the sex and target organs. Sperm analysis (motility, morphology and spermatid counts) was performed on 15 males per group of the F₀ and F₁ parental generations. Male and female mating and fertility indices, female gestation and parturition indices, and litter live birth, viability and lactation indices were calculated. Continuous data were analysed statistically using analysis of variance (ANOVA) and Dunnett's *t*-test, quantal data by the chi-squared test followed by Fisher's exact test, and non-parametric data by Kruskal-Wallis test followed by the Dunnett test.

In an ancillary sperm cell parameter study, groups of 30 previously unmated male Sprague-Dawley-derived rats (Tif:RAIf, SPF strain, age 6–7 weeks) were fed thiamethoxam in the diet for 10 weeks at a nominal concentration of 0, 10, 30, 1000 or 2500 ppm. Body weight, feed consumption and clinical signs were recorded. After 10 weeks, the animals were killed and necropsied, the testes, epididymides, prostate and seminal vesicles (with coagulating glands) were weighed, and epididymis sperm cells and/or testis spermatids were evaluated for number, motility and morphology. Changes in procedure from the first study were implemented to reduce and standardize the time for sperm collection, to refine the technique for opening the cauda epididymis and to randomize the order in which sperm evaluations were performed to minimize between-day bias.

Analysis of representative diet samples demonstrated that thiamethoxam was stable in the diet for at least 5 weeks at room temperature. Homogeneity varied in the range –4% to +5% of nominal. Analysis of representative diet samples from 7 of 12 mixes showed the overall mean concentrations to be 9.6, 29.1, 1022 and 2590 ppm, in order of increasing concentration.

There were no treatment-related deaths or clinical signs of an adverse reaction to treatment. Body weight gain was significantly reduced by 10.4% throughout the study in males at 2500 ppm. There was no significant effect on weight gain in the other male groups or in any female group, either before mating or during gestation and lactation. Feed consumption was transiently reduced by 7.5%

in males at 2500 ppm during the first 2 weeks of the first pre-mating treatment period, but not later. No other groups of either sex were affected.

There were no treatment-related effects on absolute or relative organ weights at any dose level. Statistically significant, slightly higher relative weights of spleen, heart and liver in males at 2500 ppm are considered to be related to slightly lower terminal body weights and not a direct effect of treatment.

At autopsy, there was no effect on the gross appearance of organs in male or female F_0 parental animals at any dose level. There were no effects on the microscopic appearance of the reproductive organs of F_0 males and females at 2500 ppm or in non-pregnant females and males that failed to mate. An increased incidence of minimal to marked hyaline change in renal tubule cells was observed in male rats at 1000 and 2500 ppm, and a slightly increased incidence of renal tubular casts was observed in males at 2500 ppm. Both findings were attributed to the treatment.

Sperm analyses revealed no treatment-related changes in the concentration of spermatids in testes at any dose level. Sperm morphology was unaffected by treatment.

Sperm motility in all thiamethoxam-treated groups was reduced significantly by approximately 20%. However, variability, reflected in large standard deviations, was high in all groups, indicating probable technical flaws. In view of the absence of effects on spermatid concentration, histological morphology, sperm morphology and fertility during both pairings and the absence of a dose-response relationship for reduced motility, the toxicological relevance of these results is considered equivocal. One technical flaw considered to have contributed to the variability between samples was poor standardization of the interval between sacrifice and sperm evaluation. A flaw probably accounting for the significant differences among groups was the conduct of sperm evaluations in group order rather than in randomized order. In a second sperm analysis study, reported below, procedural changes were implemented to reduce and standardize the time for sperm collection, to refine the technique for opening the cauda epididymis and to randomize the order in which sperm evaluations were performed to minimize between-day bias. This second sperm analysis study showed no effect of treatment on any sperm parameters, suggesting that these apparent changes in sperm motility were not an effect of treatment.

There were no treatment-related differences in reproductive parameters among the groups (Table 28). The number of animals that mated, the pregnancy incidence, the mean time to mating and the number of males and females that failed to mate or mated unsuccessfully were comparable in all treated and control groups for both the first and second matings, with the exception of the group treated at 1000 ppm, which showed slightly lower insemination and pregnancy incidences on both occasions. There was no effect of treatment at any dose level, at either mating, on the duration of gestation or on gestation and parturition indices.

In both F_{1a} and F_{1b} , the numbers of litters were lower in the 1000 ppm group, but were unaffected in either the lower or 2500 ppm dose groups, indicating that this was not a treatment-related response. A consequence was that a lower total number of pups was born, whereas the mean litter sizes were unaffected at the 1000 ppm dose level. Mean litter sizes at birth were slightly, but not significantly, lower than the control group at 2500 ppm in the F_{1a} (13.6 versus 11.4) and F_{1b} (13.8 versus 12.1) generations. In addition, the loss of one litter (nine stillborn pups) was responsible for an increased number of stillborn pups in the F_{1b} 1000 ppm group. No similar observation was made in the F_{1a} 1000 ppm group or in any other group in this study. Mean birth weight was unaffected by treatment at any dose level, but slightly reduced pup weight gain occurred in both F_{1a} and F_{1b} at 2500 ppm during the last 2 weeks of lactation. Sex ratios, viability and lactation indices, clinical signs, achievement of physical/behavioural developmental landmarks and macroscopic findings at necropsy were unaffected by treatment.

There were no treatment-related deaths or clinical signs in the F_1 parental animals, although two rats at 2500 ppm and one at 10 ppm were killed in a moribund condition. The overall weight gain

Table 28. Reproductive parameters: F_0 parental animals

	Dietary concentration (ppm)				
	0	10	30	1000	2500
First mating					
Females placed with males (<i>n</i>)	30	30	30	30	30
Males placed with females (<i>n</i>)	30	30	30	30	30
No. of days until evidence of mating	5.3	4.9	4.0	3.7	4.4
Total inseminated (<i>n</i>)	28	29	29	26	29
Total pregnant (<i>n</i>)	25	28	28	23	27
Pregnant females died or sacrificed moribund (<i>n</i>)	0	0	1	0	0
Duration of gestation (days)	22.1	21.8	22.2	22.0	22.0
Total delivering with liveborn pups (<i>n</i>)	25	28	27	23	27
Second mating					
Females placed with males (<i>n</i>)	30	30	29	30	30
Males placed with females (<i>n</i>)	30	30	29	30	30
Days until evidence of mating	3.3	3.0	4.3	4.1	3.2
Total inseminated (<i>n</i>)	28	29	29	25	29
Total pregnant (<i>n</i>)	24	26	27	22	27
Duration of gestation (days)	22.2	22.0	22.1	21.9	22.2
Pregnant females died or sacrificed moribund (<i>n</i>)	0	0	0	0	0
Total delivering with liveborn pups (<i>n</i>)	24	26	27	22	27

From Doubovetzky (1998)

of the selected F_1 parental rats was unaffected by treatment. The weight gain in male rats was reduced at the end of the first mating at 2500 ppm, but had recovered by the end of the second mating period. The early weight gain deficit reflects the lower starting weight in males of this group (Table 29). Feed consumption was also unaffected by treatment. Minimally higher apparent feed consumption at 2500 ppm, occasionally observed throughout the treatment period, is considered likely to reflect increased wastage.

Organ weights of F_1 parental rats are given in Table 30. Statistically significant increases in relative (to body weight) spleen (10.5%) and liver (9.1%) weights were observed in male rats, but not in females. Absolute spleen and liver weights were not significantly affected by treatment. Combined weights of the testes were significantly lower in 2500 ppm rats compared with controls, but microscopic examination showed no dose–response relationship in the incidences of tubular atrophy. Lower thymus weights occurred in females at 30, 1000 and 2500 ppm, but again there was no histological correlate, suggesting that the differences were incidental to treatment. The thymus histology of all female rats surviving to the study completion was subjected to morphometric assessment for atrophy and reported separately (Weber, 2000e). The analysis revealed mean percentages of thymic cortical area of approximately 65% in all dose groups. There was no statistically significant difference compared with control; in addition, there was no indication of an increasing or decreasing dose-related trend (Weber, 2000e).

The gross appearance of tissues and organs at autopsy in F_1 parental rats was unaffected by treatment. No treatment-related histological lesions were observed in the reproductive organs of parental rats at 2500 ppm or in non-pregnant females and males that failed to mate. Tubular atrophy of the testes was increased in the treated groups, but the incidences were not dose related and were

Table 29. Body weight development: F_1 parental animals

Day	Time point	Parameter	Dietary concentration (ppm)				
			0	10	30	1000	2500
Males							
134	Start premating	Mean body weight (g)	162.0	153.2	163.3	161.6	147.8
204	End 1st premating	Mean body weight (g)	470.2	469.5	456.9	453.6	437.9*
		Cumulative body weight gain (g)	308.2	316.3	293.6	292.0	290.1
		Body weight gain (% of control)	—	+2.6	−4.7	−5.3	−5.9
323	End 2nd postmating	Mean body weight (g)	603.8	612.2	598.2	588.8	589.2
		Cumulative body weight gain (g)	441.8	459.0	434.9	427.2	441.4
		Body weight gain (% of control)	—	+3.9	−1.6	−3.3	−0.1
Females							
134	Start premating	Mean body weight (g)	146.5	134.3	141.7	142.3	134.7
204	End 1st premating	Mean body weight (g)	294.1	281.4	284.4	288.9	279.0
		Cumulative body weight gain (g)	147.6	147.1	142.7	146.6	144.3
		Body weight gain (% of control)	—	−0.3	−3.3	−0.7	−2.2
334	End 2nd lactation	Mean body weight (g)	372.5	357.2	358.7	371.3	369.0
		Cumulative body weight gain (g)	226.0	222.9	217.0	229.0	234.3
		Body weight gain (% of control)	—	−1.4	−4.0	+1.3	+3.7

From Doubovetzky (1998)

* $P \leq 0.05$, ANOVA + Dunnett**Table 30. Organ weights: F_1 parental animals**

Organ	Organ weight ^a									
	Males					Females				
	Dietary concentration (ppm)									
	0	10	30	1000	2500	0	10	30	1000	2500
Carcass										
- Absolute	582	593	578	575	573	350	335	341	342	339
Spleen										
- Absolute	0.88	0.86	0.87	0.85	0.96	0.63	0.62	0.61	0.65	0.64
- Relative	15.19	14.50	15.03	14.96	16.79**	17.99	18.65	18.07	18.96	18.82
Liver										
- Absolute	22.59	22.74	22.12	22.92	24.43	14.21	13.21	13.41	13.74	14.17
- Relative	388.54	382.82	382.77	396.94	423.72**	405.77	394.17	394.01	401.67	418.52
Testes/ovaries										
- Absolute	4.48	4.42	4.24	4.41	4.08*	0.23	0.20**	0.22	0.22	0.23
- Relative	77.36	75.31	74.09	77.30	72.43	6.67	6.11	6.37	6.43	6.77
Thymus										
- Absolute	0.45	0.44	0.40	0.41	0.41	0.32	0.29	0.28*	0.27**	0.26**
- Relative	7.72	7.41	6.98	7.22	7.18	9.19	8.77	8.08	7.80*	7.65**

From Doubovetzky (1998)

* $P \leq 0.05$; ** $P \leq 0.01$, ANOVA + Dunnett^aAll absolute weights in grams; relative organ weights are % of body weight $\times 100$.

considered not to be of toxicological significance. Treatment of males at 1000 or 2500 ppm produced increased incidences of casts and minimal to marked hyaline changes in the renal tubule similar to those observed in F_0 male rats, which have no human significance.

In view of the potential importance of tubular atrophy, this lesion was re-examined. The re-examination involved additional histopathology of the testes from the F_0 and F_1 generation rats in order to discriminate between the minute focal tubular changes and diffuse tubular atrophy. In F_0 rats, the incidence and severity of both findings were similar in control and treated groups. In F_1 rats, there was an increase in the incidence and severity of diffuse tubular atrophy in the group fed 1000 ppm, but not at higher or lower dose levels. The diffuse tubular atrophy of the testes at 1000 ppm, but not at higher or lower dose levels, is unlikely to be treatment related (Dobovetzky, 1999).

Sperm analyses revealed no treatment-related changes at any dose level in morphology or the concentration of spermatids in testes. Sperm motility at 10 and 2500 ppm was reduced significantly by approximately 18%; however, variability, as reflected by large standard deviations, was high in all groups and was probably attributable to technical flaws in the method of sperm analysis, an issue that has been discussed above. In view of the absence of effects on spermatid concentration, histological morphology, sperm gross morphology and fertility during both pairings, the toxicological relevance of these results is considered equivocal, but led to a specific sperm analysis study, reported below. This study revealed no effect of treatment on any sperm parameters.

There were no treatment-related effects on reproductive parameters at any dose level in F_1 parental rats. The number of rats that mated, the pregnancy incidence, the mean time to mating and the number of males and females that failed to mate or mated unsuccessfully were comparable in all treated and control groups at both mating periods, with the exception of the group treated at 30 ppm, which showed slightly lower insemination and pregnancy incidences on both occasions. There was no effect of treatment at any dose level, at either mating, on the duration of gestation or on gestation and parturition indices.

Although F_2 birth weights were unaffected, the weight gain of the F_{2a} pups was slightly reduced throughout lactation at 2500 ppm and minimally reduced during the latter part of lactation for F_{2a} and F_{2b} pups at 1000 and 2500 ppm (Table 31). The observed reduction in pup weight gain was at day 14 after birth, there being no significant differences at day 7. The pups start to consume the same diet as the dam from around day 11 after birth. This indicates that the effect is most likely occurring as a result of direct exposure of the pups to thiamethoxam via diet, rather than via milk while suckling. The effects represent general toxicity associated with the much higher dose (per unit body weight) received by pups during this period compared with that received by the adults and is thus interpreted as not indicative of a developmental effect.

Litter size at birth, mean birth weight, sex ratios, viability and lactation indices, clinical signs and macroscopic findings at autopsy were unaffected by treatment at all dose levels. Whereas achievement of physical/behavioural developmental landmarks was also unaffected by treatment in the F_{2a} groups, in the F_{2b} groups, the mean time to establishment of the surface righting reflex was slightly, but significantly, longer in pups at 2500 ppm (2.3 days) compared with the other groups, including the controls (2.1–2.2 days), possibly reflecting lower body weights in this group (Dobovetzky, 1998).

In the second sperm cell study, the homogeneity of representative diet samples was shown by analysis to be acceptable and in the range from –7% to +6% of nominal. Analysis of representative diet samples showed the overall mean concentrations to be 0, 9.7, 31.8, 1044 and 2540 ppm. Overall achieved dose levels were 0, 0.5–0.7, 1.6–2.3, 53.4–74.8 and 141.5–174.7 mg/kg bw per day, respectively.

There were no treatment-related deaths or clinical signs of an adverse effect of treatment. Significantly lower body weight gains occurred during weeks 1 through 3 at 2500 ppm, resulting in

Table 31. Litter data: F_{2a} and F_{2b} litters

Parameter	Dietary concentration (ppm)				
	0	10	30	1000	2500
F_{2a}					
Number of litters	28	27	25	26	27
Total pups born	387	364	348	359	351
Mean live litter size	13.8	13.3	13.6	13.6	13.0
Live birth index	99.7	98.4	98.0	98.6	100.0
Viability index	97.4	96.6	96.8	99.2	97.7
Sex ratio (% females day 0)	52.6	50.6	54.5	48.0	53.8
Mean pup weight (g)					
- Day 0	6.3	6.2	6.0	6.1	6.1
- Day 4 (pre-cull)	9.3	9.2	8.8	9.0	8.8
- Day 4 (post-cull)	9.5	9.4	9.0	9.1	8.9
- Day 7	15.5	15.0	14.5	14.7	14.4
- Day 14	31.5	30.5	30.2	30.0	28.9**
- Day 21	54.4	52.1	51.9	51.3*	48.7**
F_{2b}					
Number of litters	28	25	21	28	25
Total pups born	396	360	301	387	353
Mean live litter size	14.1	13.8	14.1	13.8	13.9
Live birth index	99.7	96.1	98.3	99.5	98.6
Viability index	97.0	94.8	98.6	93.5	95.1
Sex ratio (% females day 0)	49.6	48.8	53.0	55.3	47.7
Mean pup weight (g)					
- Day 0	6.3	6.1	6.1	6.0	6.2
- Day 4 (pre-cull)	9.2	9.2	9.2	8.8	8.6
- Day 4 (post-cull)	9.3	9.3	9.4	8.9	8.7
- Day 7	15.2	15.1	15.3	14.3	14.1
- Day 14	32.1	31.6	31.5	29.8	30.4
- Day 21	56.5	55.1	54.8	52.0*	52.0*

From Doubovetzky (1998)

* $P \leq 0.05$; ** $P \leq 0.01$ (ANOVA + Dunnett)

a 14% reduction in weight at the end of treatment. Overall weight gain was unaffected by treatment at lower dose levels, although at 1000 ppm, transiently reduced weight gain was observed in week 2 only. Feed consumption was slightly reduced at 2500 ppm during the first 6 weeks of treatment.

There were no treatment-related effects on sex organ weights. Higher mean relative testis and right cauda epididymis weights at 2500 ppm are attributed to the reduced mean exsanguinated body weights of this group.

There was no effect on the gross appearance of organs at autopsy. There were no effects on the proportion of motile sperm, sperm morphology, concentration of spermatids in the testes or number of sperm cells per milligram cauda epididymis fluid (Table 32). The standard deviations calculated for the number of motile epididymal sperm (4–12) were substantially lower than in the original study (14–21), suggesting that technical flaws were the cause of the apparently reduced numbers of motile

Table 32. Sperm parameters

Parameter	Dietary concentration (ppm)									
	0		10		30		1000		2500	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Number used for evaluation</i>	30		30		30		30		30	
Total spermatids ($\times 10^6$ /g testis)	59.6	9.6	58.2	10.7	55.0	10.8	57.5	10.0	55.8	12.9
% abnormal sperm	12.2	6.3	11.7	5.8	11.6	4.9	12.2	5.1	11.0	5.3
Total sperm/mg cauda liquid $\times 10^6$	2.37	0.67	2.67	0.83	2.31	0.88	2.43	0.83	2.39	0.73
% motile sperm cells	73.1	12	75.5	4	73.0	7	73.5	7	74.5	8

From Doubovetzky (1998)

SD, standard deviation

Table 33. Overall mean dose of thiamethoxam received by rats in a two-generation study of reproduction with thiamethoxam

Generation/sex	Time	Mean dose received (mg/kg bw per day)			
		Dietary concentration (ppm)			
		20	50	1000	2500
F ₀ males	Weeks 1–27	1.2	3.0	61.7	155.6
F ₁ males	Weeks 1–29	1.5	3.7	74.8	191.5
F ₁ satellite males	Weeks 1–29	1.4	3.5	71.9	181.1
F ₀ females	Weeks 1–10	1.7	4.3	84.4	208.8
F ₁ females	Weeks 1–10	2.1	5.6	110.1	276.6
F ₀ females	Gestation	1.2	3.1	62.2	158.9
F ₁ females	Gestation	1.4	3.6	71.0	181.8
F ₀ females	Postpartum	3.2	8.3	172.2	415.3
F ₁ females	Postpartum	3.7	9.9	194.2	484.9

From Moxon (2004)

sperm in the multigeneration study. Sperm motility values were consistent with other published data (Chapin et al., 1992) from rats (Doubovetzky, 1998).

The NOAEL for parental toxicity in the multigeneration study in rats was 30 ppm, equal to 1.4 mg/kg bw per day for male rats, based on hyaline change and casts in renal tubules in males at 1000 ppm, equal to 45.6 mg/kg bw per day. This observation has no human relevance. The relevant NOAEL for parental toxicity was 1000 ppm, equal to 45.6 mg/kg bw per day, based on significantly reduced body weight gain at 2500 ppm, equal to 117.6 mg/kg bw per day, in F₀ generation males. The NOAEL for reproductive toxicity in the multigeneration study in rats was 2500 ppm, equal to 117.6 mg/kg bw per day for F₀ males, the highest dose tested. The overall NOAEL for pups and developing offspring was 30 ppm, equal to 1.4 mg/kg bw per day for males, based on reduced body weight gains of pups during F₁ lactation at 1000 ppm, equal to 45.6 mg/kg bw per day for males (Doubovetzky, 1998).

It is concluded that thiamethoxam was not a selective reproductive toxin in this study.

In the second two-generation study of reproduction in rats, groups of male and female Sprague-Dawley-derived Tif:RAIf, SPF strain rats were administered thiamethoxam (batch No. P.506006; purity 98.6%) continuously in the diet at dose levels of 0, 20, 50, 1000 or 2500 ppm throughout the two generations. These dietary concentrations were equal to the doses given in Table 33.

The F_0 generation comprised 26 males and 26 females in each dose group. The F_0 generation received the treated diet for 10 weeks before pairing. The rats were allowed to rear the ensuing F_{1A} litters to weaning. The breeding programme was repeated with F_1 parents selected from the F_{1A} pups to produce the F_{2A} litters, after a 10-week pre-mating period of exposure to thiamethoxam. In addition, 14 males per group in the F_1 generation were selected from the remaining pups and retained to generate histological data on the testis only (and referred to as satellite males). The growth of both parental generations, reproductive function, mating behaviour, conception, gestation, parturition, lactation and weaning and the growth and development of the pups were monitored and recorded.

There was no effect of thiamethoxam on the clinical condition of the F_0 or F_1 parent animals or the F_1 satellite males.

Body weights were lower for the F_0 males given 2500 ppm in comparison with the control group. This effect was accompanied by a reduction in feed consumption and feed utilization during weeks 1–4. The lower body weights of the F_0 males given 1000 ppm were not statistically significantly different from the control body weights (with one exception at week 4) and were not associated with any reduction in feed consumption; they were therefore considered to be of no toxicological significance. No similar effects of 1000 or 2500 ppm thiamethoxam on body weight or feed consumption were observed in the F_1 males or in the F_0 and F_1 females during the pre-mating period, gestation or postpartum. Nevertheless, feed consumption was statistically significantly reduced in the 2500 ppm group in comparison with the control group for week 3 postpartum for the F_0 females with litters. It was noted, however, that feed consumption was similar in the 2500 ppm and 20 ppm groups, suggesting that the reduction is more likely to reflect normal intergroup variation than to be an effect of treatment.

Study of reproductive function indicated that there was no effect of thiamethoxam in either the F_0 or F_1 females on mean cycle length, the number of cycles in a 25-day period, the pre-coital interval, length of the gestation period or mating success (i.e. the production of at least one live pup on the day of littering).

For the F_0 females, the proportion and percentage of successful matings were lower in the 20 and 2500 ppm groups (18/26) in comparison with the control group (21/26), but were not statistically significantly different. Also, there was no effect of thiamethoxam on the proportion of successful matings of the F_1 females.

Up to five whole-litter losses occurred in each group of the F_0 and F_1 generations. Four whole-litter losses occurred in the F_0 control group, whereas none occurred in the F_1 control group. The incidence of litters affected was not dose related and was considered to be incidental to treatment with thiamethoxam.

There was no effect of thiamethoxam on the proportion or percentage of liveborn pups or on the proportion of litters with all pups born alive for either the F_{1A} or F_{2A} litters. No treatment-attributable effect on mean litter size or pup survival on day 1, 5, 8, 15 or 22 was observed for either the F_{1A} or F_{2A} litters.

With the exclusion of whole-litter losses, the proportions of F_{1A} and F_{2A} pups surviving in the 50, 1000 and 2500 ppm groups were comparable with those of the control groups; the lowest percentages of pups surviving in each generation were recorded for the 20 ppm groups. With the inclusion of whole-litter losses, the proportions of pups surviving in the 50, 1000 and 2500 ppm groups were higher than that in the control group for the F_{1A} generation and lower than that in the control group for the F_{2A} generation (where there were no whole-litter losses in the control group). In the absence of a dose-response relationship, the lower pup survival in the 20 ppm groups was considered to be unrelated to treatment.

There was no effect of thiamethoxam on the sex ratio, body weights or clinical condition of the F_{1A} or F_{2A} pups.

On days 15 and 22, total litter weight of the F_{1A} pups was statistically significantly lower in the 2500 ppm group in comparison with the control group. This was due to a slightly smaller litter size in the 2500 ppm group combined with a slightly lower pup body weight (rather than a slightly higher pup body weight typically associated with smaller litters). As the effect was not also observed earlier in postnatal development, it was concluded that the slightly lower total litter weight was due to the direct consumption of diet containing 2500 ppm by the pups, rather than a developmental effect. However, there were no statistically significant differences in total litter weight for the F_{2A} pups.

There was no effect of thiamethoxam on the mean day of age when preputial separation or vaginal opening occurred.

Postmortem examination of organ weights of parental rats showed that there was no effect of thiamethoxam on the weight of the brain, right cauda epididymis, ovaries, prostate gland, seminal vesicles or uterus with cervix.

For the F_0 males, relative (to body weight) adrenal and kidney weights were statistically significantly greater in the 2500 ppm group in comparison with the control group; however, no statistically significant differences in these relative organ weights were observed for F_0 females, F_1 males or F_1 females in any of the thiamethoxam-treated groups.

Liver weights were significantly increased in F_0 male rats of the 1000 ppm group, and relative liver weights were significantly increased in F_1 male and F_1 female rats of the 2500 ppm group. No other statistically significant liver differences were recorded. Kidney weight relative to body weight was significantly increased in F_0 males of the F_0 generation, but not in the F_1 generation.

Pituitary weights were significantly increased in F_0 females of the 1000 and 2500 ppm groups, but not in any other group.

The mean spleen weight was notably higher for the F_0 males given 2500 ppm, but this was due to one male (number 120) with an enlarged spleen (weight 4.569 g). For the F_1 males given 2500 ppm, the relative (to body weight) spleen weight was statistically significantly greater than in the control group.

Absolute and relative (to body weight) weights of the epididymides were unaffected by treatment in the F_0 groups. In the F_1 generation, however, the absolute weight of the combined epididymides was statistically significantly greater in the 2500 ppm group than in the control group, and the relative weights of the combined epididymides were statistically significantly greater than in the control group in both the 1000 and 2500 ppm groups.

Testis weights were unaffected by treatment in the F_0 generation, but they were significantly increased, relative to body weight, in the F_1 20, 1000 and 2500 ppm groups, but not in the 50 ppm group. Comparison of the F_0 and F_1 control group testis weights would suggest that a reason for the significantly higher relative weights in the F_1 generation 20 and 1000 ppm groups was a particularly low control group testis weight (Table 34).

Sperm parameter data were analysed with and without the males listed in Table 35, which, upon gross examination, were found to have an abnormal testis and/or cauda epididymis and presented poor sperm samples. The data excluding the abnormal sperm samples are probably the more appropriate for evaluation, because the distribution of the males with gross abnormalities of these organs showed no dose–response relationship.

There was no effect of thiamethoxam on the number of sperm in the right testis of the F_0 males, but for the F_1 males given 50, 1000 or 2500 ppm, the total number of sperm and the number of sperm per gram of right testis were statistically significantly lower than in the control group.

There was no effect of thiamethoxam on the number of sperm in the right cauda epididymis of the F_0 males, but for the F_1 males given 2500 ppm, the total number of sperm and the number of sperm per gram of right cauda epididymis were statistically significantly higher than in the control group.

Table 34. Organ weights (adjusted for final body weight) in male rats of a two-generation dietary study of reproduction with thiamethoxam

Organ	Adjusted organ weight (g)				
	Dietary concentration (ppm)				
	0	20	50	1000	2500
F₀					
Kidney	3.00	3.03	3.01	2.95	3.11*
Testis	4.13	4.03	4.25	4.04	4.18
Epididymis	1.638	1.545	1.619	1.582	1.674
F₁					
Kidney	2.83	2.88	2.85	2.81	2.85
Testis	3.90	4.12*	4.01	4.13*	4.21**
Epididymis	1.584	1.617	1.615	1.656*	1.668*

From Moxon (2004)

* $P < 0.05$; ** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)

Table 35. Abnormal testis samples observed in a two-generation dietary study of reproduction in rats with thiamethoxam

Dietary concentration (ppm)	Male number	Comments
F₀		
0	8	Right cauda reduced, no sperm visible. Testis reduced, few sperm visible.
20	34	Right cauda reduced, no sperm visible. Testis reduced, few sperm visible.
	48	Right cauda reduced, no sperm visible. Testis reduced, few sperm visible.
1000	80	Right cauda reduced, no sperm visible. Testis reduced, few sperm visible.
	101	Right cauda reduced, no sperm visible. Testis reduced, few sperm visible.
2500	109	Right cauda reduced, no sperm visible. Testis reduced, few sperm visible.
	117	Right cauda reduced, few sperm visible. Testis reduced, few sperm visible. Sperm abnormal.
	129	Mass on right cauda. Sperm abnormal.
F₁		
2500	122	Right cauda reduced. Sperm abnormal.

From Moxon (2004)

There was no effect of thiamethoxam on the percentage of motile sperm in any treatment group.

For the F₀ males, there was no effect of thiamethoxam on straight line, curvilinear or average path velocities. For the F₁ males given 2500 ppm, the straight line, curvilinear and average path velocities were statistically significantly lower in comparison with the control group (Table 36). There was no effect of thiamethoxam on straight path velocities.

Table 36. Sperm velocity in F_1 parent males of a two-generation study of reproduction in rats with thiamethoxam

Observation	Dietary concentration (ppm)				
	0	20	50	1000	2500
Curvilinear velocity	305.0	297.2	302.4	297.7	289.6**
Average path velocity	123.9	122.1	123.1	120.4	116.2**

From Moxon (2004)

** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)

Gross postmortem examination revealed no abnormalities in any tissue from the F_0 or F_1 rats that could be attributed to treatment with thiamethoxam. Furthermore, there was no significant difference in the number of uterine implantation sites or postimplantation losses for either the F_0 or F_1 rats.

Microscopic examination of the kidneys revealed increased tubule cell hyaline droplet formation in the majority of F_0 males given 2500 ppm thiamethoxam and in a few given 1000 ppm. Similar findings were observed in F_1 males, except that the incidence in F_1 males given 1000 ppm (17/26) was much higher than in the F_0 generation (3/26). This type of change was not observed in males given 20 ppm or 50 ppm in either generation or in any females. Other minor (i.e. minimal or slight) kidney changes observed at an increased incidence in males given 2500 ppm were hyaline eosinophilic casts, tubular dilatation with granular eosinophilic casts at the cortico-medullary junction, tubule cell basophilia and interstitial mononuclear cell infiltration. Slight increases in the incidences of hyaline eosinophilic casts and tubule cell basophilia compared with controls were also observed in F_1 males given 1000 ppm.

Germ cell loss, recorded under four morphologies, according to degree and distribution, was observed in the testis of some treated and control males. As the four categories clearly represented variable degrees of a single pathological process, they were merged to a single finding (i.e. germ cell loss/disorganization with or without Sertoli cell vacuolation) for the purposes of tabulation and evaluation.

An increased incidence (14/26) of minimal germ cell loss/disorganization with or without Sertoli cell vacuolation was observed in the testis of F_1 males given 2500 ppm compared with the control and lower dose groups (1/26 – 3/26). The change was extremely minor, affecting a few scattered tubules, minimal change being defined as affecting an average of not more than 10 tubules per section for the four sections examined from each testis. It was characterized by variable germ cell loss ranging from complete depletion (i.e. Sertoli cell-only tubules) to minimal loss of a few germ cells in one segment of a tubule cross-section. In some instances, partial germ cell loss was associated with germ cell disorganization and Sertoli cell vacuolation, indicating a degenerative process. The change was not associated with any discernible reduction in epididymal sperm or increase in epididymal desquamated germ cells. The average total number of tubule cross-sections affected in all four sections per testis examined from affected males receiving 2500 ppm was 10.2, which represents approximately 0.4% of tubule cross-sections in four average testis sections. This calculation is based on an approximate count of tubule cross-sections in the four standard testis sections from a representative control male conservatively estimated at 2500. The change was most frequently observed in regions adjacent to the rete testis.

A small number of F_0 males given 20 ppm (2/26) or 2500 ppm (3/26) showed severe germ cell loss, affecting nearly all tubules, which amounted to nearly total atrophy of the tubules. These were considered to be spontaneous lesions, unrelated to treatment, because the incidence was low, unrelated to dose and not repeated in the F_1 generation and the changes were identical to those observed in control rats of this strain (quoted in Moxon, 2004).

Table 37. Incidence of rats with testicular tubules showing germ cell loss or disorganization, with or without Sertoli cell vacuolation

	Dietary concentration (ppm)				
	0	20	50	1000	2500
Total number of F ₁ males (main study + satellites)	40 (26 + 14)	40 (26 + 14)	40 (26 + 14)	40 (26 + 14)	40 (26 + 14)
Main study: unilateral/bilateral status unknown	3/26	1/26	1/26	3/26	15/26
Satellites: unilateral	1/14	4/14	2/14	3/14	0/14
Satellites: bilateral	1/14	0/14	0/14	1/14	5/14
Total incidence	5/40	5/40	3/40	7/40	20/40

From Moxon (2004)

For the main study males, only one testis was available for histological examination, and so it was not possible to determine whether changes were unilateral or bilateral. For this reason, a satellite group of F₁ males was used from which both testes were available. Minimal testicular tubule changes were observed as on the main study. However, a treatment-related increase in incidence of germ cell loss/disorganization with or without Sertoli cell vacuolation was confined to minimal bilateral change at 2500 ppm only. The average number of tubule cross-sections affected per testis in the four sections per testis examined from satellite males receiving 2500 ppm thiamethoxam that showed bilateral change was 9.1—that is, similar to the corresponding figure (10.2) from the main study F₁ males and again representing approximately 0.4% of tubule cross-sections in the four sections examined.

Unilateral change (in the satellite groups) was observed at a low incidence and unrelated to dose in control and treated groups, suggesting that, in contrast to the bilateral change, this was a spontaneous lesion. As mentioned above, it was not possible to distinguish between unilateral and bilateral changes in the main study rats because only one testis was available for examination. Therefore, the incidence of lesions reported for the main study rats represents a summation of spontaneous and treatment-related lesions.

The testicular histology data from main study F₁ and satellite F₁ males are summarized in Table 37 and show the overall incidence of germ cell loss/disorganization with or without Sertoli cell vacuolation in both groups. When the incidences of change in both groups are added together, regardless of unilateral or bilateral status, the data confirm that there is an increase at 2500 ppm thiamethoxam, with a clear no-effect level at 1000 ppm. Although the incidence was clearly increased at 2500 ppm, the nature and severity of the lesions were identical to those observed at a low incidence in control and lower dose group F₁ animals and in all groups of F₀ males.

No changes were detected in any other tissue from F₀ or F₁ rats that could be attributed to treatment with thiamethoxam. Also, the numbers of small follicles in the ovaries of F₁ females receiving 2500 ppm thiamethoxam were comparable to the numbers observed in control females.

Postmortem examination of the F_{1A} or F_{2A} pups did not reveal any gross morphological changes or brain, spleen or thymus weight changes that could be ascribed to thiamethoxam treatment.

The NOAEL for parental toxicity in the second multigeneration study in rats was 50 ppm, equal to 3.0 mg/kg bw per day for male rats, based on hyaline change and casts in renal tubules in males at 1000 ppm, equal to 61.7 mg/kg bw per day. This observation has no human relevance. The relevant NOAEL for parental toxicity was 1000 ppm, equal to 61.7 mg/kg bw per day, based on significantly reduced body weight gain at 2500 ppm, equal to 155.6 mg/kg bw per day, in F₀ generation males.

The NOAEL for reproductive toxicity in the multigeneration study in rats was 1000 ppm, equal to 74.8 mg/kg bw per day for F₁ males, based on minimal testicular germ cell loss or disorganiza-

tion, with or without Sertoli cell vacuolation (and unaccompanied by any reduction in epididymal sperm numbers), at 2500 ppm, equal to 191.5 mg/kg bw per day. These effects were not observed in the earlier study (Dobrovetzky, 1998, 1999), a difference that could be attributed to a refinement of the methods used in Moxon (2004) for opening the cauda epididymis and randomizing the order in which sperm observations were performed, in order to minimize between-day bias in the ancillary sperm cell parameter study, in which only F₀ males were investigated.

The NOAEL for pups and developing offspring was 1000 ppm, equal to 74.8 mg/kg bw per day for males and 110.1 mg/kg bw per day for females, based on reduced body weight gains of pups during F₁ lactation at 2500 ppm, equal to 191.5 mg/kg bw per day for males and 276.6 mg/kg bw per day for females (Moxon, 2004).

It is concluded that thiamethoxam was not a selective reproductive toxin in this study.

(b) *Developmental toxicity*

Rats

Groups of 24 mated female Sprague-Dawley rats (Tif:RAIf, SPF strain, at least 8 weeks old, supplied by Ciba-Geigy Ltd, Switzerland) were treated orally, by gavage, with thiamethoxam (batch No. P.506006; purity 98.6%) in 0.5% aqueous carboxymethylcellulose at a dose level of 0, 5, 30, 200 or 750 mg/kg bw from day 6 through 15 of gestation (day 0 = plug or sperm observed in vaginal smear). Mortality was recorded twice daily, and clinical signs and body weight were recorded daily. Feed consumption was measured throughout gestation. Dams were killed on day 21 and the fetuses delivered by caesarean section. The uterine tract and contents were removed and weighed. Gross examination was made of main organs of the thoracic and abdominal cavities, and ovaries (including corpora lutea count), uteri (implantation site count) and placentae were examined macroscopically. Fetuses were sexed, examined for external malformations and variations and then weighed. Approximately one half of the fetuses from each litter were examined for soft tissue malformations and variations after fixation in Bouin's solution and micro-dissection of the head, thoracic and abdominal viscera. The remaining fetuses were subjected to skeletal evaluation using Dawson's technique and examined for skeletal malformations, anomalies and variations. A malformation was defined as a very rare, permanent structural change that may adversely affect fetal survival, development or function. A distinction was made between skeletal anomalies and skeletal variations. A skeletal anomaly is a rare, slight to moderate, permanent or reversible structural change that is not considered to impair fetal survival, development or function. A variation is a relatively frequent, transient structural deviation from normal development that is considered not to have any detrimental effect on fetal survival, development or function. Variations occur regularly in fetuses from untreated rats. Continuous data were analysed statistically using ANOVA and Dunnett's *t*-test, quantal data by the chi-squared test followed by Fisher's exact test, and non-parametric data by Kruskal-Wallis non-parametric ANOVA followed by the Mann-Whitney U test.

Analysis of formulations demonstrated that thiamethoxam was stable in the vehicle and was homogeneously distributed (relative standard deviations in the range -4.0% to +5.0%). Analysis showed mean achieved concentrations to be 98.1%, 94.7%, 95.0% and 99.5% of the nominal values, in order of increasing dose level.

No treatment-related deaths occurred, but one dam at 750 mg/kg bw per day was killed on day 9 for humane reasons. Transient hypoactivity, piloerection and, in two females, regurgitation of dosing material were the only clinical findings related to treatment. Maternal body weight gain (uncorrected for the uterus and its contents) was moderately depressed during the treatment period at 750 mg/kg bw per day, and body weight change was decreased (-46.5%) at this dose and minimally decreased (-15.8%) at 200 mg/kg bw per day over treatment days 6-15. No effects on body weight were observed at lower dose levels. Also, no significant body weight reductions were observed when

Table 38. Intrauterine data from rats treated with thiamethoxam in a developmental toxicity study

	Dose (mg/kg bw per day)				
	0	5	30	200	750
Dams with implants	22	23	23	22	23
Dams with viable fetuses	22	23	23	22	22
Dams with resorptions (%)	8 (33)	9 (38)	6 (25)	5 (21)	6 (25)
Dams with affected implants ^a (%)	8 (33)	9 (38)	6 (25)	5 (21)	7 (29)
Corpora lutea/dam	15.5 ± 2.2 ^b	14.7 ± 2.8	15.0 ± 2.2	16.0 ± 1.7	15.4 ± 2.8
Implants/dam	14.5 ± 3.2	13.5 ± 2.6	14.4 ± 2.1	14.9 ± 1.4	14.3 ± 3.9
Preimplantation loss ^c (%)	7.1 ± 16.5	8.3 ± 7.0	3.5 ± 6.8	6.7 ± 7.6	9.3 ± 19.5
Postimplantation loss ^d (%)	3.3 ± 4.9	3.1 ± 4.2	2.9 ± 5.3	2.2 ± 4.4	2.8 ± 5.3
Live fetuses/dam	14.0 ± 3.1	13.1 ± 2.7	14.0 ± 2.0	14.6 ± 1.6	13.9 ± 4.0
Dead fetuses/dam	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Resorptions/dam: early	0.5 ± 0.7	0.4 ± 0.5	0.4 ± 0.8	0.3 ± 0.6	0.4 ± 0.7
Resorptions/dam: late	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
Malformed live fetuses ^e (%)	0.0 ± 0.2	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Affected implants ^f (%)	3.7 ± 4.9	3.1 ± 4.2	3.6 ± 5.4	2.2 ± 4.4	2.8 ± 5.3
Sex ratio (% females)	48.9	57.0 ± 0.2	51.9 ± 1.0	46.4 ± 1.0	52.8 ± 1.0
Mean pup body weight (g)	5.3 ± 0.3	5.3 ± 0.3	5.2 ± 0.3	5.2 ± 0.3	4.8** ± 0.3
Reproductive tract weight (g)	99.0 ± 21.8	93.7 ± 15.8	98.3 ± 13.6	102.6 ± 11.4	90.2 ± 24.2
Uncorrected body weight (g)	360.1	358.9	358.5	354.8	335.4*
Corrected body weight ^g (g)	261.1	265.2	260.2	252.2	245.2*
Corrected body weight change: gestation ^h (g)	63.3	68.8	64.2	55.7	49.2
Uncorrected body weight change: treatment (g)	63.4	62.0	63.8	53.4**	33.9**
Corrected body weight change: treatment ⁱ (g)	33.2	37.3	33.7	26.1	31.0

From Winkler (1996a)

* $P \leq 0.05$; ** $P \leq 0.01$ (two-tailed)^a Affected implants include dead fetuses, resorptions and malformed live fetuses.^b Mean ± standard deviation. Mean value determined for each female.^c 100 minus the percentage of corpora lutea that are reflected in the implantation sites.^d 100 minus the percentage of implants that are viable at the time of intrauterine inspection.^e (Number of malformed live fetuses/total live fetuses) × 100.^f [(Dead fetuses + resorptions + malformed live fetuses)/total implants] × 100.^g Body weight (gestation day 21) – weight of reproductive tract plus contents.^h Body weight (gestation day 21 – gestation day 0) – weight of reproductive tract plus contents.ⁱ Body weight (gestation day 21 – gestation day 7) – weight of reproductive tract plus contents.

maternal body weights corrected for uterine contents were compared for the treatment period. Feed consumption was depressed during the treatment period at 750 mg/kg bw per day.

Pregnancy incidence, mean number of corpora lutea, preimplantation loss, number of implantation sites, early and late postimplantation losses, mean numbers of live fetuses and the sex ratio were unaffected by treatment at all dose levels (Table 38). A significant reduction in the mean body weight of live fetuses occurred at 750 mg/kg bw per day. This was attributed to the maternal toxicity (reduced body weight gain) observed at this dose level.

In view of the possibly treatment-related thymus atrophy in the F₁ females in one of the two-generation rat studies described above (Dobrovetsky, 1998; Weber, 2000e), the thymus weights of

Table 39. Incidence of malformations and treatment-related anomalies and variants in rats treated with thiamethoxam in a developmental toxicity study

	Dose (mg/kg bw per day)				
	0	5	30	200	750
Litters evaluated	22	23	23	22	22
Fetuses evaluated	309	302	322	321	305
Malformations					
External malformations					
- Runt fetus	1	0	2	0	0
- Mouth: agnathia	0	0	1	0	0
- Skin: generalized oedema	1	0	0	0	0
Visceral malformations					
- Diaphragmatic hernia	0	0	0	0	1
Skeletal malformations					
- Unossified mandibula and os pubis	0	0	1	0	0
Total no. of fetuses with any malformation	1	0	2	0	1
Skeletal anomalies					
Total no. (%) of fetuses with any anomaly	23 (14.9)	14 (9.3)	16 (10.1)	12 (7.2)	40 (26.7)
No. (%) of fetuses with:					
- Asymmetric sternebra 6	4 (2.6)	3 (2.0)	2 (1.3)	3 (1.8)	11 (7.3)
- Irregular/absent ossification of occipital bone	3 (1.9)	0	2 (1.3)	2 (1.2)	12 (8.0)*
Skeletal variants					
Total no. (%) of fetuses with any variant	154 (100)	150 (100)	158 (100)	166 (100)	150 (100)
No. (%) of fetuses with:					
- Poor ossification of sternebra 5	0	0	1 (0.6)	0	10 (6.7)**
- Shortened 13th rib	13 (8.4)	5 (3.3)	4 (2.5)*	21 (12.7)	27 (18.0)*
- Absent ossification of metatarsal 1	15 (9.7)	31 (20.7)*	24 (15.2)	16 (9.6)	51 (34.0)**

From Winkler (1996a)

* $P < 0.05$; ** $P \leq 0.01$

the fetuses were recorded (Dobrovetzky, 2000). No specific effect on the prenatal development of the thymus was identified.

The incidence and type of external, visceral and skeletal malformations were not affected by treatment at any dose level (Table 39). Increased incidences of skeletal anomalies and variants occurred at 750 mg/kg bw per day. Treatment-related anomalies were asymmetric sternebrae and irregular, poor or absent ossification of the occipital bone. Other commonly occurring skeletal anomalies were present at similar incidences in all treated and control groups. Skeletal variants were recorded in all fetuses from all dose groups. Treatment-related increases in the incidence of skeletal variants occurring at 750 mg/kg bw per day were poor ossification of sternebra 5, shortened 13th rib, absent ossification of metatarsal 1, and poor or absent ossification of one or more phalanges (commonly absence of proximal phalanges, incidences not shown in Table 39). These findings are considered to represent a treatment-related delay of ossification, secondary to reduced pup weight, in turn a reflection of maternal toxicity. The incidences of skeletal variants were unaffected by treatment at dose levels lower than 750 mg/kg bw per day (Winkler, 1996a).

The NOAEL for maternal toxicity in rats was 30 mg/kg bw per day, based on slightly decreased body weight gain in dams, providing a lowest-observed-adverse-effect level (LOAEL) of 200 mg/kg

bw per day. The NOAEL for fetotoxicity was 200 mg/kg bw per day, based on mild reduction in mean fetal body weight at 750 mg/kg bw per day. Further evidence of fetotoxicity at this dose was increased incidences of skeletal anomalies (irregular or absent ossification of the occipital bone) and skeletal variants (poor ossification of sternebra 5, shortened 13th rib and non-ossification of metatarsal 1). Thiamethoxam was not teratogenic in the developmental toxicity study in rats (Winkler, 1996a).

Rabbits

Groups of 19 mated female rabbits (Russian Chbb:HM strain, at least 3 months old) were treated orally, by gavage, with thiamethoxam (batch No. P.506006; purity 98.6%) in 0.5% aqueous carboxymethylcellulose from day 7 through day 19 of gestation (day 0 = plug or sperm in smear) at a daily dose level of 0, 5, 15, 50 or 150 mg/kg bw per day. Females were observed twice daily for mortality and once daily for clinical signs. Body weights were recorded daily. Feed consumption was recorded on days 4, 7, 12, 16, 20, 24 and 29. Dams were killed on day 29 and the reproductive tract plus contents removed by hysterectomy and weighed. Examinations were made of the main organs of the thoracic and abdominal cavities as well as ovaries, uteri and placentae, and the numbers of corpora lutea were counted. In dams that died or were killed before scheduled autopsy, the number and location of implantation and/or abortion sites in the uterus were recorded. In dams killed at scheduled autopsy, the number and location of live and dead fetuses or early and late (embryonic or fetal) losses were recorded. Fetuses were numbered, tagged individually, weighed, sexed and examined for external malformations and variations. All fetuses were examined for thoracic/abdominal soft tissue or skeletal malformations, anomalies and variations. A distinction was made between skeletal anomalies and skeletal variations, as defined above (see section on rats). Approximately one half of the fetuses were examined for cranial soft tissue malformations. All fetal trunks and approximately half of the fetal heads per litter were assigned to skeletal assessment according to the staining technique of Dawson. Continuous data were analysed statistically using ANOVA and Dunnett's *t*-test, quantal data by the chi-squared test followed by Fisher's exact test, and non-parametric data by Kruskal-Wallis non-parametric ANOVA followed by the Mann-Whitney U test.

Analysis of dose formulations showed that the test article was stable in the vehicle and homogeneously dispersed (−2% to +3% mean concentration). Overall mean achieved concentrations were within the range 97.2–101.4% of the nominal value.

Three rabbits treated at 150 mg/kg bw per day died or were killed for humane reasons at the end of the treatment period or shortly thereafter. Treatment-related clinical signs at 150 mg/kg bw per day comprised a bloody discharge in the perineal area of 13 rabbits, including all those that died or were killed. Deaths and clinical signs of adverse effects of treatment were not apparent at lower dose levels. Overall mean body weight was markedly reduced by treatment at 150 mg/kg bw per day, due primarily to weight loss during the early part of the treatment period (Table 40). Weight gain reduction at 50 mg/kg bw per day during treatment was minimal and non-significant. Weight gain recovery at 150 mg/kg bw per day was rapid in the post-treatment period on days 20–29 of gestation. Feed consumption was significantly and dose-dependently reduced in the 50 and 150 mg/kg bw groups during the treatment period (days 7–12 and 12–16) and from days 16 to 20 in the 150 mg/kg bw per day group. A significant, compensatory increase in feed consumption was recorded in the 150 mg/kg bw per day group in the post-treatment period (Table 40). Feed consumption and body weight gain were unaffected by treatment at lower dose levels.

At autopsy, the uterine contents of the three rabbits that died or were killed before schedule in the 150 mg/kg bw per day group were haemorrhagic, and one rabbit also showed a haemorrhagic vagina. There were no other treatment-related autopsy findings.

Total resorption occurred in three rabbits at 150 mg/kg bw per day and in one rabbit at 50 mg/kg bw per day. Therefore, there was a treatment-related reduction in the number of rabbits with live fetuses and a concomitant increase in postimplantation loss at 150 mg/kg bw per day. The mean pup

Table 40. Mean body weight gain and feed consumption in rabbits treated with thiamethoxam in a developmental toxicity study

	Dose (mg/kg bw per day)				
	0	5	15	50	150
Mean body weight (g)					
- Day 0	2733	2833	2840	2833	2718
- Day 7	2726	2854	2834	2819	2740
- Day 20	2778	2899	2873	2838	2667
- Day 29	2840	2998	3001	2952	2777
Mean body weight gain (g)					
- Days 0–6 (pretreatment)	–7	21	–6	–14	22
- Days 7–12 (treatment)	–26	–23	–17	–30	–105**
- Days 12–16 (treatment)	57	69	73	47	38
- Days 16–20 (treatment)	21	–2	–17**	3	–6
- Days 20–24 (post-treatment)	–2	19	20	24	28
- Days 24–29 (post-treatment)	64	81	108	91	83
- Overall (gestation period)	107	165	161	119	59
Feed consumption (g/day)					
- Days 0–4 (pretreatment)	93.5 ± 14.4 ^a	94.1 ± 19.1	91.6 ± 17.4	95.4 ± 10.0	98.3 ± 11.4
- Days 4–7 (pretreatment)	88.6 ± 11.2	97.9 ± 20.0	95.3 ± 12.6	92.0 ± 13.2	100.4 ± 17.3
- Days 7–12 (treatment)	88.7 ± 14.1	92.4 ± 15.1	88.5 ± 15.8	69.2** ± 9.9	21.4** ± 9.4
- Days 12–16 (treatment)	86.7 ± 13.6	91.2 ± 17.4	79.1 ± 20.7	64.2** ± 19.1	38.5** ± 21.0
- Days 16–20 (treatment)	96.3 ± 21.7	95.9 ± 20.2	88.5 ± 22.0	80.6 ± 20.6	56.8** ± 27.7
- Days 20–24 (post-treatment)	96.3 ± 28.7	99.2 ± 21.1	93.1 ± 17.2	100.9 ± 21.2	125.1* ± 16.6
- Days 24–29 (post-treatment)	93.4 ± 26.1	96.8 ± 20.4	100.3 ± 14.5	103.3 ± 23.9	121.6* ± 17.2

From Winkler (1996b)

* $P \leq 0.05$; ** $P \leq 0.01$ ^a ± standard deviation.

weights of both sexes were reduced as a result of treatment at 150 mg/kg bw per day. Gravid uterus weight, number of corpora lutea, preimplantation loss, number of implantation sites and number of dead fetuses were unaffected by treatment (Table 41).

No external or skeletal fetal malformations occurred in the study, and no treatment-related visceral malformations were evident. In the 0, 5, 15, 50 and 150 mg/kg bw per day groups, respectively, there were 0, 1, 2, 0 and 1 fetuses with flexure of the forepaw, probably due to restriction of movement within the uterus; it is not a malformation, and the incidence was not dose related. The distribution of visceral malformations did not indicate an effect of treatment at any dose level. Small gall bladder was noted in some groups, but the incidence was haphazard and did not show any dose–response relationship. The fetal incidence of the skeletal anomaly fused sternbrae was increased at 150 mg/kg bw per day, but not at lower dose levels. This is considered to be treatment related as a consequence of reduced birth weight, which is in turn a reflection of maternal toxicity. The distribution of other skeletal anomalies did not indicate an effect of treatment. Skeletal variants occurred in a majority of fetuses from most litters in all treatment groups. The overall fetal incidences were 70.9%, 78.4%, 79.4%, 79.5% and 75.6% in order of increasing dose level. One skeletal variation, absent ossification of the medial phalanx of anterior digit 5, occurred at a fetal incidence of 8.9% at 150 mg/kg bw per day, whereas the control group incidence was 0%. Although the observed incidence was

Table 41. Intrauterine data from rabbits treated with thiamethoxam in a developmental toxicity study

	Dose (mg/kg bw per day)				
	0	5	15	50	150
Does with implants	15	19	19	19	18
Does with live fetuses	15	19	19	18	12
Does with resorptions (%)	9 (47)	4 (21)	9 (47)	8 (42)	13 (68)
Does with affected implants ^a (%)	10 (53)	8 (42)	10 (53)	10 (53)	16 (84)
Corpora lutea/doe	6.7 ± 1.2 ^b	7.2 ± 1.2	7.2 ± 1.4	6.9 ± 1.3	7.1 ± 1.6
Implants/doe	4.7 ± 2.1	5.4 ± 1.2	5.9 ± 1.7	5.4 ± 2.0	5.4 ± 2.1
Preimplantation loss ^c (%)	32.6 ± 25.2	23.1 ± 21.7	17.5 ± 12.2	23.7 ± 18.4	25.1 ± 19.0
Postimplantation loss ^d (%)	21.0 ± 24.3	6.3 ± 16.1	9.9 ± 12.8	16.3 ± 25.9	45.6 [§] ± 35.4
Live fetuses/doe	3.7 ± 2.2	5.1 ± 1.5	5.4 ± 1.7	4.6 ± 2.2	3.0 ± 2.4
Dead fetuses/doe	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Resorptions/doe: early	1.0 ± 1.1	0.3 ± 0.6	0.5 ± 0.7	0.6 ± 1.0	2.4 ± 2.7
Resorptions/doe: late	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.2 ± 0.5	0.0 ± 0.0
Malformed live fetuses ^e (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Affected implants ^f (%)	1.0 ± 1.1	0.3 ± 0.6	0.6 ± 0.7	0.8 ± 1.1	2.4 ± 2.7
Sex ratio (% females)	43.6	50.5	54.9	46.5	51.1
Mean pup body weight (g)					
- All	44.0 ± 4.3	41.5 ± 3.2	41.7 ± 2.9	42.1 ± 3.9	37.5** ± 4.5
- Males	44.4 ± 4.9	41.7 ± 3.6	43.0 ± 3.3	42.2 ± 4.1	38.8** ± 4.9
- Females	41.8 ± 2.0	40.9 ± 3.2	40.8 ± 3.2	41.1 ± 4.3	36.6** ± 5.4
Reproductive tract weight (g): all	226 ± 115	303 ± 82	316 ± 80	294 ± 99	210 ± 100
Corrected body weight ^g	2614	2695	2685	2658	2567
Corrected body weight change: gestation ^h	-119	-138	-155	-175	-151
Corrected body weight change: treatment ⁱ	-112	-159	-149	-161	-173

From Winkler (1996b)

[§] Not significant ($P = 0.386$); ** $P \leq 0.01$ ^a Affected implants include dead fetuses, resorptions and malformed live fetuses.^b Mean ± standard deviation. Mean value determined for each female.^c 100 minus the percentage of corpora lutea that are reflected in the implantation sites.^d 100 minus the percentage of implants that are viable at the time of intrauterine inspection.^e (Number of malformed live fetuses/total live fetuses) × 100.^f [(Dead fetuses + resorptions + malformed live fetuses)/total implants] × 100.^g Body weight (gestational day 29) – weight of reproductive tract plus contents.^h Body weight (gestational day 29 – gestational day 0) – weight of reproductive tract plus contents.ⁱ Body weight (gestational day 29 – gestational day 7) – weight of reproductive tract plus contents.

within the historical control range of 0–9.2%, the finding may be an indication of treatment-related delayed ossification (Table 42) (Winkler, 1996b).

The NOAEL for maternal toxicity in rabbits was 15 mg/kg bw per day, based on reduction in body weight gain and feed consumption during the treatment period in dams at 50 mg/kg bw per day. The NOAEL for fetotoxicity in rabbits was 50 mg/kg bw per day, based on increased postimplantation loss and reduction in fetal body weights at 150 mg/kg bw per day. Additional evidence of fetotoxicity at this dose was increased incidences in fetuses of delayed ossification, absent ossification and anomalies (fused sternbrae) (Winkler, 1996b).

Table 42. Fetal incidence and nature of malformations and treatment-related skeletal anomalies and variations in rabbits treated with thiamethoxam in a developmental toxicity study

	Dose (mg/kg bw per day)				
	0	5	15	50	150
Litters evaluated	15	19	19	18	12
Fetuses evaluated	55	97	102	88	45
Visceral malformations					
Renal aplasia	1	0	0	0	0
Uretral aplasia	1	0	0	0	0
Gall bladder aplasia	0	1	0	0	0
Diaphragmatic hernia	0	0	0	1	0
Skeletal malformations	0	0	0	0	0
External malformations	0	0	0	0	0
Total no. of malformed fetuses (% fetal incidence)	1 (1.8)	1 (1.0)	0 (0)	1 (1.1)	0 (0)
(% litter incidence)	(6.7)	(5.3)	(0)	(5.6)	(0)
Visceral anomalies					
Testicular hypoplasia, right	0	0	0	1	0
Small gall bladder	0	6	1	0	3
Skeletal anomalies					
Total no. (%) of fetuses with any skeletal anomaly	8 (14.5)	9 (9.3)	7 (6.9)	5 (5.7)	11 (24.4)
No. (%) of fetuses with:					
- Fused sternebrae 2 and 3	0	0	1 (1.0)	0	3 (6.7)
- Fused sternebrae 3 and 4	0	0	2 (2.0)	1 (1.1)	5 (11.1)*
- Fused sternebrae 4 and 5	1 (1.8)	0	3 (2.9)	1 (1.1)	2 (4.4)
Skeletal variations					
Total no. (%) of fetuses with any skeletal variation	39 (70.9)	76 (78.4)	81 (79.4)	70 (79.5)	34 (75.6)
Absent ossification of the medial phalanx	0	1 (1.0)	0	0	4 (8.9)

From Winkler (1996b)

* $P \leq 0.05$

2.6 Special studies

(a) Neurotoxicity

In a single-dose neurotoxicity study, groups of fasted Sprague-Dawley rats (CrI:CD® BR strain, 10 of each sex per group, approximately 7 weeks old at dosing) were administered a single dose orally by gavage of thiamethoxam (batch No. 9600110; purity 98.7%) in 0.5% methylcellulose solution at a dose level of 0, 100, 500 or 1500 mg/kg bw. Records were made of morbidity and mortality twice daily, clinical observations daily and detailed physical examinations weekly. Body weights were recorded on the day prior to treatment and weekly thereafter. The rats were subjected to neurological function tests and observations (functional observational battery [FOB]) and assessment of locomotor activity prior to treatment, on day 1 (approximately 2–3 hours after dosing) and 1 and 2 weeks later. The tests were performed blind, under red light/white noise conditions. The FOB included home cage/hand-held and open-field observations, response observations in a testing arena and performance measures (including grip strength, foot splay on landing, rectal temperature, tail flick latency and auditory startle response). At termination on day 16, whole-body perfusion fixation was performed on six rats of each sex per group, and central and peripheral nervous system tissues

(brain, four levels of spinal cord, pituitary, cervical and lumbar dorsal root ganglia, cervical and lumbar dorsal and ventral root nerves, Gasserian ganglia [or trigeminal ganglia, important in rats as the first part of the pathway from whiskers to the brain], sciatic, tibial and sural nerves and eyes with optic nerves) were fixed. Anterior tibialis and gastrocnemius skeletal muscles and gross lesions were also fixed. The central and peripheral nervous system tissues from control and high-dose rats were prepared for microscopic evaluation. The remaining four rats of each sex per group were subjected to autopsy, their tissues and organs examined postmortem and the tissues discarded. Sections of all nerve tissues collected from control and high-dose groups were examined microscopically. As there was no histological evidence of neurotoxicity in high-dose rats of either sex, the intermediate- and low-dose groups were not examined.

Three females at 1500 mg/kg bw died within 24 hours of dosing. Treatment-related clinical signs were observed at 1500 mg/kg bw on the day of dosing only. The signs were partial closure of eyes in some rats of both sexes and, in females only, tremors, hypoactivity and coldness to the touch. All other rats survived to the end of the study without clinical signs of an adverse reaction to treatment. Body weight gain was significantly reduced in males at 1500 mg/kg bw during the first week after dosing, but the animals gained more weight than the controls during the remainder of the study.

Treatment-related effects in the FOB/locomotor activity tests were limited to the evaluation performed 2–3 hours after dosing. There were no effects at any dose level during the week 1 and week 2 evaluations. At 2–3 hours after dosing, results of the home cage/hand-held observations revealed an increased incidence of moderate ease of removal from the home cage, active or rigid handling/body tone, tremors and ptosis at 1500 mg/kg bw. In addition, a slightly drooped palpebral closure was noted at 500 and 1500 mg/kg bw. Slight lacrimation occurred in females at 1500 mg/kg bw, and mildly impaired respiration was observed in 1500 mg/kg bw rats and in one male at 500 mg/kg bw. A dark-coloured nasal discharge was seen in one male at 500 mg/kg bw and a further male at 1500 mg/kg bw. Open-field observations on the 1500 mg/kg bw rats revealed a longer latency to first step, an increased incidence of a crouched-over posture in females, a mild to moderate impairment of gait, tremors and hypoarousal, and reduced rearing behaviour. A mild impairment of gait also occurred in one male at 500 mg/kg bw. Response and performance observations showed an increased incidence of uncoordinated landing in the righting reflex test, lower mean rectal temperatures and significantly higher mean forelimb grip strength values for males at 500 and 1500 mg/kg bw. The auditory startle response test required a significantly higher mean input stimulus value for males at 1500 mg/kg bw. Automated measurement of locomotor activity showed significantly lower activity during the first 15–20 minutes of testing at 500 and 1500 mg/kg bw.

Gross pathological findings in the three females that died prior to the scheduled end of the experiment revealed that all had dark-brown lobes of the liver and one had mottled dark-red lobes of the lung. In rats that survived to the end of the study, there were no treatment-related postmortem findings at any dose level. Microscopic examination of tissues from whole-body perfused rats revealed no treatment-related histological alterations in the tissues of the central and peripheral nervous systems. The lesions observed were of the type, incidence and severity anticipated in rats of this age and strain and occurred in both the control and high-dose groups.

In the absence of treatment-related neurohistological changes and persistent functional changes, the transient functional effects observed are considered to be signs of overt toxicity and/or signs of pharmacological overstimulation.

The NOAEL in the single-dose neurotoxicity study in rats was 100 mg/kg bw, based on transient behavioural changes at 500 mg/kg bw (Minnema, 1997).

In a subchronic neurotoxicity study, five groups of 10 male and 10 female Sprague-Dawley rats (CrI:CD® BR strain, approximately 6 weeks old) were administered thiamethoxam (batch No. 9600110; purity 98.7%) orally, in the diet, at concentrations of 0, 10, 30, 500 or 1500 ppm (males)

and 0, 10, 30, 1000 or 3000 ppm (females) for 13 weeks. These concentrations delivered mean doses equal to 0, 0.7, 1.9, 31.8 and 95.4 mg/kg bw per day (males) and 0, 0.7, 2.1, 73.2 and 216.4 mg/kg bw per day (females). Records were made of morbidity and mortality twice daily, clinical observations daily and detailed physical examinations weekly. Body weights were recorded 1 day before treatment commenced and weekly thereafter. Feed consumption was measured weekly. Ophthalmoscopic examinations were performed prior to treatment and during week 13. The rats were subjected to a battery of neurological function tests and observations (FOB) and assessment of locomotor activity prior to treatment and during weeks 4, 8 and 13. The tests were performed blind, under red light/white noise conditions. The FOB included home cage/hand-held and open-field observations, response observations in a testing arena and performance measures (including grip strength, foot splay on landing, rectal temperature, tail flick latency and auditory startle response). After at least 13 weeks of treatment, whole-body perfusion was performed on the first six rats of each sex per group. Central and peripheral nervous system tissues (brain, four levels of spinal cord, pituitary, cervical and lumbar dorsal root ganglia, cervical and lumbar dorsal and ventral root nerves, Gasserian ganglia, sciatic, tibial and sural nerves, eyes with optic nerves) were fixed in formalin. Anterior tibialis and gastrocnemius skeletal muscles, kidneys and gross lesions were also fixed. The central and peripheral nervous system tissues from control and high-dose animals were prepared for histological evaluation as paraffin-embedded 5 µm sections and/or plastic-embedded 1 µm sections, as appropriate. As there was no histological evidence of neurotoxicity in the high-dose rats of either sex, tissues of the low- and intermediate-dose groups were not examined. All remaining rats were subjected to autopsy. Kidneys and gross lesions were fixed from non-perfused rats, but all other tissues were discarded.

Analysis of representative diet samples demonstrated acceptable homogeneity (variation between samples up to 14%), acceptable stability (stable for 7 days at room temperature) and acceptable achieved concentrations (all within 10% of nominal concentrations, with some minor exceptions).

No deaths occurred, there were no treatment-related clinical signs of an adverse effect of treatment, and body weight development and feed consumption throughout the study were unaffected by treatment at all dose levels. After 13 weeks of treatment, there was no evidence of ocular toxicity at any dose level.

Neurobehavioural assessment of animals at all dose levels and at all time points revealed no treatment-related effects on any of the home cage/hand-held and open-field observations, response observations in the testing arena or performance measures (grip strength, foot splay on landing, rectal temperature, tail flick latency and auditory startle response). Locomotor activity was also unaffected by treatment at all dose levels and at all time points.

There were no treatment-related gross pathological findings. Microscopic examination of central and peripheral nervous system tissues indicated that no treatment-related alterations were present in rats of the high-dose groups. Lesions that were observed were of the type, incidence and severity anticipated in rats of this age and strain, and they occurred in both the control and high-dose groups.

The NOAEL for systemic toxicity and neurotoxicity in the 13-week neurotoxicity study was 1500 ppm, equal to 95.4 mg/kg bw per day, in males and 3000 ppm, equal to 216.4 mg/kg bw per day, in females, the highest doses tested, based on the absence of treatment-related effects at these doses (Minnema, 1998).

(b) Developmental neurotoxicity study in rats

In a postnatal developmental neurotoxicity study conducted according to OECD guideline 426 (2003 draft), groups of 30 time-mated, pregnant female Alpk:APfSD (Wistar-derived) rats received thiamethoxam (batch No. 506006; purity 98.8%) in the diet at a concentration of 0, 50, 400 or 4000 ppm, respectively, from gestation day 7 through postnatal day 22. These dietary concentrations delivered doses equal to 0, 4.3, 34.5 and 298.7 mg/kg bw per day during gestation and 0, 8.0, 64.0 and

593.5 mg/kg bw per day postpartum, assuming that the feed consumption of pups was zero. While this assumption is correct for postpartum life up to about day 11, it is not correct for days 11–22, during which solid food is taken. Pups were allocated to the F_1 phase of the study on postpartum day 5, separated from the dam on day 29 and allowed to grow to adulthood.

Observations made on maternal rats were for clinical signs twice daily and for body weights and feed consumption immediately prior to administration of test diets on day 7, on days 15 and 22 of gestation and on days 1, 5, 8, 12, 15 and 22 postpartum. Additionally, feed consumption was measured on day 1, and body weights were measured on the day of termination.

The maternal rats were subjected to a battery of neurological function tests (FOB) conducted blind on days 10 and 17 of gestation and days 2 and 9 of lactation. The FOB included, but was not limited to, the following:

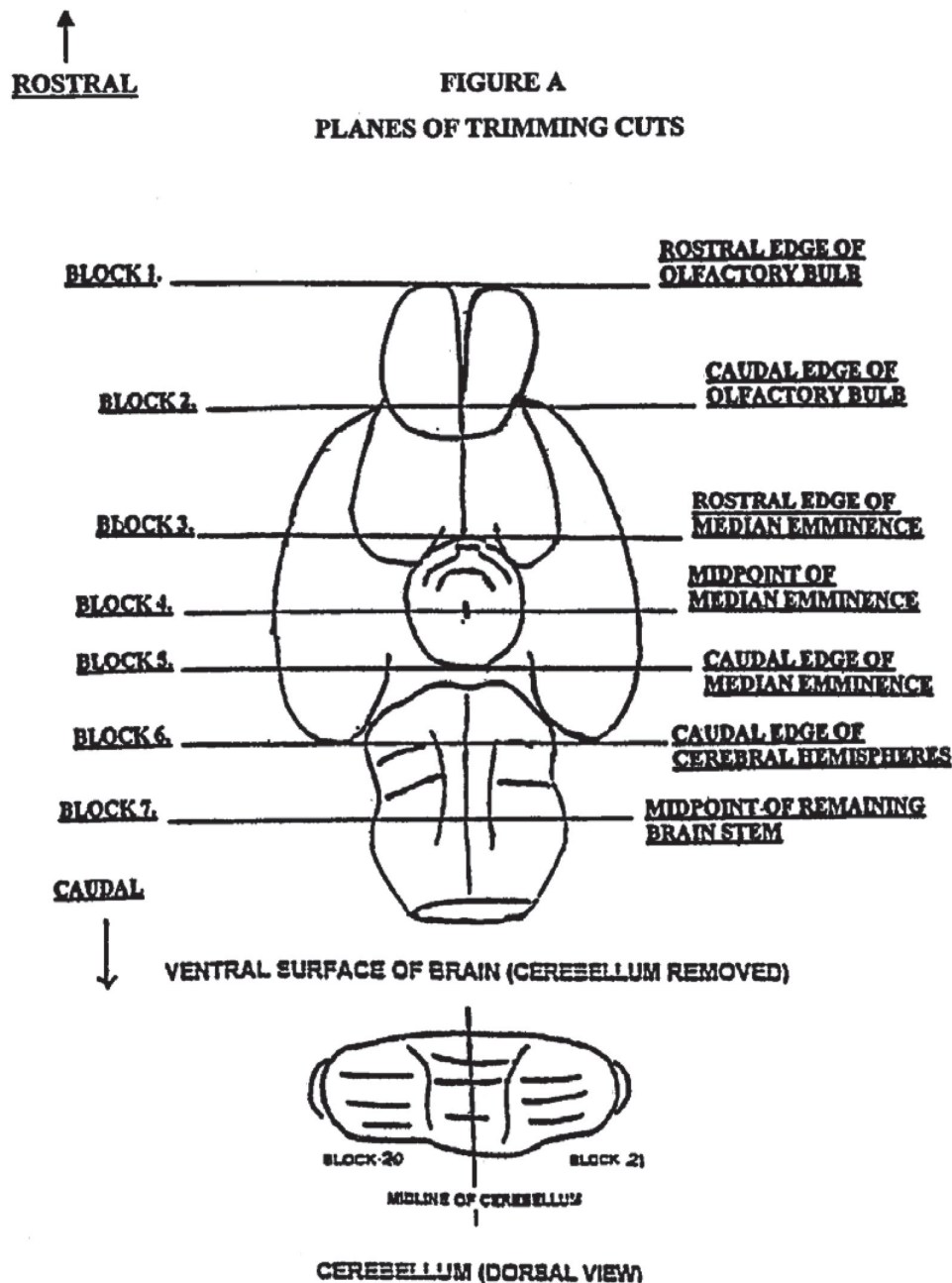
- assessment of signs of autonomic function (e.g. lacrimation, ptosis, pupillary function, exophthalmus, salivation, piloerection, urination and defecation);
- description, incidence and severity of any convulsions, tremors or abnormal movements;
- description and incidence of posture and gait abnormalities;
- description and incidence of any unusual or abnormal behaviour, excessive or repetitive actions and general signs of toxicity, including thin, dehydrated, altered muscle tone and altered fur appearance (including staining around the nose or mouth).

The offspring in each litter were examined as soon as possible after parturition was complete (day 1 postpartum) and always within 24 hours. On days 1 and 5 postpartum, the sex, weight and clinical condition of each pup were recorded, after which litters were standardized to eight randomly selected pups, although sexes were represented as equally as possible. Litters of seven or eight pups with at least three male and three female pups were used for selection of the F_1 generation. Pups not selected for the F_1 generation on day 5 postpartum were killed and discarded.

Postpartum observations for clinical signs were made on F_1 pups at least once a day and any significant findings recorded until day 5, after which detailed clinical observations and body weights were recorded for the selected F_1 rats on days 5, 12, 18, 22, 29, 36, 43, 50 and 57 postpartum and prior to termination on day 63 postpartum. The selected F_1 pups were examined for specific developmental landmarks that included day of age on which vaginal opening occurred in females, from day 29, and when preputial separation occurred in males, from day 41. Body weights were recorded on these days. In addition, the selected pups were designated for FOB tests, as described for the maternal rats above, on days 5, 12, 22, 36, 46 and 61, motor activity testing on days 14, 18, 22 and 60 and auditory startle reflex on days 23 and 61. The pups were also assessed for learning and memory. The test used a “Y”-shaped water maze with one escape ladder. The time taken by the pup to find the escape ladder was recorded for each trial. The rats were given six trials on either day 21 or day 59. In addition, a straight “maze” (channel) was used to evaluate swimming speed. Each pup completed one trial in the straight channel immediately following the six trials in the “Y”-shaped water maze. Three days later, on day 24 or day 62, the pups were retested using the same procedures.

Gross postmortem examinations were conducted on parental females that failed to produce litters, females with litters not required for selection and, on day 29 postpartum, the parental female rats whose litters had been selected. Females with total litter loss were killed and discarded without examination. Gross postmortem examinations were also conducted on pups (one of each sex per litter, to give at least 10 of each sex per group) on day 12 and day 63. The brains of these rats were weighed, fixed and stored. In addition, at least 10 rats of each sex per group were deeply anaesthetized by intra-peritoneal injection of sodium pentobarbitone and killed by perfusion fixation with formol saline. The rats were perfused with a volume of fixative approximately equivalent to their estimated body

Figure 3. Planes of trimming cuts



weight. Organs and tissues selected and fixed, processed and examined from these rats were brain, eye (with optic nerve and retina), spinal cord (including cervical and lumbar swellings), spinal nerve roots (dorsal and ventral root fibres) at cervical and lumbar swellings, dorsal root ganglia at cervical and lumbar swellings, proximal sciatic nerve, proximal tibial nerve, distal tibial nerve (tibial nerve calf muscle branches) and gastrocnemius muscle. In the first instance, blocks from the control and high-dose rats were sectioned and stained. Subsequently, selected areas of the dorsal cortex, thalamus and hippocampus were processed from animals in the intermediate-dose groups.

Brain morphometric measurements were made using an image analyser at levels 2–5 in the brain and a section of cerebellum (Figure 3).

Table 43. Distribution of parental females killed ahead of schedule in a developmental neurotoxicity study in rats treated with thiamethoxam

	Dietary concentration (ppm)			
	0 (control)	50	400	4000
Failed to litter		0	0	1
Total litter loss	—	1	1	1
Insufficient pups ^a	8	6	2	2

From Brammer (2003)

^a Sufficient pups = at least three males and three females in a litter of at least seven pups.

Table 44. Intergroup comparison of maternal body weights during gestation (selected time points; adjusted mean values shown for day 15 onwards) in a developmental neurotoxicity study in rats treated with thiamethoxam

Day	Dietary concentration (ppm)			
	0 (control)	50	400	4000
1	262.0	259.1	261.9	257.9
15	335.3	335.1	334.4	320.7**
22	417.0	415.6	417.8	394.9**

From Brammer (2003)

** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)

All analyses were carried out in SAS.¹ Fisher's exact tests were used for the proportion of whole-litter losses, proportion of pups born alive, proportion of pups surviving, proportion of litters with all pups alive, proportion of litters with all pups surviving, proportion of male pups and the developmental landmarks; ANOVA was used for most body weight analyses and for feed consumption data, motor activity measurements, startle response tests, swimming times, time to preputial separation or vaginal opening, brain weights and brain morphometry measurements; and analyses of covariance were used for mean pup body weights subsequent to day 1, litter-based body weights (separately for males and females) subsequent to day 5 and brain weight as a covariant of final body weight.

Among the parental rats, a number of females in each group were killed prior to the scheduled termination for the reasons given in Table 43. The female that failed to litter was found not to be pregnant.

No treatment-related clinical signs were seen either during general observations of the females or during the FOBs on days 10 and 17 of gestation and days 2 and 9 of lactation.

The body weights of the dams fed 4000 ppm thiamethoxam were statistically significantly lower than those of controls on days 15 and 22 of gestation, by 4–5% (equivalent to approximately 18% lower body weight gain from days 7 to 22 of gestation) (Table 44). This lower body weight was maintained from days 1 to 22 postpartum, although there was no effect of treatment on postpartum weight gain (Table 45). Feed consumption of the dams fed 4000 ppm thiamethoxam was also statistically significantly lower than that of controls (by 17–20%) on days 7–22 of gestation, but this reduced feed consumption continued throughout the postpartum period.

¹ SAS Institute Inc. SAS/STAT 9.1 user's guide. Cary, NC, USA, SAS Institute Inc., 2004.

Table 45. Intergroup comparison of maternal body weights postpartum (selected time points; adjusted mean values shown for day 15 onwards) in a developmental neurotoxicity study in rats treated with thiamethoxam

Day	Dietary concentration (ppm)			
	0 (control)	50	400	4000
1	314.6	313.1	313.2	291.7**
15	369.1	362.5	362.0	344.9**
29	353.1	350.2	353.2	352.4

From Brammer (2003)

* $P < 0.05$; ** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)

Table 46. Litter size and viability in a developmental neurotoxicity study in rats treated with thiamethoxam

Observation	Dietary concentration (ppm)			
	0 (control)	50	400	4000
Proportion of litters with all pups born live	28/30	25/30	27/30	27/29
Proportion of male pups (day 1)	161/358	179/363	217/386**	189/380
% of male pups (day 1)	44.8	50.4	56.6**	50.0
Proportion of male pups (day 5)	158/344	166/336	204/362**	181/354
% of male pups (day 5)	45.7	50.8	56.7*	51.3
Mean litter size (day 1)	11.9	12.0	12.8	13.1
Mean litter size (day 5)	11.5	11.6	12.5	12.6
Proportion of pups surviving (days 1–5)	344/358	336/347	362/372	354/367

From Brammer (2003)

* $P < 0.05$; ** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)

There was no effect of treatment with thiamethoxam on the duration of gestation (mean of 22 days in all groups) or on the proportion of male pups; the statistically significant difference from control in the 400 ppm group was within the range of typical variability and was clearly unrelated to treatment (Table 46). There was no effect of maternal treatment with thiamethoxam on the clinical observations made on pups. The proportion of pups born live was comparable for all groups, and there was no effect of treatment on litter size on day 1. The whole-litter losses (in one female from each thiamethoxam-treated group) were considered to be unrelated to treatment.

The body weights of male and female pups born to dams fed 4000 ppm thiamethoxam were statistically significantly lower than those of controls on day 1 (by approximately 8%) and day 5 (pre-cull) (by approximately 12%), but there were no treatment-related effects on pup weights at lower doses or on total litter weight on day 1 or day 5 (pre-cull) at any dose.

In the selected F_1 rats, no treatment-related clinical signs were observed, and there were no effects of treatment detected in the FOBs on days 5, 12, 22, 36, 46 and 61. Three rats were killed intercurrently due to clinical signs, all of which were considered to be incidental to treatment with thiamethoxam. Body weights in the 4000 ppm group were statistically significantly lower than those of controls on day 5 (by approximately 12%) and remained lower than those of controls throughout the study (with a maximum effect on day 18 of 13%).

Preputial separation was delayed by an average of 1.5 days relative to the controls in the 4000 ppm males, and the body weight on the day of preputial separation was lower than that of controls in this group, whereas there was no treatment-related effect on the day of vaginal opening.

Table 47. Intergroup comparison of startle amplitude: selected time points in a developmental neurotoxicity study in rats treated with thiamethoxam

	Startle amplitude (V)							
	Males				Females			
	Dietary concentration (ppm)							
	0	50	400	4000	0	50	400	4000
Day 23 repetitions 1–10	604.0	382.9*	405.3*	429.6	600.8	450.2	533.9	377.1*
Day 61 repetitions 11–20	715.8	846.2	1048.1*	930.9	896.8	932.7	844.8	810.0
Day 61 repetitions 21–30	664.6	746.2	970.5*	858.5	803.2	700.5	778.3	668.4
Day 61 repetitions 31–40	596.5	731.5	931.8**	761.5	685.0	748.3	766.5	626.8

From Brammer (2003)

* $P < 0.05$; ** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)**Table 48. Intergroup comparison of startle response: selected time points in a developmental neurotoxicity study in rats treated with thiamethoxam**

	Time to maximum amplitude (ms)							
	Males				Females			
	Dietary concentration (ppm)							
	0	50	400	4000	0	50	400	4000
Day 61 repetitions 21–30	25.0	22.2*	22.6	21.8*	22.5	22.7	23.4	23.7
Day 61 repetitions 41–50	23.5	23.3	24.2	23.6	21.7	22.3	22.5	24.2*

From Brammer (2003)

* $P < 0.05$ (statistically significant difference from control group mean; Student's t -test, two-sided)

Locomotor activity was unaffected by treatment. A statistically significant difference from control values observed only on day 14 of the 50 ppm group was clearly not dose related.

There was no effect of maternal treatment with thiamethoxam on startle amplitude or the time to maximum amplitude of the F_1 rats on day 23 or 61. Some statistically significant differences from control (especially in the 400 ppm group) provided no evidence for any treatment-related effects (Tables 47 and 48).

There was no effect of maternal treatment with thiamethoxam on the ability to learn or on memory on days 21 and 24 and on days 59 and 62. Learning in all groups at both time points was demonstrated by a decreased time taken to complete the “Y”-shaped water maze task between trials 1 and 6 in the learning phase (on day 21 or 59). Typically, the time taken to complete trial 6 was less than half the time taken on trial 1. Memory was demonstrated by the difference in time taken to complete trial 1 between the learning and memory phases. For all groups at all time points, group mean time for trial 1 (memory phase) was much less than that for trial 1 (learning phase). A small number of isolated statistically significant differences from the control group showed no consistent pattern and were therefore considered incidental to treatment.

Absolute (fresh) brain weight was lower than that of controls on days 12 and 63 in males and females of dams fed 4000 ppm thiamethoxam. There were no differences from control following adjustment for terminal body weight (Table 49). Post-perfusion on day 63, brain weight was lower than that of controls in males and females of dams fed 4000 ppm thiamethoxam and in females of dams fed 400 ppm thiamethoxam, but again, this effect was not apparent following adjustment for terminal body weight.

At autopsy, no gross effects of treatment were observed.

Table 49. Intergroup comparison of brain weights: F_1 rats in a developmental neurotoxicity study in rats treated with thiamethoxam

	Brain weight (g)							
	Males				Females			
	Dietary concentration (ppm)							
	0	50	400	4000	0	50	400	4000
Day 12								
Absolute brain weight	1.15	1.16	1.13	1.10*	1.11	1.09	1.10	1.06*
Brain weight adjusted for body weight	1.15	1.14	1.12	1.13	1.10	1.08	1.10	1.09
Day 63								
Absolute brain weight	2.07	2.02	2.04	1.99*	1.90	1.89	1.86	1.86
Brain weight adjusted for body weight	2.06	2.02	2.03	2.01	1.88	1.90	1.87	1.87

From Brammer (2003)

* $P < 0.05$ (statistically significant difference from control group mean; Student's t -test, two-sided)

A detailed microscopic examination of the nervous system revealed no effects of maternal treatment with thiamethoxam at doses up to 4000 ppm. Minimal demyelination was observed in the peripheral nerves in both control and treated rats. Although the incidence of this lesion showed both small increases and decreases in treated rats, this is a common spontaneous change in this age and strain of rat, and there were no consistent trends to suggest a relationship with treatment. The remaining microscopic findings were considered to be spontaneous and unrelated to treatment.

Brain morphometry at day 12 did not reveal any treatment-related effects. A small number of statistically significant differences in some morphometric parameters at 4000 ppm were not apparent after adjustment for body weight. These differences are therefore considered to be a consequence of the reduced birth weight and lower body weights seen in the 4000 ppm group. The only statistically significant difference after adjustment for body weight was an increase in the width of the thalamus at level 5 in females in the 4000 ppm group. However, in the absence of differences in the adjusted or unadjusted values in males or in the thalamus measurements at other brain levels, this is considered to be incidental to maternal treatment with thiamethoxam.

Brain morphometry at day 63 revealed a number of statistically significant differences in males and females in the 4000 ppm group in several parameters at levels 3–5 prior to adjustment for body weight. Many of these were not apparent after adjustment for body weight, suggesting that they are a consequence of the reduced birth weight and lower body weights seen at this dose level. Nevertheless, following adjustment for body weight, the statistically significant differences from control values described below remained.

At 4000 ppm in males, there were decreases at level 5 in the width of the thalamus and in the width of the hippocampus. In females at 4000 ppm, there were decreases at level 4 in the width of the thalamus and in the total brain width and at level 5 in the width of the thalamus. The decrease in total width at level 4 can be accounted for by the decrease in thalamus width. There were no statistically significant differences noted in these brain regions examined in the lower dose groups.

In all the male groups and females at 4000 ppm, a statistically significant difference from the control value was present, after adjustment for body weight, at level 3 in the height of the dorsal cortex parallel to the mid-sagittal line. The values for all treated groups were within the range of historical control mean values (Table 50). In addition, there was no dose–response relationship. These differences are therefore considered to be incidental to treatment.

There was a request to provide supplementary data on selected brain morphometry from pups of the 50 and 400 ppm dose levels of this experiment at days 12 and 63 (Brammer, 2007). This

Table 50. Brain morphometry at postnatal day 63 in a developmental neurotoxicity study in rats treated with thiamethoxam

	Brain morphometry (mm)							
	Males				Females			
	Dietary concentration (ppm)							
	0	50	400	4000	0	50	400	4000
Level 3 dorsal cortex 1 (3AB) mean	1.58	1.35*	1.37*	1.40*	1.51	1.50	1.48	1.46
Adjusted mean	1.58	1.35*	1.37*	1.40*	1.54	1.51	1.48	1.42*
Historical control values range								
High mean ± standard deviation	1.53 ± 0.11				1.46 ± 0.11			
Low mean ± standard deviation	1.25 ± 0.16				1.31 ± 0.07			

From Brammer (2003)

* $P < 0.05$

Table 51. Intergroup comparison of brain morphometry (selected measurements/adjusted mean): F_1 animals (day 12) in a developmental neurotoxicity study in rats treated with thiamethoxam

Sex	Parameter	Brain morphometry ^a			
		Dietary concentration (ppm)			
		0 (control)	50	400	4000
Males	Cerebellum length (8L)	4.40	4.11*	4.09**	4.34
Males	Cerebellum – prepyramidal fissure – thickness of molecular layer (8PPFM)	63.7	59.2	61.7	59.5
Females	Level 4 – thalamus – width (4E)	7.64	7.19**	7.34	7.29*

From Brammer (2007)

* $P < 0.05$; ** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)

^a Units of measurement for brain morphometry are in millimetres (mm), except the cerebellum-preculminate fissure thickness and prepyramidal fissure thickness (8PCF1 to 8PPF0), which are in micrometres (μ m).

involved examination of day 12 slides from brain level 4 (corpus callosum thickness and thalamus width) and the cerebellum (length and prepyramidal fissure – thickness of the molecular layer) and day 63 slides from brain levels 3 (dorsal cortex 2 thickness and piriform cortex thickness), 4 (dorsal cortex thickness, corpus callosum thickness, thalamus height and width, thalamus/cortex overall width and hippocampus, width of dentate gyrus) and 5 (dorsal cortex thickness and hippocampus overall width).

In day 12 males or females, a small number of brain morphometry parameters were statistically significantly different from control at 4000 ppm thiamethoxam.

At 4000 ppm thiamethoxam only, the thickness of the molecular layer of the cerebellar prepyramidal fissure was statistically significantly lower than control (by 12.2%) in day 12 males, but not statistically significant for adjusted mean (by 6.6%) (Table 51), and not in females. Adjusted mean cerebellum length was statistically significantly lower by 7.0% compared with control at 400 ppm in males, but not at 4000 ppm (1.4%). These measures of the cerebellum are clearly quite variable at this growth stage, but a treatment-related effect at 4000 ppm cannot be excluded.

Level 4 thalamus width was statistically significantly lower in all three treated groups for females (Table 51), but there was no coherent dose–response relationship, and the magnitude of the differences was small (maximum 5.9% at 50 ppm). There was no difference in this thalamus parameter in males at any dose level, and there were no differences in any related structures in the

females, including level 5 thalamus width, level 4 thalamus height and level 4 thalamus/cortex overall width. These small differences are therefore considered not to be treatment related.

Corpus callosum thickness was not statistically significant at any dose level. However, examination of this parameter in the intermediate-dose groups was requested, because the value in 4000 ppm females was 15.5% lower than in controls. There were no statistically significant differences from control in males at 4000 ppm or in the intermediate-dose groups on day 12. Considering the lack of any statistically significant differences and the small differences from control in all additional measurements, it was concluded that there was no treatment-related effect on day 12 corpus callosum thickness.

In the day 63 brain morphometry data, there were a number of statistically significant differences in males and females in the 4000 ppm group in several parameters at brain levels 3–5 prior to adjustment for body weight. Many of these were not apparent after adjustment for body weight, suggesting that they were a consequence of the reduced birth weight and lower body weights seen at this dose level (Brammer, 2003). Nevertheless, the overall pattern of lower values for a number of parameters (unadjusted for body weight) suggests that a treatment-related effect cannot be excluded at 4000 ppm. In contrast, additional analysis of the intermediate-dose groups (unadjusted for body weight) revealed only a very small number of differences from control, none of which was considered to be treatment related. Even after adjustment for body weight, most parameters were statistically significantly different from the controls in the 4000 ppm group, particularly among males, whereas statistically significant differences in the 400 and 50 ppm groups were few and not consistent with dose-related responses (Table 52).

In males, level 3 dorsal cortex 1 thickness was statistically significantly lower than control in all three treatment groups. However, comparison of concurrent with historical control data from other developmental neurotoxicity studies suggested that the concurrent control data in this study were somewhat high (Table 53). Of greater importance, there was no semblance of a dose–response relationship, there was no difference in females and the values for the treated groups (1.35, 1.37 and 1.40 mm for groups 2, 3 and 4) were all close to the mean of the historical controls for this parameter (1.34 mm).

Level 4 thalamus width values were statistically significantly lower than control values in males at 400 and 4000 ppm. Once again, it may be argued that the control group value in this experiment was high in comparison with control group values from other experiments. In addition, no changes to related parameters, such as level 4 thalamus height or level 5 thalamus width, were observed at 400 ppm in males. The level 4 thalamus width in females at 400 ppm, although statistically significantly different from the concurrent control, was higher than the control value, not lower. Therefore, the small difference in level 4 thalamus width in males at 400 ppm (4.5%) was considered not to be treatment related.

Level 4 thalamus/cortex overall width was statistically significantly lower than the control value at all three treatment levels in day 63 males. However, similar to the pattern described above for thalamus width, the concurrent control value was near the high end of the historical control data, and the mean of the historical control value (14.4 mm) was very close to the values for the 50 and 400 ppm groups (14.14 and 14.18 mm, respectively). The actual differences from control values were small (4.6%, 4.3% and 5.0%, respectively) and showed no dose-related response. The weight of evidence indicates that there was not a treatment-related effect for this parameter.

In females at day 63, level 3 dorsal cortex 2 thickness was statistically significantly lower than the control in the 50 ppm group. In the absence of any dose–response relationship, this also was not considered to be treatment related.

Table 52. Intergroup comparison of brain morphometry (selected measurements/adjusted mean): F_1 animals (day 63) in a developmental neurotoxicity study in rats treated with thiamethoxam

Parameter	Brain morphometry ^a			
	Dietary concentration (ppm)			
	0 (control)	50	400	4000
Males				
Level 3 – dorsal cortex 1 – thickness (3AB)	1.58	1.35**	1.37**	1.40**
Level 3 – dorsal cortex 2 – thickness (3BB)	1.90	1.88	1.87	1.72**
Level 3 – piriform cortex – thickness (3CB)	1.52	1.51	1.49	1.38**
Level 4 – dorsal cortex – thickness (4AB)	1.53	1.46	1.46	1.36**
Level 4 – corpus callosum – thickness (4C)	0.45	0.41	0.44	0.38*
Level 4 – thalamus – height (4D)	5.61	5.35	5.55	5.07**
Level 4 – thalamus – width (4E)	8.94	8.70	8.58*	8.46*
Level 4 – thalamus/cortex – overall width (4F)	14.81	14.12*	14.18*	14.12
Level 4 – hippocampus – width dentate gyrus (4HB)	0.64	0.60	0.61	0.59*
Level 5 – thalamus width (5C)	8.10	8.03	7.93	7.51**
Level 5 – hippocampus – overall width (5EB)	1.56	1.54	1.61	1.45*
Females				
Level 3 – dorsal cortex 1 – thickness (3AB)	1.54	1.51	1.48	1.41*
Level 3 – dorsal cortex 2 – thickness (3BB)	1.79	1.64**	1.71	1.70
Level 4 – dorsal cortex – thickness (4AB)	1.41	1.42	1.41	1.30*
Level 4 – thalamus – height (4D)	5.19	5.59*	5.40	5.25
Level 4 – thalamus – width (4E)	8.44	8.50	8.73*	8.04**
Level 4 – thalamus/cortex – overall width (4F)	14.44	14.40	14.73	13.55**
Level 5 – thalamus – width (5C)	7.86	7.64	7.74	7.31**

From Brammer (2007)

* $P < 0.05$; ** $P < 0.01$ (statistically significant difference from control group mean; Student's t -test, two-sided)

^a Units of measurement for brain morphometry are in millimetres (mm), except the cerebellum-preculminate fissure thickness and prepyramidal fissure thickness (8PCF1 to 8PPF0), which are in micrometres (μ m).

Table 53. Ranges and mean values for historical control data: selected brain morphometry measurements in a developmental neurotoxicity study in rats treated with thiamethoxam^a

Parameter	Brain morphometry measurements (mm)			
	Minimum	Maximum	Mean	Standard deviation
Day 63 – males				
Level 3 – dorsal cortex 1 – thickness	1.22	1.53	1.34	0.09
Level 4 – corpus callosum – thickness	0.31	0.46	0.37	0.05
Level 4 – thalamus – width	8.27	8.86	8.59	0.18
Level 4 – thalamus/cortex – overall width	14.2	14.9	14.4	0.2
Day 63 – females				
Level 3 – dorsal cortex 1 – thickness	1.22	1.46	1.32	0.08
Level 4 – corpus callosum – thickness	0.30	0.45	0.36	0.05
Level 4 – thalamus – width	8.19	8.71	8.41	0.16
Level 4 – thalamus/cortex – overall width	13.6	14.6	14.1	0.3

From Brammer (2007)

^a Ranges, means and standard deviations were calculated for those parameters where these historical control data are discussed in the text for males or females. Values are from control groups in developmental neurotoxicity studies conducted from 2001 to 2004 (Brammer, 2003, 2007).

Level 4 thalamus width in females was statistically significantly higher than the control value at 400 ppm and lower at 4000 ppm. At 400 ppm, this difference was small (3.2%) and opposite to the effect at the high dose and, as described for males, was considered not to be treatment related.

The NOAEL for maternal toxicity in this study of developmental neurotoxicity was 400 ppm, equal to 34.5 mg/kg bw per day, based on decreased body weight gain and feed consumption in dams throughout gestation and postpartum at 4000 ppm, equal to 298.7 mg/kg bw per day. The NOAEL for fetotoxicity in this study of developmental neurotoxicity was 400 ppm, equal to 64.0 mg/kg bw per day, based on reduced birth weight, reduced pup body weight gain and some evidence of delayed preputial separation at 4000 ppm, equal to 298.7 mg/kg bw per day. The NOAEL for developmental neurotoxicity was 4000 ppm, equal to 298.7 mg/kg bw per day, the highest dose tested, based on an absence of any quantitative histological or behavioural changes accompanying the small changes in brain morphometry (Brammer, 2007).

(c) *Other special studies*

Special studies on thiamethoxam were performed to investigate the etiology of adenoma and adenocarcinoma formation in mouse liver during the 18-month carcinogenicity study (Bachmann, 1998a). Special studies were also performed to investigate the renal tubule changes in rats. The results of these supplementary studies are summarized in [Table 54](#).

(i) *Mouse liver tumours*

The incidences of any hepatic tumours in mice after 18 months of exposure to thiamethoxam at 0, 5, 20, 500, 1250 or 2500 ppm were, in males, 11, 7, 10, 19, 22 and 44 (out of 50) and, in females, 0, 0, 0, 5, 9 and 29 (out of 50) (Bachmann, 1998b). Most were hepatocellular tumours. The incidences of primary hepatocellular adenomas in these same dose groups of 50 mice of each sex, respectively, were, for males, 9, 5, 8, 17, 21 and 39, and, for females, 0, 0, 0, 5, 8 and 28. In addition, hepatocellular carcinomas were significantly increased in males of the highest-dose group, with incidences of 3, 3, 2, 4, 4 and 16, whereas in females, there were a few hepatocellular carcinomas in the two highest dose groups only: 0, 0, 0, 0, 2 and 3. Thus, any explanation for the induction of these tumours should account for their occurrence in both male and female Tif:MAGf(SPF) mice (but particularly in male mice) from a dietary exposure concentration of 500 ppm. The genetic toxicity database for this compound indicates that it is not a genetic toxin; therefore, other modes of action were to be investigated with, most probably, a dose below which tumours would not be expected to occur (i.e. a “threshold”). Modes of action are addressed in [Appendix 1](#). Furthermore, there was no evidence for hepatocellular adenoma or carcinoma induction in rats receiving thiamethoxam in their diets at 0, 10, 30, 500 or 1500 ppm for 2 years (Bachmann, 1998a). The numbers of hepatocellular adenomas in these groups (out of 49 or 50 of each sex) were, for males, 0, 1, 0, 1 and 1, and, for females, 0, 2, 0, 2 and 2. The numbers of hepatocellular adenocarcinomas were also sporadic, being, for males, 0, 0, 1, 1 and 0; and for females, 0, 0, 1, 0 and 0. Thus, any mode of action should also account for the species specificity of the response.

A number of properties of thiamethoxam have been identified, some of which appear to be time- and dose-related key events that characterize the emergence of liver cancer in thiamethoxam-treated mice in a manner that resembles the cholesterol-lowering drugs, statins, which also induce liver tumours in mice. Thus, the mode of action of this pesticide may be thought to resemble that of the statins; however, the studies conducted so far provide no evidence for a statin-like inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, this being the rate-limiting enzyme in cholesterol biosynthesis (see below). Properties of thiamethoxam identified in mice include perturbation of cholesterol biosynthesis, hepatotoxicity, cell death (both as single-cell necrosis and as apoptosis) and a sustained increase in cell replication rates. These changes occur in a dose-dependent

Table 54. Summary of special studies conducted on thiamethoxam

Study; test system; dose levels	NO(A)EL	LO(A)EL	Main effects	Reference
In vivo liver biochemical parameters; male and female mice; 14 days of feeding; 0, 100, 500, 2500 ppm	100 ppm	500 ppm	Moderate induction of liver phase I and II xenobiotic metabolizing enzymes	Trendelenburg (1998)
In vivo hepatic cell proliferation; male and female mice; up to 59 days of feeding; 0, 100, 500, 2500 ppm	Proliferation: 100 ppm (males), 500 ppm (females) Hepatotoxicity: < 100 ppm (males), 500 ppm (females)	Proliferation: 500 ppm (males), 2500 ppm (females) Hepatotoxicity: 100 ppm (males), 2500 ppm (females)	2500 ppm: Hepatic glycogenesis and fatty change after 3+ days of treatment Increased liver weight at 2500 ppm after 7+ days of treatment Hepatic necrosis/apoptosis after 27+ days of treatment Hepatic deposition of lipogenic pigment after 59 days of treatment Increased hepatocyte proliferation after 3+ days of treatment	Weber (1998)
Changes in plasma cholesterol levels during dietary feeding studies Mice: 0, 50, 200, 500, 1250, 2500, 5000 ppm Rats: 0, 1000, 3000 ppm	200 ppm for cholesterol reduction in mice	Reduction in plasma cholesterol in mice at 500 ppm and above	Sustained and dose-dependent reduction in plasma cholesterol in mice but not in rats	Green (2003b)
Comparative hepatotoxicity in weanling and adult mice; 0, 500, 1250, 2500 ppm	Adults < 500 ppm; weanlings 500 ppm	Adults 500 ppm; weanlings 1250 ppm	Significant reduction in plasma cholesterol in adults at 500 ppm and above, in weanlings at 1250 ppm and above; internal dose in weanlings approximately 2× adults; thus, weanlings are less sensitive to the hepatic effects of thiamethoxam	Green (2003c)
Hepatic cell proliferation and apoptosis in male mice fed thiamethoxam for up to 50 weeks; 0, 50, 200, 500, 1250, 2500, 5000 ppm	200 ppm	500 ppm	General toxicity at 2500 and 5000 ppm; time- and dose-related hepatotoxicity; increased AST and ALT at 1250 ppm and above, increased apoptosis and necrosis at 500 ppm and above; increased cell proliferation at 1250 ppm and above	Weber (2003)
Comparative toxicity of thiamethoxam and metabolites in two strains of mouse; 2500 ppm thiamethoxam; 2000 ppm CGA 322704; 500 ppm CGA 265307	Highest dose tested	N/A	In contrast to the effects of thiamethoxam, CGA 322704 and CGA 265307 had no significant effects on liver; both strains responded similarly	Noakes (2003a)
Sublobular assessment of hepatic cell proliferation; 0, 200, 500, 1250 ppm	200 ppm	500 ppm	Thiamethoxam induces increases in hepatocellular labelling at 500 and 1250 ppm	Soames (2003)

Table 54 (continued)

Study; test system; dose levels	NO(A)EL	LO(A)EL	Main effects	Reference
Hepatotoxicity of metabolites fed for up to 50 weeks; 2500 ppm thiamethoxam; 2000 ppm CGA 322704; 500 ppm CGA 265307; 500, 1000 ppm CGA 330050	Thiamethoxam: N/A; CGA 330050: after 10 weeks, 500 ppm; CGA 322704 and CGA 265307: highest doses tested	Thiamethoxam: 2500 ppm, only concentration tested; CGA 330050: after 10 weeks, 1000 ppm	Thiamethoxam and CGA 330050 induced significant hepatic effects; CGA 322704 and CGA 265307 had no significant effects on liver; metabolite CGA 330050 has a key role in the mechanism of thiamethoxam hepatotoxicity	Green (2003d)
Role of nitric oxide in mouse hepatotoxicity	N/A	N/A	CGA 265307 inhibits nitric oxide synthase; this inhibition of the normal protective effect of nitric oxide exacerbates the liver toxicity induced by CGA 330050	Green (2003e)
Hepatic cell proliferation and apoptosis in female rats fed thiamethoxam for up to 50 weeks; 1000, 3000 ppm	3000 ppm, highest concentration tested	N/A	No effect on hepatocyte proliferation detected, as measured by increased S-phase DNA synthesis or apoptosis	Noakes (2003b)
Biochemical parameters in rat liver after 1 and 10 weeks; 1000, 3000 ppm thiamethoxam	N/A	N/A	No remarkable inducing effect on xenobiotic metabolizing enzymes; no effect on hepatic glutathione concentration or on activity of γ -glutamylcysteine synthetase	Waechter (2003)
Comparative metabolism in mice and rats in vivo and in mouse, rat and human liver fractions in vitro	N/A	N/A	Production of key metabolites in vivo much higher in mice than rats; induction of metabolism in mice over time, whereas reduction seen in rats; results in plasma concentrations of CGA 265307 in mice after 10 weeks that are 108-fold higher than in rats; a similar difference is seen in vitro, with rate conversions in mouse liver being much higher than those in rat liver fractions; the rate conversions in human liver were even lower than in rat liver, indicating that human metabolism of thiamethoxam to the key metabolites would be minimal	Green (2002)
Metabolism in rats and mice in dietary feeding studies 50 week mouse: 500, 1250, 2500 ppm thiamethoxam 50 week rat: 1000, 3000 ppm thiamethoxam 20 week mouse: 2500 ppm thiamethoxam, 200 ppm CGA 322704, 500 ppm CGA 265307 1 week mouse: 1000 ppm CGA 330050	N/A	N/A	Following administration of thiamethoxam, the plasma concentrations of parent were similar in rats and mice, but the concentrations of CGA 322704, CGA 265307 and CGA 330050 were much higher in mice than in rats, with evidence for induction of metabolism in mice, but not rats; following administration of CGA 322704 to mice, the only major metabolite detected was CGA 265307; as CGA 322704 is not carcinogenic in the mouse, CGA 265307 is unlikely to be responsible for the carcinogenicity of thiamethoxam in the mouse;	Green (2003a)

Table 54 (continued)

Study; test system; dose levels	NO(A)EL	LO(A)EL	Main effects	Reference
			CGA 330050 is the only major metabolite identified that occurs in significantly greater quantities in mice than in rats and that is unique to thiamethoxam; it is therefore likely to have a key role in the carcinogenicity of thiamethoxam in the mouse	
In vivo hepatic cell proliferation; male rats; 28 days feeding; 0, 10 000 ppm	10 000 ppm (~710.6 mg/kg bw per day), highest dose tested	N/A	No effect on hepatocyte proliferation detected, as measured by increased S-phase DNA synthesis	Persohn (1995)
In vitro cytotoxicity; rat/mouse hepatocytes; 0, 10–5000 µmol/l (9 concentrations)	5000 µmol/l (rat and mouse), highest concentration tested	N/A	No cytotoxicity detected in either species	Bouis (1997)
In vivo acute hepatic cell damage; male mice; 500 mg/kg bw	N/A	500 mg/kg bw	Cytoplasmic condensation of mostly periportal hepatocytes at 6 h, suppression of hepatocellular mitotic activity at 24 h after dosing	Weber (1999a)
In vivo hepatic cell apoptosis; male mice; up to 59 days of feeding, 0, 100, 500, 2500 ppm; 9 months of feeding, 0, 2500 ppm	100 ppm (~15.8 mg/kg bw per day)	500 ppm	Increase in apoptotic activity after 59 days, still present after 9 months (data only at 2500 ppm)	Weber (1999b)
Immunohistochemical assessment of α_{2u} -globulin in the rat kidney upon administration of CGA 293343 for 28 days, 90 days, 12 months, 24 months	N/A	N/A	Renal tubular hyaline change	Weber (2000a,b,c,d)

LO(A)EL, lowest-observed-(adverse-)effect level; N/A, not applicable; NO(A)EL, no-observed-(adverse-)effect level

manner and are seen in their entirety only at the dose levels at which tumour incidences are increased. At least some of them may therefore constitute key events in a mode of (carcinogenic) action, although it is perhaps difficult to understand a key role for interference with cholesterol metabolism. An important question is whether they all occur at least at all dose levels (but not necessarily all examination times) at which tumour incidences are increased. It is, of course, possible that a key event acting in a minimal fashion at a lower dose level might be insufficient to trigger succeeding steps in the process. Generally, it is not possible to refer back to the standard toxicity studies on mice (3-month feeding study: Bachmann, 1996a; 18-month feeding study: Bachmann, 1998a) for support of the investigation results, because the required measurements and observations were not made. In spite of the statin-like properties, the inductions noted in liver phase I and phase II enzymes had similarities with those produced by phenobarbital (Trendelenburg, 1998). This similarity is possibly misleading, however, as the effect on these enzymes is small, and it has been suggested (Trendelenburg, 1998) that it is insufficient to account for the emergence of liver tumours.

Table 55. Successive changes in the livers of mice treated with thiamethoxam for up to 50 weeks

Study week	Observation
1–10	Liver dysfunction: Decreased protein synthesis, glycogen and lipid accumulation, reduced cholesterol, cytochrome P450 induction and hepatocellular hypertrophy
10–50	Hepatotoxicity: Increased ALT/AST, pigmentation, inflammatory cell infiltration, hepatocellular hypertrophy, single-cell necrosis and increased apoptosis
20–50	Sustained increase in cell replication rates

From Trendelenburg (1998); Weber (1998, 2003); Green (2003b,c); Noakes (2003a)

Several progressive steps in the hepatotoxicity of thiamethoxam in mice include an early-stage mild hepatic dysfunction caused by the metabolite CGA 330050 (characterized by lowering of plasma cholesterol, induction of cytochrome P450 isoenzymes, decreased protein synthesis and glycogen and lipid accumulation), leading to hypertrophy, lymphocytic infiltration and pigmentation of hepatocytes and Kupffer cells. These processes may be exacerbated by another specific thiamethoxam metabolite, CGA 265307. This metabolite does not seem to be hepatotoxic, but it is a competitive inhibitor with arginine, the natural substrate of nitric oxide synthase *in vitro* (Green, 2003e). Nitric oxide has a regulatory role in liver in modulating the adverse effects of tumour necrosis factor alpha (TNF α) released from endothelial cells during chemical-induced hepatotoxicity. Consequently, there is likely to be exacerbation of cytotoxicity, single-hepatocyte necrosis and apoptosis, which are believed to lead to increased cell proliferation. Much of this (but not all) could constitute key events in a mode of action that finally leads to the formation of hepatic tumours.

The hepatic changes preceding the emergence of tumours have been studied in experiments in which mice were fed diets containing 0–5000 ppm thiamethoxam for periods from 1 to 50 weeks (Trendelenburg, 1998; Weber, 1998, 2003; Green, 2003b,c; Noakes, 2003a). The results of these studies suggest the progression of changes shown in Table 55.

In this study of up to 50 weeks' duration, hepatocellular neoplasms did develop in small numbers, even in controls, in weeks 40–50, but the higher incidences were observed only in the 79-week carcinogenicity study.

The results from these six reports are summarized below.

1–10 weeks: Liver dysfunction

The earliest stages of thiamethoxam's effect on the mouse liver (within 1 week of dosing) are characterized by histological changes, including accumulation of glycogen and lipid in centrilobular regions of the liver. These histological changes are accompanied by several significant biochemical changes, including those described below.

Protein synthesis: A statistically significant reduction in plasma total protein was observed in male Tif:MAGf mice (females not studied) fed on diets containing 2500 ppm thiamethoxam for 2 and 11 weeks, but with signs of recovery at 21 weeks. This reduction was not due to reduced albumin concentration and was not induced by the major metabolites, CGA322704 at 2000 ppm or CGA265307 at 500 ppm (Noakes, 2003a).

Cytochrome P450 induction: A significant increase in hepatic cytochrome P450, principally of the 2B family, was seen in mice fed on diets containing 0, 100, 500 or 2500 ppm thiamethoxam for 14 days (Trendelenburg, 1998). Thiamethoxam was found to be a moderate (up to 9-fold) inducer of mouse CYP2B isoenzymes and both 7-pentoxoresorufin *O*-depentylase (PROD) and 7-benzoyloxyresorufin *O*-debenzylase (BROD) activities. However, these moderate increases alone were considered insufficient to be causally linked to the development of liver cancer. The enzymatic changes found are summarized in Table 56.

Table 56. Summary of enzyme activities

Assay	Result
Protein content	No effect in either sex at any dose in supernatant and cytosolic fractions 24% increase in females at 2500 ppm in microsomal fraction
Cytochrome P450	59% increase in males at 2500 ppm 52% increase in females at 2500 ppm
7-Ethoxyresorufin <i>O</i> -deethylase	No effect in males at all dose levels 45% increase in females at 500 ppm 157% increase in females at 2500 ppm
7-Pentoxyresorufin <i>O</i> -deethylase	42% increase in males at 500 ppm 725% increase in males at 2500 ppm 51% increase in females at 100 ppm 103% increase in females at 500 ppm 711% increase in females at 2500 ppm
7-Benzoyloxyresorufin <i>O</i> -debenzylase	72% increase in males at 500 ppm 1021% increase in males at 2500 ppm 53% increase in females at 100 ppm 109% increase in females at 500 ppm 782% increase in females at 2500 ppm
Coumarin 7-hydroxylase	55% increase in males at 2500 ppm No effect in females at any dose level
Regioselective and stereoselective hydroxylation of testosterone	Total oxidation rates increase in males by 41% due to increased hydroxylation at positions 2 β , 6 α , 6 β , 15 α and oxidation to androstenedione; total oxidation rates increase in females by 37% due to increased hydroxylation at positions 6 α , 16 α , 16 β and oxidation to androstenedione
Lauric acid 11-hydroxylation	47% increase in males at 2500 ppm 104% increase in females at 2500 ppm
Lauric acid 12-hydroxylation	No effect in males at any dose level No effect in females at any dose level
Uridine diphosphate-glucuronosyltransferase	33% increase in males at 2500 ppm 31% increase in females at 2500 ppm
Glutathione <i>S</i> -transferase	61% increase in males at 2500 ppm 198% increase in females at 2500 ppm
Epoxide hydrolase	50% increase in males at 2500 ppm 133% increase in females at 2500 ppm
Cyanide-insensitive peroxisomal- β -oxidation	No effect in males at any dose level No effect in females at any dose level

From Trendelenburg (1998)

Differences in thiamethoxam metabolism in mice and rats in vivo and mice, rats and humans in vitro: Following dietary administration of thiamethoxam to mice and rats at 2500 ppm and 3000 ppm, respectively, for 1 or 10 weeks, quantitative differences were seen in the concentrations of metabolites in plasma that increased with time of exposure. Over the duration of the study, there was evidence of induction of the metabolism of thiamethoxam in mice, whereas a reduction in metabolism of thiamethoxam was observed in rats. After 10 weeks, the concentration of CGA 265307 in plasma was 108-fold higher in mice than in rats (Table 57).

Table 57. Concentration of thiamethoxam and its metabolites in plasma in rats and mice fed on diets containing thiamethoxam for up to 50 weeks

	Concentrations (µg/ml)					
	Mouse			Rat		
	Dietary concentration (ppm)					
	2500			3000		
	Weeks on diet					
	1	10	50	1	10	50
Thiamethoxam	11.82	14.86	9.71	7.01	19.21	7.91
CGA 322704	2.54	5.31	3.38	0.96	0.63	1.20
CGA 265307	1.98	7.05	4.20	0.09	0.05	0.05
CGA 330050	0.86	1.50	1.12	0.14	0.10	0.12

From Green (2003a)

The species differences seen in vivo were reflected in the in vitro rate comparisons (Figures 4–7).

The mean rate for the conversion of thiamethoxam to CGA 265307 via CGA 322704 was 54-fold lower in rat liver than in mouse liver, and via CGA 330050 was 87-fold lower in rat liver than in mouse liver.

The rates for each of the metabolic transformations of thiamethoxam by its major pathways were significantly lower in microsomal preparations from human liver than from mouse liver. The mean rate for the conversion of thiamethoxam to CGA 265307 via CGA 322704 was 371-fold lower in human liver than in mouse liver, and the conversion via CGA 330050 was 238-fold lower in human liver than in mouse liver. The remarkably high Michaelis-Menten constant (K_m) values seen in human livers for the metabolism of thiamethoxam to CGA 322704 and CGA 330050 suggest that metabolism of thiamethoxam by these pathways in humans would be minimal at expected exposure levels (Green, 2002). A proposed interconversion pathway for thiamethoxam and these metabolites is shown in Figure 8.

The trends in metabolism in mice and rats first observed over the early weeks of exposure to thiamethoxam persisted in subsequent studies in which the metabolism of thiamethoxam was investigated in blood and liver samples from mice and rats following dietary administration of thiamethoxam and its major metabolites CGA 322704, CGA 265307 and CGA 330050 for up to 50 weeks.

Following administration of thiamethoxam to mice at 500, 1250 or 2500 ppm, the dose–response relationship was approximately linear over the dose range tested, with no evidence of saturation of metabolic pathways at higher dietary dose levels (Table 58).

Following administration of thiamethoxam to mice at 2500 ppm in the diet, the concentrations of thiamethoxam and its metabolites in plasma and liver were similar. Plasma concentrations are therefore a reasonable reflection of the concentrations in liver, the target organ for carcinogenicity in the mouse.

Following administration of thiamethoxam to mice and rats at 2500 ppm and 3000 ppm in the diet, respectively, the concentration of thiamethoxam in plasma was similar in the mouse and the rat, demonstrating that the internal dose of the test substance was similar. As the concentration of thiamethoxam in plasma reflects the concentration in liver, the absence of liver tumours in rats suggests that parent thiamethoxam is not responsible for the liver tumours seen in mice.

Figure 4. The metabolism of thiamethoxam to CGA 322704 in mouse, rat and human liver microsomes (Green, 2002)

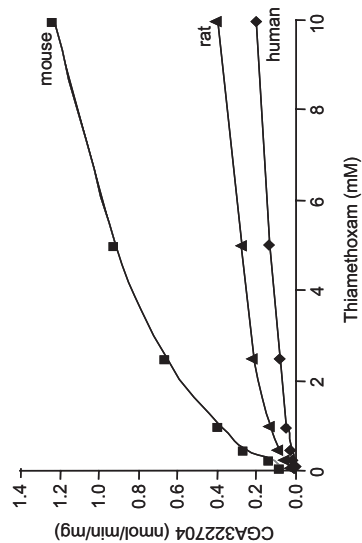


Figure 5. The metabolism of CGA 322704 to CGA 265307 in mouse, rat and human liver microsomes (Green, 2002)

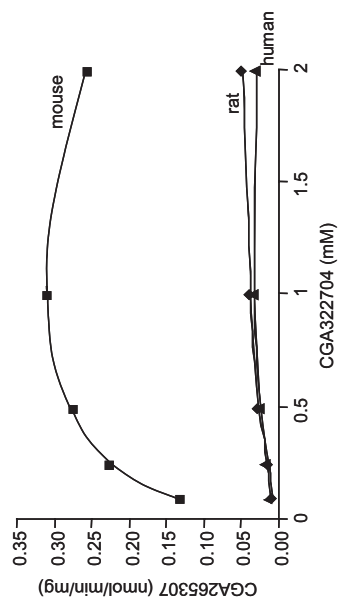


Figure 6. The metabolism of thiamethoxam to CGA 330050 in mouse, rat and human liver microsomes (Green, 2002)

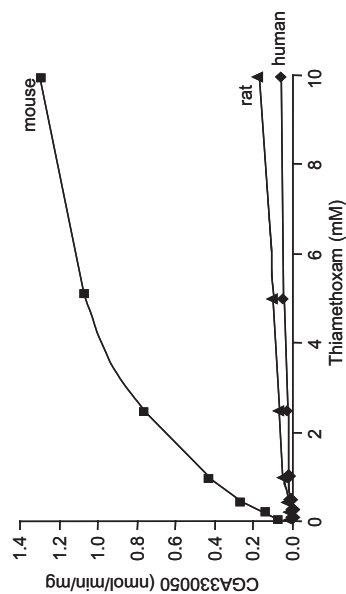


Figure 7. The metabolism of CGA 330050 to CGA 265307 in mouse, rat and human liver microsomes (Green, 2002)

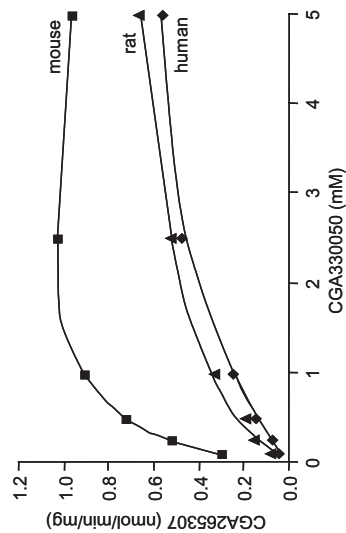


Figure 8. Proposed metabolic pathway of thiamethoxam in the mouse and rat (Green, 2002)

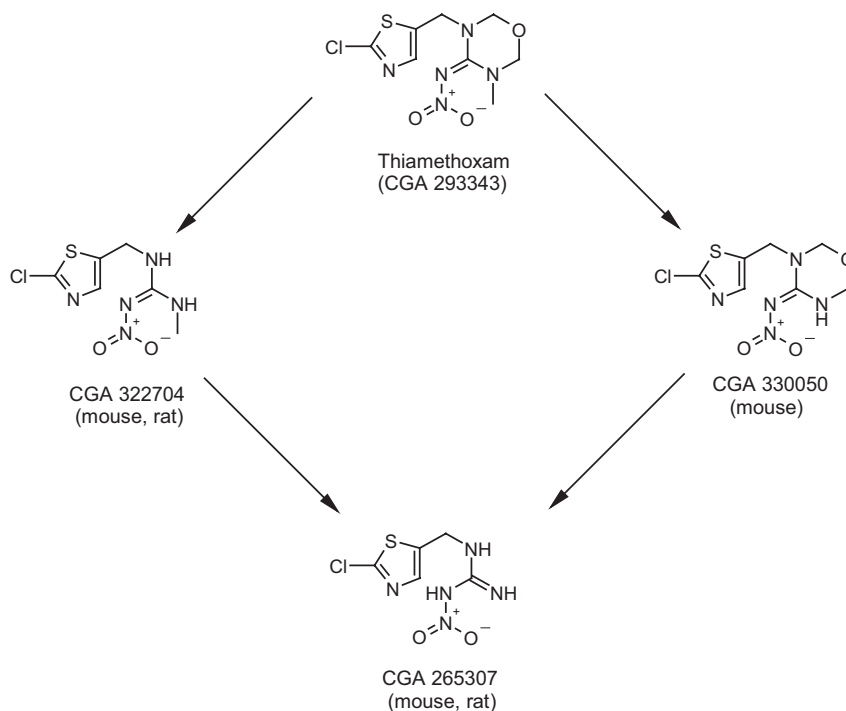


Table 58. Concentration of thiamethoxam and its metabolites in plasma in mice fed on diets containing thiamethoxam for 10 and 50 weeks

	Concentration (µg/ml)					
	Dietary concentration (ppm)					
	500	1250	2500	500	1250	2500
	Weeks on diet					
	10			50		
Thiamethoxam	3.78	8.63	14.86	2.26	5.54	9.71
CGA 322704	1.08	2.63	5.31	0.80	1.99	3.38
CGA 265307	1.68	3.62	7.05	1.48	3.31	4.20
CGA 330050	0.53	0.91	1.50	0.40	0.85	1.12

From Green (2003a)

The concentrations of the three metabolites CGA 322704, CGA 265307 and CGA 330050 in plasma were considerably higher (8-fold, 140-fold and 15-fold higher, respectively, after 10 weeks) in mice than in rats. The increase in plasma concentration of CGA 265307 after 1 week and 10 weeks in mice indicates that there is induction of the pathway(s) leading to the formation of this metabolite in this species. The differences in concentrations of CGA 265307 and CGA 330050 between mice and rats are sufficient to suggest that they may account for the species differences in carcinogenicity. These findings are also consistent with the study (above) in which the rate constants for this pathway were measured in vitro in rat, mouse and human liver fractions (Green, 2002).

Metabolite CGA 322704 (clothianidin) has been tested in rodent carcinogenicity studies; however, tumour incidences were not increased in either mice (CD-1 strain) or rats at levels up to 3000 ppm and 2000 ppm, respectively (Federal Register, 2003). The possibility that different mouse strains respond differently to thiamethoxam was investigated; however, no difference was seen in the metabolism of thiamethoxam, CGA 322704 or CGA 265307 in TifMAGf and CD-1 strains, so it is considered unlikely that the differences in the carcinogenicity of thiamethoxam and CGA 322704 were due to the use of different strains of mouse (Green, 2003a).

The evidence suggesting liver enzyme induction in mice, but not in rats, was investigated further. After treatment of female Tif:RAIf (SPF) rats with thiamethoxam, some of the investigated biochemical and immunochemical liver parameters were slightly increased, whereas others decreased. However, the observed changes gave no indication for any remarkable inducing effect of thiamethoxam on the hepatic phase I and phase II xenobiotic metabolizing enzymes. In addition, treatment did not affect the hepatic glutathione concentration or the activity of γ -glutamylcysteine synthetase, the rate-limiting enzyme in glutathione biosynthesis (Waechter, 2003). This is in contrast to similar studies in the mouse, in which thiamethoxam was a moderate inducer of hepatic xenobiotic metabolizing enzymes and caused an increased hepatic glutathione concentration as well as an increased activity of γ -glutamylcysteine synthetase (Trendelenburg, 1998).

Plasma cholesterol changes: The earliest and most significant change in mice fed on diets containing thiamethoxam is a marked reduction in plasma cholesterol levels. This was demonstrated in a study by Noakes (2003a), in which blood cholesterol levels were statistically significantly reduced by 4%, 38% and 35%, respectively, in male Tif:MAGf mice (females not studied) fed diets containing 2500 ppm thiamethoxam for 2, 11 and 21 weeks. The early reduction in plasma cholesterol level was confirmed in a separate experiment by Green (2003b), in which plasma cholesterol level was reduced 31% in mice receiving 2500 ppm thiamethoxam for 4 weeks. Return to control diet in this second Green (2003b) experiment for another 2 or 4 weeks non-significantly changed the cholesterol concentration in comparison with the controls by +19% and -8%, respectively. Even after just 1 week on diets containing thiamethoxam at 1250 and 2500 ppm, plasma cholesterol levels were reduced in weanling mice by 15% and 21%, respectively, but not at 500 ppm (+4.5%), whereas 1 week on the 500, 1250 or 2500 ppm diets reduced plasma cholesterol levels in adult mice by 22%, 32% and 28%, respectively (Green, 2003c). Other studies showed that the effect on cholesterol was persistent and occurred at all dietary concentrations associated with increases in liver tumour incidence in mice. Dietary administration of thiamethoxam to Tif:MAGf mice at 0, 50, 200, 500, 1250, 2500 or 5000 ppm for up to 50 weeks statistically significantly reduced plasma cholesterol levels at all sampling times (10, 20, 30, 40 and 50 weeks) in the 5000 ppm group and at most sampling times in the 2500 and 1250 ppm groups. Cholesterol level was also significantly reduced in the 500 ppm group at 10 and 20 weeks by 30% and 28%, respectively, but not at later times. Cholesterol was not reduced at any sampling time in the 200 or 50 ppm groups (Green, 2003b). The mechanism by which cholesterol is reduced in mice has not been established. Based on the assumption that thiamethoxam was acting like statins, the effect of thiamethoxam on 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis, was investigated, but the enzyme was not inhibited in mice treated with 2500 ppm thiamethoxam diets for 20 weeks, and neither thiamethoxam nor its metabolites CGA265307 and CGA330050 at 300 μ mol/l for 20 minutes inhibited the enzyme in vitro (Green, 2003b). These concentrations were of the order 10-fold higher than the normal substrate concentrations.

In comparisons of the activity of thiamethoxam with those of some of its metabolites, it was found that the concentration of plasma cholesterol in mice was not induced by CGA 322704 at 2000 ppm or by CGA 265307 at 500 ppm given for up to 20 weeks, whereas 2500 ppm thiamethoxam reduced the values at 1, 10 and 20 weeks by 19%, 38% and 35%, respectively. In contrast, the metabolite CGA 330050 reduced plasma cholesterol levels after 1, 4 or 10 weeks by 14%, 18% and 16%,

respectively, at 500 ppm and by 16%, 25% and 19%, respectively, at 1000 ppm (Green, 2003b). This same study showed that feeding 1000 or 3000 ppm thiamethoxam to rats for up to 50 weeks did not lead to cholesterol level reductions at 1, 10, 20, 30, 40 or 50 weeks.

In considering whether this effect on circulating cholesterol concentrations might constitute a key event in hepatocarcinogenesis in mice, it is necessary to recall the effects of thiamethoxam on this parameter in rats in 28-day (Bachmann, 1995) and 90-day (Bachmann, 1996b) studies. There were statistically significant increases in cholesterol concentrations of 22%, 26% and 43%, respectively, in the 28-day study in male rats given diets containing 1000, 2500 or 10 000 ppm thiamethoxam and of 70% in female rats given 10 000 ppm thiamethoxam. In the 90-day study, 5000 ppm thiamethoxam was associated with increases in cholesterol of 22% in males and 24% in females.

There is strong evidence that changes in lipid metabolism in rodents lead to an increase in liver tumours. A large number of drugs have been developed in recent years for the control of lipids (triglycerides and cholesterol) and the prevention of coronary heart disease. These fall into two main classes: the hypolipidaemic drugs, such as the fibrates, and the more recent statins. Both classes have been evaluated in rodent carcinogenicity studies during their development, and the majority of drugs in each class cause liver cancer, particularly in mice, but also in rats (MacDonald et al., 1988; Gerson et al., 1989; Newman & Hulley, 1996; von Keutz & Schluter, 1998). Thiamethoxam is not a peroxisome proliferator, and it has minimal effects on plasma triglycerides *in vivo*. In specifically lowering plasma cholesterol, its mode of action might resemble that of the statin family of cholesterol-lowering drugs. Changes in cholesterol biosynthesis and/or metabolism are early events, but it is unclear whether they constitute key events in the mode of action of thiamethoxam as a mouse liver carcinogen.

10–50 weeks: Hepatotoxicity

At this stage of continuous dosing in mice (15 examined per group), frank signs of hepatotoxicity were seen (Noakes, 2003a; Weber, 2003), and the statistically significant changes described below were observed.

Plasma cholesterol changes: As described above, reductions in plasma cholesterol were sustained throughout this period.

Plasma clinical chemistry changes: The median AST activity was increased at 2500 ppm in weeks 20 and 40 (but not week 10, 30 or 50) and at 5000 ppm in all weeks from 10 to 50. The median ALT activity was significantly increased at 1250 ppm in weeks 40 and 50 (but not at earlier times) and at 2500 and 5000 ppm in all weeks from 10 to 50. No changes were observed in the activity of alkaline phosphatase (Weber, 2003). On the basis of the dose levels at which the activities of these plasma enzymes are affected, it would appear that this is not a key event in mouse liver carcinogenesis.

Hepatocellular hypertrophy: Hepatocellular hypertrophy incidences and/or mean severity (in parentheses) were statistically significantly increased from the control group value of 0/15 to 7/15 (1.00), 12/15 (1.17) and 12/15 (1.67), respectively, at weeks 30, 40 and 50 for the 2500 ppm group and to 12/15 (1.75), 13/15 (2.15), 14/15 (2.00), 13/15 (2.23) and 14/15 (1.50) at weeks 10, 20, 30, 40 and 50 for the 5000 ppm group. This change was characterized by enlarged centrilobular/midzonal hepatocytes with increased amounts of cytoplasmic glycogen, fat and smooth endoplasmic reticulum (Weber, 2003). The increase in hepatocellular hypertrophy was confirmed in a separate study in which male TifMAGf mice were given diets containing 0 or 2500 ppm thiamethoxam for up to 20 weeks. The incidences (and severity) at week 10 were 0/12 at 0 ppm and 7/12 (1.14) at 2500 ppm and, at week 20, 1/11 (2.00) at 0 ppm and 7/12 (1.29) at 2500 ppm. In this same experiment, neither of the metabolites CGA 322704 (2000 ppm) and CGA 265307 (500 ppm) induced hypertrophy at any time up to 20 weeks (Noakes, 2003a).

Table 59. Intergroup comparison of selected microscopic findings in the liver: total incidence across all time points in mice treated with thiamethoxam

Finding	Dietary concentration (ppm)						
	0	50	200	500	1250	2500	5000
Hepatocellular hypertrophy	0	1	0	2	5	35*	66*
Hepatocellular necrosis	9	11	13	27*	44*	66*	71*
Inflammatory cell infiltration	18	21	18	31*	33*	51*	47*
Hepatocellular apoptosis	0	0	1	6*	28*	51*	63*
Pigmentation	0	1	1	4	8*	38*	57*
Fatty change	30	37	38	52*	61*	57*	12*

From Weber (2003)

* $P < 0.05$ (statistically significant difference from control group mean)

Pigmentation: Pigmentation was statistically significantly increased in incidence and/or mean severity at 500 ppm (week 50), at 2500 ppm (from weeks 20 to 50) and at 5000 ppm (all time points). Yellow/brown pigment granules in the cytoplasm of centrilobular hepatocytes characterized this lesion. Occasionally, pigmented Kupffer cells were observed (Weber, 2003). The pigment was not identified. This increase in pigmentation at 2500 ppm was confirmed in a separate study after 20 weeks of exposure, in which the incidences were 1/11 in the 0 ppm group and 5/12 in the 2500 ppm group (Noakes, 2003a). The pigment could possibly have been haemosiderin or lipofuscin. In the absence of effects on blood cells, the latter is more likely.

Hepatic single-cell necrosis: Hepatic single-cell necrosis was increased significantly in incidence and/or mean severity at 500 ppm in week 40, at 1250 ppm in weeks 20, 30, 40 and 50 and at 2500 and 5000 ppm in weeks 10, 20, 30, 40 and 50. Hepatocellular necroses affected single cells or small groups of cells with mainly centrilobular localization and were often accompanied by inflammatory cells (Weber, 2003).

Inflammatory cell infiltration: Inflammatory cell infiltration was increased significantly in incidence and/or mean severity at 500 ppm in week 50, at 1250 ppm in weeks 40 and 50, at 2500 ppm in weeks 20, 40 and 50 and at 5000 ppm in weeks 30, 40 and 50 (Weber, 2003).

Hepatocellular apoptosis: Hepatocellular apoptosis, as assessed by standard histology, was increased in incidence and/or mean severity at 1250 ppm thiamethoxam in weeks 20 and 30 and at 2500 ppm and 5000 ppm in weeks 10, 20, 30, 40 and 50. Hepatocellular apoptoses affected single cells or small groups of cells with mainly centrilobular localization. Quantitative assessment of the extent of apoptosis using terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate (dUTP) nick end labelling (TUNEL) (Gavrieli, Sherman & Ben Sasson, 1992) area density showed an increase at 500 ppm in weeks 30 and 40, at 1250 ppm in weeks 30 and 40, at 2500 ppm in weeks 30, 40 and 50 and at 5000 ppm in weeks 10, 20, 30, 40 and 50 (Weber, 2003). Largely consistent with these data, Noakes (2003a) did not show any increase in TUNEL in TifMAGf mice treated for up to 20 weeks with thiamethoxam at 2500 ppm, CGA 322704 at 2000 ppm or CGA 265307 at 500 ppm.

These hepatic manifestations of effects of thiamethoxam, all time points combined, are summarized in Table 59.

In vitro study of cytotoxicity in primary hepatocyte cultures from rats and mice failed to demonstrate any effect of thiamethoxam or the metabolite CGA 256084 at concentrations of up to 5000 $\mu\text{mol/l}$ (Bouis, 1997).

20–50 weeks: Sustained increased cell replication rate

An increased median 5-bromo-2'-deoxyuridine (BrdU) labelling index was observed at 1250 ppm in week 40, at 2500 ppm in weeks 30, 40 and 50 and at 5000 ppm in weeks 10, 30, 40 and 50. These increases were determined across the whole liver lobule (Weber, 2003). These observations were confirmed in a separate study after 20 weeks of exposure to 2500 ppm, but no increase was observed after 10 weeks (Noakes, 2003a). When the increases in cell replication rates were measured specifically in centrilobular hepatocytes, the region of the liver in which single-cell necrosis and apoptosis were concentrated, a statistically significant increase in cell replication rates was also observed in the 500 ppm dose group. The labelling indices (%) increased from 0.15 ± 0.10 in the control group to 0.36 ± 0.31 at 500 ppm and 0.48 ± 0.45 at 1250 ppm. There was no increase (0.10 ± 0.07) at 200 ppm (Soames, 2003). The metabolites CGA 322704 at 2000 ppm and CGA 265307 at 500 ppm did not increase BrdU labelling after 10 or 20 weeks (Noakes, 2003a). An assessment of DNA replicative synthesis in male rats fed thiamethoxam for 28 days failed to demonstrate any significant change in the numbers of proliferating cell nuclear antigen-positive hepatocyte nuclei at dietary concentrations up to 10 000 ppm (Persohn, 1995).

Hepatocellular neoplasia developed to some degree in Tif:MAGf mice even in the small groups in the comparatively short 50-week dietary experiments with thiamethoxam. At week 40, there were 0, 0, 0, 1, 1, 2 and 1 hepatocellular neoplasms in the 0, 50, 200, 500, 1250, 2500 and 5000 ppm groups, respectively, whereas at 50 weeks, the numbers were 2, 1, 1, 0, 2, 3 and 4, respectively. Most were adenomas, but some carcinomas were also reported.

Carcinogenicity and toxic properties of metabolites: Both the hepatic tumours and the events described above were seen in the Tif:MAGf mouse. The long-term studies with thiamethoxam metabolite CGA 322704 (tested as clothianidin) used another strain, believed to be the CD-1 mouse, in which no carcinogenesis in liver or elsewhere was observed. In order to investigate possible strain effects on the outcome of these studies, both male Tif:MAGf mice and male CD-1 mice were fed on diets containing 0 or 2500 ppm thiamethoxam for 1, 10 or 20 weeks. The end-points monitored in this study were identical to those in the 50-week studies: namely, liver biochemistry, histopathology, apoptosis (TUNEL) and cell replication rates (Noakes, 2003a).

Decreases in plasma cholesterol levels were seen at all time points after exposure to the metabolite CGA 322704. At 10 and 20 weeks, aminotransferase activities were increased, and the histopathological changes noted at these time points in the 50-week mouse study were observed. An increase in cell replication rates was seen at 20 weeks. The magnitude of the changes seen in this study was comparable to those seen in the 50-week mouse study. All of these changes were seen in both Tif:MAGf and CD-1 mice to a similar degree. Thus, the events, at least some of which may be key to a mode of action, are not mouse strain specific.

Two other thiamethoxam metabolites investigated in these studies, CGA 330050 and CGA 265307, were dosed to Tif:MAGf mice at levels selected to mimic systemic exposure to these metabolites following high-dose feeding levels of thiamethoxam. These were 500 and 1000 ppm for CGA 330050 and 500 ppm for CGA 265307. The dose levels of CGA 330050 induced the same changes in the livers of mice as those seen after dietary administration of thiamethoxam. Plasma cholesterol levels were reduced significantly, and the histopathological examination of the livers revealed an increase in hypertrophy, single-cell necrosis, apoptosis and increased cell replication at 10 weeks. Plasma cholesterol was statistically significantly reduced at week 1 by 14% and 16% in the 500 and 1000 ppm groups, respectively, whereas at weeks 4 and 10, there were statistically significant reductions only in the 1000 ppm group of 25% and 31%, respectively. Hepatocellular hypertrophy incidences were 0/12 in all groups at 1 week, but after 10 weeks, they were 0/12, 0/12 and 11/12 (8/12 being of minimal severity) in the 0, 500 and 1000 ppm groups, respectively. Single-cell necrosis/apoptosis did not occur at 1 week, but at 10 weeks, the incidences were 2/12, 2/12 and 6/12 (higher,

but not significant) in the 0, 500 and 1000 ppm CGA 330050 groups. However, BrdU labelling of hepatocyte nuclei was statistically significantly increased after 10 weeks at 1000 ppm. The percentages in the 0, 500 and 1000 ppm groups were 0.20 ± 0.11 , 0.36 ± 0.24 and 0.57 ± 0.61 , respectively. Therefore, CGA 330050 is seen to play a role in the development of hepatic lesions (Green, 2003d).

CGA 265307 (dosed at 500 ppm in the diet, a dose level selected to mimic systemic exposure to this metabolite following a feeding level of 2500 ppm thiamethoxam) did not induce any of the hepatic lesions seen in thiamethoxam-treated mice when tested in a 20-week study alongside thiamethoxam and CGA 322704 (Noakes, 2003a).

The metabolic relationships of these metabolites (CGA 322704, CGA 330050 and CGA 265307) to each other are shown in Figure 8 above. It is possible for CGA 265307 to be produced from thiamethoxam by two routes, one of which is found in mouse (via CGA 330050), whereas the other pathway can occur in both mice and rats (via CGA 322704).

It is proposed that the key events in a mode of (carcinogenic) action of thiamethoxam in mice are as follows:

1. Exposure to thiamethoxam, its absorption and metabolism in the liver, in which metabolites CGA 322704 and CGA 330050 are produced, both of which are further metabolized to CGA 265307. There are significant quantitative species differences in the metabolism of thiamethoxam.
2. Hepatocellular toxicity induced by CGA 330050 and exacerbated by CGA 265307, seen as increases in hepatocellular single-cell necrosis and apoptosis. Changes in plasma aminotransferase activities are not seen at all critical doses.
3. Sustained cell proliferation in the liver of mice. Adenomas develop from these proliferating cell populations by a non-genotoxic process that has not been identified.

Metabolism to the critical metabolites proceeds in mice to an extent that far exceeds that in rats in vivo. Experiments with mouse, rat and human hepatocytes in vitro indicate close similarities of rat and human hepatocytes and the dissimilarity of mouse hepatocytes. The species differences are not absolute, all metabolites being found in rats as well as in mice, but the quantitative differences are of paramount importance in the induction of toxic and neoplastic effects. As thiamethoxam does not induce hepatocellular neoplasms in rats, it is reasonable to conclude that the compound will not do so in exposed humans either.

(ii) *Effects on kidney in rats*

Subchronic and chronic studies with thiamethoxam in rats indicated the kidney to be a target organ in males. Histopathologically, renal tubular hyaline change was observed at high doses (≥ 250 ppm) in subchronic studies. In the chronic study, increased incidences of regenerative changes in renal tubules (12-month interim sacrifice) and chronic nephropathy (24-month terminal sacrifice) were the main findings at high doses (≥ 500 ppm) in male animals. The morphological appearance of the predominant finding "renal tubular hyaline change" in subchronic studies as well as its exclusive treatment-related induction in the kidney of male rats suggested this effect to be an α_{2u} -globulin nephropathy. The sponsor of the compound also suggested that the chronic nephropathy that was observed and that seemed to be exacerbated by treatment was also dependent upon α_{2u} -globulin. This assumption is unlikely to be correct, as chronic progressive nephropathy is a separate entity and is not male rat specific, although it has a tendency to be more common in males than in females.

A number of studies were conducted with the objective of providing evidence for α_{2u} -globulin involvement in the renal pathology observed, but there seems to have been little consideration of chronic progressive nephropathy as a component of the renal lesions. Chronic progressive nephropathy develops as a naturally occurring disease of rats that is particularly pronounced in males (Elema

& Arends, 1975). Not only is it a degenerative disease, but it is also highly regenerative, in that cell proliferation is increased in many of the affected tubules. It is important to recognize that this disease is not the result of any chemical treatment, and it is necessary to distinguish its regenerative aspects from preneoplasia (atypical hyperplasia) (Hard & Seely, 2005). Incidence and severity can be influenced by a number of factors, which include strain, sex (Elema & Arends, 1975), diet (Rao, 2002) and hormonal status (Baylis, 1994; Tanaka et al., 1995), in addition to chemical treatment. Chronic progressive nephropathy begins at about 2 months of age, when some rats develop basophilic renal tubules with a thickened basement membrane. Progression involves an increase in the number of tubules affected, tubule degeneration and atrophy and an ongoing renal tubule cell proliferation in which mitotic figures may be frequent (Hard & Seely, 2005). By the time that end-stage (grade 8) chronic progressive nephropathy is reached, there are virtually no normal tubules remaining, and death from renal failure is highly probable. In these advanced stages of this disease and in the same areas of the kidney, there is the development of atypical hyperplasia. Atypical hyperplasia is distinguished from foci of regeneration by the absence of thickening of the tubule basement membranes and the observation of more disorganization and crowding, sometimes with stratification, of tubule epithelial cells. In untreated rats, it does not commonly develop to extreme stages within 2-year studies. Exacerbation of chronic progressive nephropathy currently has no identified biochemical events equivalent to those that occur in α_{2u} -globulin nephropathy, and the process is not easily investigated by experiment. However, this process is becoming more widely recognized as a potential mode of action by which chemicals may influence the incidence of kidney tumours in rats (although this was not an issue with thiamethoxam). Clearly, chronic progressive nephropathy does occur in untreated rats, its severity does increase with advancing age, and it is exacerbated by exposure to some chemicals in comparison with untreated rats. Importantly, however, it has no human counterpart (Hard, Johnson & Cohen, 2009) and therefore should not be taken into consideration in human health risk assessment.

In the 90-day dietary study in rats (Bachmann, 1996b), chronic lesions of the renal tubules were already apparent and were at a higher incidence in the higher dose group rats of both sexes. In the 0, 25, 250, 1250, 2500 and 5000 ppm groups, respectively, the incidences in groups of 10 male rats were 0, 1, 3, 6, 10 and 9 and, in female rats, 4, 5, 7, 7, 9 and 10. Pairwise comparison of the incidences would indicate 250 ppm as a NOAEL in male rats, but, as mentioned above, the lesion has no human significance, and so it may be discounted.

With regard to α_{2u} -globulin nephropathy, there have been extensive investigations and a large number of publications pointing to it being male rat specific and—again—of no human significance. It is emphasized once more that, in the context of thiamethoxam, the issue is only of non-neoplastic renal lesions that are treatment related. There is no requirement to formulate a mode of action for tumour induction. The key events considered key to an α_{2u} -globulin-associated nephropathy are as follows:

- increase in the number and size of hyaline droplets in the renal cortex;
- identification of the protein accumulating in hyaline droplets as α_{2u} -globulin;
- reversible binding of thiamethoxam or a metabolite to α_{2u} -globulin;
- a characteristic sequence of histopathological changes in shorter-term studies (necrosis in the tubule epithelium, exfoliation of cells into proximal tubule lumen, granular cast formation and linear mineralization of the papillary tubules);
- sustained regenerative tubule cell proliferation or foci of tubule hyperplasia.

It may not be necessary to consider all of these events, but at least the early ones should be manifest.

The incidences of hyaline change in groups of 10 rats treated with 0, 25, 250, 1250, 2500 or 5000 ppm thiamethoxam, respectively, in the 90-day study were, in males, 1, 0, 4, 8, 10 and 10 and, in females, 0 in all groups. Furthermore, in males, the severity of the hyaline lesion was scored, on average, in these same groups, respectively, as 1.0, 0, 1.3, 1.5, 1.7 and 2.3. Thus, while there are no specific data on number and size of the hyaline droplets, a dose-related severity increase was recorded by the pathologist. It is significant that there was no report of hyaline droplets in the kidneys of female rats in this experiment (Bachmann, 1996b) and, furthermore, that there was no mention of them in mice, although kidneys were examined (Bachmann, 1996a).

Four retrospective immunohistochemical studies (with a specific anti- α_{2u} -globulin antibody) were performed with the intention of identifying and quantifying the renal accumulation of α_{2u} -globulin in male rats. These studies used material from a 28-day study, a 3-month study, and the 12-month and the 24-month terminations of a carcinogenicity and chronic toxicity study in rats with thiamethoxam. The results showed the treatment-related increase in renal α_{2u} -globulin accumulation in male rats (Weber, 2000a,b,c,d), particularly after 28 days and 3 months of treatment. Another important diagnostic tool for altered α_{2u} -globulin in hyaline droplets is the presence of crystalline material, but this is better seen if Mallory-Heidenhain stain is used, which was not the case in these studies. It was concluded that the renal changes observed in male rats treated with high doses of thiamethoxam represent a mild α_{2u} -globulin nephropathy. As such, it is a male rat-specific lesion that has no human relevance and should not be considered in risk assessment of this compound.

3. Toxicology of metabolites

Clothianidin (the *E*-isomer of CGA 322704) was evaluated at this Meeting of the JMPR.

The acute toxicity of CGA 322704 has been investigated in a rat LD₅₀ assay and in a bacterial mutation assay. No other investigations were submitted; however, the carcinogenicity of this compound has been evaluated previously by regulatory authorities (Federal Register, 2003). No indication of carcinogenicity was observed in CD-1 mice.

In an acute oral toxicity study conducted according to OECD guideline 401 (1987), groups of five male and five female fasted Wistar HanIbm:WIST strain rats were each administered CGA 322704 technical (batch No. RV-2793/6; purity 99%) suspended in 0.5% methylcellulose solution in aqueous 0.1% polysorbate 80, at a dose volume of 10 ml/kg bw by gavage, as a single oral dose of 0, 1500 or 2000 mg/kg bw. Mortality, clinical signs and body weight changes were recorded. Autopsies were performed on rats at termination on day 14.

There were no deaths during the study. Tremor, piloerection and hunched posture were observed in all rats in the 2000 mg/kg bw group on the day of treatment, but all rats appeared normal by day 1. No remarkable clinical observations were seen among animals in the 0 and 1500 mg/kg bw groups. Body weights and body weight changes of rats in all groups were not affected by treatment. Autopsy examinations did not reveal any treatment-related abnormalities.

The acute oral LD₅₀ of CGA 322704 in male and female rats was greater than 2000 mg/kg bw (Cantoreggi, 1998).

There was no evidence of mutagenic activity in a bacterial reverse mutation assay performed over a dose range of up to 5000 µg/plate. The assay was performed in the presence and absence of an exogenous metabolic activating system (S9 mix) prepared in the standard way from male rats treated with Aroclor 1254 and using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 and *Escherichia coli* WP2uvrA. At the highest dose, the numbers of revertants per plate were reduced in *S. typhimurium* TA102 and *E. coli* WP2uvrA in the presence of S9 mix (Deparade, 1998).

4. Observations in humans

As a new compound, there is little information on exposure of workers to thiamethoxam, and none on exposure of the general population. There have so far been no reported incidents of adverse reactions during the manufacture or formulation of thiamethoxam. Twelve cases of adverse reactions related to thiamethoxam are recorded in the Syngenta adverse effects database up to July 2009. The symptoms reported were rash, pruritus, erythema and dermal irritation, all of which were transitory.

Comments

Biochemical aspects

In rats given [^{14}C]thiamethoxam labelled in either the thiazole or oxadiazine ring as a single oral dose of 0.5 or 100 mg/kg bw, the radiolabel was rapidly and completely absorbed, based on the recoveries in excreta. The time to reach maximum concentrations in plasma was 1–4 hours. Distribution to the tissues was generally non-selective, but resulted in higher concentrations in liver and blood. Tissue residues in rats amounted to 0.3% of the total applied dose after 7 days.

The depletion from the tissues followed first-order kinetics, with half-lives in all tissues in the range of 2–6 hours, independent of the dose level, the site of the label and the sex of the rats. Seven days after oral administration of 0.5 mg/kg bw, the tissue residues were very low. The absorbed material was rapidly excreted from rats, predominantly in the urine. The routes of elimination and the urinary pattern of the rapidly excreted thiamethoxam and its metabolites were complex and independent of the route of administration, the dose level, pretreatment with non-radiolabelled thiamethoxam, the site of the label and the sex of the animals.

In rats, about 20–30% of the dose was biotransformed, whereas 70–80% was eliminated as unchanged thiamethoxam. Within 24 hours, about 90% of the dose was excreted via kidneys with recovery in urine, and about 4% via the bile with recovery in faeces; total faecal recovery was about 5%. In mice, 30–60% of the dose was biotransformed and eliminated mainly in urine, but faecal elimination accounted for about 19%.

Twenty-two metabolites were isolated from the excreta of rats and identified. The quantitatively most important metabolite was CGA 322704 (clothianidin), which accounted for about 10% of the dose. The individual contributions of all the other metabolites did not exceed 1% of the dose. Plasma concentrations of two of these minor metabolites, CGA 330050 and CGA 265307, were 15- to 140-fold higher in mouse than in rat. The major reaction involved in the biotransformation of thiamethoxam is cleavage of the oxadiazine ring to the corresponding nitroguanidine compound. Minor pathways are reduction of the nitroguanidine group, yielding a hydrazine, followed by either acylation or further reduction to a guanidine derivative, hydrolysis of the guanidine group to the corresponding urea, demethylation of the guanidine group and substitution of the chlorine of the thiazole ring by glutathione. Cleavage between the thiazole and oxadiazine ring occurs to a small extent and is mediated by either glutathione or oxidative dealkylation. The glutathione derivatives are prone to further degradation. Both the thiazole and oxadiazine moieties are susceptible to oxidative attack. These minor pathways proceed to small molecules and ultimately, probably, to carbon dioxide. The small molecules generated may enter the general metabolism. Metabolic degradation of thiamethoxam in mice proceeded via the same pathway as in rats. All major and almost all minor metabolites found in rat excreta were also detected in mouse excreta.

In vitro comparisons of thiamethoxam metabolism in mouse, rat and human liver microsomal preparations clearly support the significantly higher generation of CGA 330050 and CGA 265307 in mice compared with rats and additionally demonstrate that human liver microsomes metabolize thiamethoxam in a manner quantitatively similar to and not exceeding that of rats.

Toxicological data

The acute toxicity of thiamethoxam is low, the oral LD₅₀ being 1563 mg/kg bw in rats and 871 mg/kg bw in mice. Signs of toxicity at high doses included tonic or clonic convulsions, ptosis and reduced locomotor activity. The acute dermal LD₅₀ of thiamethoxam in rats was greater than 2000 mg/kg bw. The 4-hour acute inhalation LC₅₀ of thiamethoxam in rats was greater than 3.72 mg/l (the mean achieved concentration). Thiamethoxam was not irritating to rabbit skin or rabbit eyes. Thiamethoxam was not a skin sensitizer in the Magnusson & Kligman maximization test in guinea-pigs.

The short-term oral toxicity of thiamethoxam administered via the diet was evaluated in mice, rats and dogs. These consisted of 4-week range-finding studies in rats followed by 13-week toxicity studies in rats and dogs and a 52-week toxicity study in dogs. A 13-week range-finding study was also conducted in mice.

The liver was identified as a target organ in mice and rats. Treatment for 13 weeks induced liver hypertrophy, inflammatory cell infiltration and pigmentation of hepatocytes and Kupffer cells in both rodent species. In mice, single-cell necrosis and apoptosis occurred in parallel with these alterations.

The kidney was identified as a target organ in rats, but not in mice or dogs. Both sexes were affected, but there was a difference between the sexes in both morphology and sensitivity. In males, nephrotoxicity was characterized by tubular epithelial hyaline droplet accumulation, acute and chronic tubule lesions, basophilic proliferation and cast formation. The pattern of effects in male rat kidneys resembled α_{2u} -globulin nephropathy, which is generally accepted to be a phenomenon exclusively found in males of some strains of rats. Immunohistochemical studies on kidneys from male rats exposed to thiamethoxam for 28 days or 3, 12 or 24 months with a specific anti- α_{2u} -globulin antibody identified treatment-related increases in renal α_{2u} -globulin accumulation, particularly after 28 days and 3 months of treatment. It was concluded that the renal changes observed in male rats treated with thiamethoxam represent a mild α_{2u} -globulin nephropathy, which is male rat specific and has no human relevance. Renal lesions in females were confined to an increased incidence of chronic tubular lesions and an increase in the severity of nephrocalcinosis. Other observations, particularly basophilic proliferation, tended to be increased in all groups of females at 28 days and may represent the beginnings of chronic progressive nephropathy. However, such observations were not repeated after 3 (or 12) months of exposure. Therefore, the NOAELs of human relevance in rats were derived on the basis of effects in organs other than male kidney in the repeated-dose toxicity studies after 28 days and 3 months of treatment as well as in the 2-year study.

Other target organs and changes in rats were fatty changes in the adrenal cortex, enhanced haemosiderosis or extramedullary haematopoiesis in the spleen and follicular epithelial hypertrophy in the thyroid gland. Thymic and splenic atrophy in dogs and alterations suggestive of delayed maturation of the gonads in dogs and female mice occurred at doses causing substantial growth retardation.

The NOAELs derived from short-term studies in which thiamethoxam was administered orally were as follows:

- The NOAEL in the 90-day dietary study in mice with thiamethoxam was 100 ppm (equal to 14.3 mg/kg bw per day), based on raised platelet counts at 1250 ppm (equal to 176 mg/kg bw per day) in females. Minimal lymphocytic infiltration and hepatocyte hypertrophy were observed in males at 100 and 1250 ppm; in the absence of any other hepatic changes, these were considered an adaptive response or an early sign of mouse-specific hepatotoxicity.
- The NOAEL in the 28-day dietary study in rats was 100 ppm (equal to 8.0 mg/kg bw per day), based on increased plasma cholesterol concentrations at a dose level of 1000 ppm (equal to 81.7 mg/kg bw per day). The male rat-specific kidney effects have no human relevance, and therefore they are not considered for the NOAEL.

- The NOAEL in the 90-day dietary study in rats was 250 ppm (equal to 17.6 mg/kg bw per day in males), based on reduced body weight gain and histological findings in the adrenals at 1250 ppm (equal to 84.9 mg/kg bw per day). Observation of hyaline droplet accumulation in the kidneys of male rats was considered indicative of α_{2u} -globulin involvement, which is male rat specific and has no human significance.
- The NOAEL in the 90-day oral toxicity study in dogs was 250 ppm (equal to 8.23 mg/kg bw per day), based on prolonged thromboplastin times in both sexes at 1000 ppm (equal to 32 mg/kg bw per day).
- The NOAEL in the 52-week oral toxicity study in dogs was 750 ppm (equal to 21 mg/kg bw per day), based on prolonged thromboplastin times and reductions in testis weights at 1500 ppm (equal to 42 mg/kg bw per day).

Long-term toxicity and carcinogenicity studies were performed in mice and rats. The main target organs were the liver in mice and female rats and the kidneys in male rats. In rats, the principal findings were increased incidences of renal tubule regenerative lesions, which were considered to represent the sequelae of the rat-specific nephropathies observed in short-term studies. Minor and morphologically different changes occurred in the spleen of both rats and mice.

The NOAEL in the 78-week dietary study in mice was 20 ppm (equal to 2.63 mg/kg bw per day), based on hepatotoxic effects (i.e. increased liver weights, hepatocellular hypertrophy, pigment deposition, inflammatory cell infiltration and single-cell necrosis) at 500 ppm (equal to 63.8 mg/kg bw per day). Thiamethoxam was tumorigenic in mice and induced hepatocellular adenomas in male and female mice at a dose level of 500 ppm and hepatocellular adenocarcinomas in male mice at 2500 ppm (equal to 354 mg/kg bw per day).

Special studies on thiamethoxam (the *EZ*-isomer mixture, as used in all toxicity studies) were performed to investigate the etiology of adenoma and adenocarcinoma formation in mouse liver during an 18-month oncogenicity study. The hypothesis investigated was that the mouse specificity in this response is due to a very large species difference in metabolism of thiamethoxam, as demonstrated by 15-fold and 140-fold higher plasma concentrations of CGA 330050 and CGA 265307, respectively, in mice than in rats following 10 weeks of dosing with thiamethoxam. This large difference is supported by in vitro comparison of thiamethoxam metabolism by microsomal preparations from mouse, rat and human liver. Although these metabolites also occur with rat microsomes, their concentrations are very much lower than in mice. The data also suggested that humans were likely to be even less susceptible than rats to the hepatic effects of thiamethoxam. The mode of action proposed for development of these tumours is based on the hepatotoxicity of the metabolite CGA 330050 in particular, with CGA 265307 exacerbating its effect, and the subsequent sustained cell proliferation of mouse hepatocytes, leading to the development of a higher incidence of hepatocellular tumours. Not all elements for a mode of action have been identified, but the available data support the contention of a low risk to humans with regard to both hepatotoxicity and carcinogenicity and the absence of any genotoxic involvement. An alternative metabolic pathway to CGA 265307 in both mice and rats is via CGA 322704. The *E*-isomer of CGA 322704 was evaluated at the present Meeting, and it was concluded that it is not carcinogenic in mice or rats.

The NOAEL in the 78-week dietary study in mice for non-hepatic effects is 1250 ppm (equal to 162 mg/kg bw per day), based on reductions in body weight and effects on spleen and stomach at 2500 ppm (equal to 354 mg/kg bw per day).

The NOAEL in the 104-week dietary study in rats was 1000 ppm (equal to 50.3 mg/kg bw per day in females), based on foci of cellular alteration in the liver and increased severity of splenic haemosiderosis at 3000 ppm (equal to 155 mg/kg bw per day). Increased incidences of renal chronic tubular lesions and basophilic proliferation were observed exclusively in male rats at 500 ppm (equal

to 21.0 mg/kg bw per day). These renal lesions were considered to represent the outcome of α_2 -globulin-mediated nephropathy, which is widely acknowledged as male rat specific and not relevant in human risk assessment, and therefore they were not used to identify the NOAEL.

Thiamethoxam was tested for genotoxicity and mutagenicity in an adequate range of assays, both in vitro and in vivo. In none of these assays was there any evidence of genotoxic or mutagenic potential.

The Meeting concluded that thiamethoxam is unlikely to be genotoxic.

On the basis of the absence of genotoxicity in vivo, the absence of carcinogenicity in rats and the mode of action by which liver tumours arise in mice, the Meeting concluded that thiamethoxam is unlikely to pose a carcinogenic risk at human dietary exposure levels.

Hyaline change and casts in renal tubules were observed in male rats in the multigeneration studies at 1000 ppm (equal to 45.6 mg/kg bw per day). This observation has no human relevance. Therefore, the relevant NOAEL for parental toxicity is 1000 ppm (equal to 45.6 mg/kg bw per day), based on significantly reduced body weight gain at 2500 ppm (equal to 117.6 mg/kg bw per day in F_0 generation males). The overall NOAEL for reproductive toxicity in the multigeneration studies in rats was 1000 ppm (equal to 74.8 mg/kg bw per day for F_1 males), based on minimal testicular germ cell loss or disorganization, with or without Sertoli cell vacuolation (and unaccompanied by any reduction in epididymal sperm numbers), at 2500 ppm (equal to 191.5 mg/kg bw per day). These effects were not observed in the first study, a difference that could be attributed to a refinement of the methods used for sperm observations between the first and second studies. The overall NOAEL for offspring was 30 ppm (equal to 1.4 mg/kg bw per day for the males), based on marginal reductions in body weight gains of F_{2a} and F_{2b} pups during lactation at 1000 ppm (equal to 45.6 mg/kg bw per day for males) in the first of the two studies.

The NOAEL for maternal toxicity in the developmental toxicity study in rats was 30 mg/kg bw per day, based on slightly decreased body weight gain in dams, providing a LOAEL of 200 mg/kg bw per day. The NOAEL for fetotoxicity was 200 mg/kg bw per day, based on mild reduction in mean fetal body weight at 750 mg/kg bw per day. Further evidence of fetotoxicity at this dose was increased incidences of skeletal anomalies (irregular or absent ossification of the occipital bone) and skeletal variants (poor ossification of sternebra 5, shortened 13th rib and non-ossification of metatarsal 1).

The NOAEL for maternal toxicity in the developmental toxicity study in rabbits was 15 mg/kg bw per day, based on reduction in body weight gain and feed consumption during the treatment period in dams at 50 mg/kg bw per day. The NOAEL for fetotoxicity in rabbits was 50 mg/kg bw per day, based on increased postimplantation loss and reduction in fetal body weights at 150 mg/kg bw per day. Further evidence of fetotoxicity at this dose was increased incidence of delayed and absent ossification as well as an increased incidence of fused sternebrae in fetuses.

The Meeting concluded that thiamethoxam can cause fetotoxicity and skeletal anomalies (malformations and variants), but only at maternally toxic doses.

An acute neurotoxicity study, a 13-week neurotoxicity study and a developmental neurotoxicity study were conducted in rats. A comprehensive set of neurotoxicity end-points was investigated in these studies, including an evaluation of potential to induce neurobehavioural or neuromorphological changes. The studies did not show any specific neurotoxicity after repeated exposure of adult rats or any specific developmental neurotoxicity in the offspring, including at doses causing maternal toxicity. Acute administration of thiamethoxam at dose levels approaching the LD_{50} produces a range of transient neurobehavioural effects, including tonic or clonic convulsions, ptosis and reduced locomotor activity. The NOAEL in the single-dose neurotoxicity study in rats was 100 mg/kg bw, based on transient behavioural changes at 500 mg/kg bw. The NOAEL for systemic toxicity and neurotoxicity in the 13-week neurotoxicity study was 1500 ppm (equal to 95.4 mg/kg bw per day) in males and 3000 ppm (equal to 216.4 mg/kg bw per day) in females, the highest doses tested, based on the

absence of treatment-related effects at these doses. The NOAEL for systemic toxicity in a study of developmental neurotoxicity was 400 ppm (equal to 34.5 mg/kg bw per day), based on decreased body weight gain and feed consumption in dams throughout gestation and postpartum, as well as reduced birth weight, reduced pup body weight gain, some evidence of delayed preputial separation and small changes in brain morphometry, but without any quantitative histological or behavioural changes, at 4000 ppm (equal to 298.7 mg/kg bw per day). The NOAEL for developmental neurotoxicity was 4000 ppm (equal to 298.7 mg/kg bw per day), the highest dose tested.

The Meeting concluded that thiamethoxam is not a neurotoxin in mammals at the tested dose levels, although it is a member of the neonicotinoid chemical class, the biological effects of which in target species are mediated primarily by an interaction with nicotinic acetylcholine receptor sites.

No information on medical surveillance or poisoning incidents was available.

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

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.08 mg/kg bw on the basis of a NOAEL of 250 ppm (equal to 8.23 mg/kg bw per day) in a 90-day study of toxicity in dogs, based on prolonged thromboplastin time. A safety factor of 100 was applied. This ADI is protective of the hepatotoxic and hepatocarcinogenic effects observed in mice, which were not observed in rats because of marked species differences in metabolism. It is also protective of the marginally toxic effects observed in a multigeneration study in rats at 46 mg/kg bw per day.

A number of blood chemistry and haematology parameters in dogs were considered as a basis for the ADI, but the only consistently altered parameter was measures of blood coagulation. Other end-points that received consideration included feed consumption reduction in a gavage study of developmental toxicity in rabbits. Renal changes observed in rats arose by processes not relevant for risk assessment at human dietary exposure levels.

An acute reference dose (ARfD) of 1 mg/kg bw was established on the basis of a NOAEL of 100 mg/kg bw in a single-dose study of neurotoxicity in rats. A safety factor of 100 was applied. The transient functional changes in rats appeared to be mild signs of overt toxicity rather than neurotoxicity. The neurotoxicity study was supported by a single-dose study of toxicity in mice, in which clinical signs of toxicity were observed at 500 mg/kg bw, the lowest dose tested.

The metabolite of thiamethoxam, CGA 322704 (clothianidin), was evaluated separately by the present Meeting.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day range-finding study of toxicity	Toxicity	100 ppm, equal to 14.3 mg/kg bw per day	1250 ppm, equal to 176 mg/kg bw per day
	Eighteen-month study of toxicity and carcinogenicity	Toxicity ^a	20 ppm, equal to 2.63 mg/kg bw per day	500 ppm, equal to 63.8 mg/kg bw per day
		Carcinogenicity ^b	20 ppm, equal to 2.63 mg/kg bw per day	500 ppm, equal to 63.8 mg/kg bw per day
Rat	Single-dose study of neurotoxicity	Toxicity	100 mg/kg bw	500 mg/kg bw

Species	Study	Effect	NOAEL	LOAEL
	Ninety-day study of toxicity	Toxicity ^c	250 ppm, equal to 17.6 mg/kg bw per day	1250 ppm, equal to 84.9 mg/kg bw per day
	Twenty-four-month studies of toxicity and carcinogenicity	Toxicity ^c	1000 ppm, equal to 50.3 mg/kg bw per day	3000 ppm, equal to 155 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 155 mg/kg bw per day ^d	—
	Two-generation study of reproductive toxicity (1)	Reproductive toxicity	2500 ppm, equal to 117.6 mg/kg bw per day ^d	—
		Parental toxicity ^c	1000 ppm, equal to 45.6 mg/kg bw per day	2500 ppm, equal to 117.6 mg/kg bw per day
		Offspring toxicity	30 ppm, equal to 1.4 mg/kg bw per day	1000 ppm, equal to 45.6 mg/kg bw per day ^e
	Two-generation study of reproductive toxicity (2)	Reproductive toxicity	1000 ppm, equal to 74.8 mg/kg bw per day	2500 ppm, equal to 191.5 mg/kg bw per day
		Parental toxicity ^c	1000 ppm, equal to 61.7 mg/kg bw per day	2500 ppm, equal to 155.6 mg/kg bw per day
		Offspring toxicity	1000 ppm, equal to 74.8 mg/kg bw per day	2500 ppm, equal to 191.5 mg/kg bw per day
	Developmental toxicity study	Maternal toxicity	30 mg/kg bw per day	200 mg/kg bw per day
		Embryo and fetal toxicity	200 mg/kg bw per day	750 mg/kg bw per day
Rabbit	Developmental toxicity study	Maternal toxicity	15 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	150 mg/kg bw per day
Dog	Ninety-day study of toxicity	Toxicity	250 ppm, equal to 8.23 mg/kg bw per day	1000 ppm, equal to 32 mg/kg bw per day

^a Mouse particularly susceptible to hepatotoxicity, based on the mode of action of thiamethoxam.

^b No carcinogenicity relevant to humans based on mode of action considerations of tumorigenic effects in mice.

^c Toxicity relevant to humans, not including nephrotoxicity specific for male rats.

^d Highest dose tested.

^e Marginal LOAEL.

Estimate of acceptable daily intake for humans

0–0.08 mg/kg bw

Estimate of acute reference dose

1 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption

Rapid and extensive, > 90%

Distribution

Distributed throughout the body; higher concentrations in liver and blood

Potential for accumulation	None
Rate and extent of excretion	High, > 90% within 24 h
Metabolism in animals	22 metabolites identified in rats
Toxicologically significant compounds (animals, plants and environment)	Parent; CGA 330050; CGA 322704 (clothianidin)
<i>Acute toxicity</i>	
Rat, LD ₅₀ , oral	1563 mg/kg bw
Mouse, LD ₅₀ , oral	871 mg/kg bw
Rat, LC ₅₀ , inhalation	> 3.7 mg/l (4 h)
Rat, LD ₅₀ , dermal	> 2000 mg/kg bw
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (Magnusson & Kligman test)
<i>Short-term studies of toxicity</i>	
Target/critical effect	Coagulation in dogs
Lowest relevant oral NOAEL	8.23 mg/kg bw per day (3-month study in dogs)
Lowest relevant dermal NOAEL	60 mg/kg bw per day (liver, 4-week study in rats)
Lowest relevant inhalation NOAEC	No data
<i>Long-term studies of toxicity and carcinogenicity</i>	
Target/critical effect	Liver, spleen, stomach (mice)
Lowest relevant NOAEL	2.63 mg/kg bw per day (18-month study in mice)
Carcinogenicity	Carcinogenic in mice, but unlikely to pose a risk at human dietary exposure levels, based on the proposed mode of action
<i>Genotoxicity</i>	
	Not genotoxic
<i>Reproductive toxicity</i>	
Reproductive target/critical effect	Prewaning weight gain
Lowest relevant reproductive NOAEL	74.8 mg/kg bw per day (rat)
Developmental target/critical effect	Rat: Not teratogenic; reduced live birth weight, delayed ossifications Rabbit: Reduced pup body weight, delayed ossifications and increased incidences of skeletal abnormalities
Lowest relevant developmental NOAEL	50 mg/kg bw per day (rabbit)
<i>Neurotoxicity/delayed neurotoxicity</i>	
	No signs of neurotoxicity
<i>Other toxicological studies</i>	
	Mechanistic studies relevant to hepatotoxicity and hepatocarcinogenicity in mice and renal toxicity in rats
<i>Medical data</i>	
	No reports of toxicity in workers exposed during manufacture or use

Summary

	Value	Study	Safety factor
ADI	0–0.08 mg/kg bw	Ninety-day study of toxicity in dogs	100
ARfD	1 mg/kg bw	Neurotoxicity study in rats supported by a single-dose toxicity study in mice	100

References

- Altmann B (1996) CGA 293343 technical—3-month subchronic dietary toxicity study in Beagle dogs. Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942107, 15 October 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Altmann B (1998) CGA 293343 tech.—12-month chronic dietary toxicity study in Beagle dogs. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Unpublished report No. 942108, 22 July 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Bachmann M (1995) CGA 293343 tech.: 28-days range finding study in rats (administration in food). Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942088, 5 May 1995. Submitted to WHO by Syngenta Crop Protection AG.
- Bachmann M (1996a) CGA 293343 tech.—3-month range finding toxicity study in mice (administration in food). Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942105, 13 August 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Bachmann M (1996b) CGA 293343 tech.—3-month oral toxicity study in rats (administration in food). Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942089, 23 January 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Bachmann M (1998a) CGA 293343 tech.—24-month carcinogenicity and chronic toxicity study in rats. Novartis Crop Protection AG, Experimental Toxicology, Stein, Switzerland. Unpublished report No. 942110, 27 July 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Bachmann M (1998b) CGA 293'343 tech.: 18-month oncogenicity study in mice. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Unpublished report No. 942109, 2 June 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Baylis C (1994) Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. *Journal of Clinical Investigation*, 94:1823–1829.
- Boobis AR et al. (2006) IPCS framework for analysing the relevance of a cancer mode of action for humans. *Critical Reviews in Toxicology*, 36:781–792.
- Bouis P (1997) The effects of CGA 293343 tech. and CGA 256084 in primary cultured rat and mouse hepatocytes. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB97/36, 20 November 1997. Submitted to WHO by Syngenta Crop Protection AG.
- Brammer A (2003) Thiamethoxam: Developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Unpublished laboratory report No. RR0936, 29 May 2003. Submitted to WHO by Syngenta Crop Protection AG.
- Brammer A (2007) Thiamethoxam: Supplement to developmental neurotoxicity study in rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Unpublished laboratory report No. RR0936-REG-S1, 15 January 2007. Submitted to WHO by Syngenta Crop Protection AG.
- Briswalter C (1999) The metabolism of [thiazol-2-¹⁴C] CGA 293'343 after multiple oral administration to mice—further identification of metabolites. Novartis Crop Protection, Basel, Switzerland, Amendment 1 to Study No. 027AM09, 23 August 2000. Submitted to WHO by Syngenta Crop Protection AG.

- Briswalter C (2002a) Metabolism of [oxadiazin-4-¹⁴C] CGA 293343 in the mouse after oral administration. Syngenta Crop Protection AG, Basel, Switzerland. Unpublished report No. 027AM16. Submitted to WHO by Syngenta Crop Protection AG.
- Briswalter C (2002b) Blood kinetics of CGA 293343 and its metabolites in male rats after oral administration of [oxadiazin-4-¹⁴C] CGA 293343. Syngenta Crop Protection AG, Basel, Switzerland. Submitted to WHO by Syngenta Crop Protection AG.
- Briswalter C (2002c) Blood kinetics of CGA 293343 and its metabolites in male mice after oral administration of [oxadiazin-4-¹⁴C] CGA 293343. Syngenta Crop Protection AG, Basel, Switzerland. Unpublished report No. 027AM14; Syngenta File No. CGA 293343/1806. Submitted to WHO by Syngenta Crop Protection AG.
- Briswalter C (2002d) Absorption, distribution and excretion of [oxadiazin-4-¹⁴C] CGA 293343 in the mouse after oral administration. Syngenta Crop Protection AG, Basel, Switzerland. Unpublished report No. 027AM15; Syngenta File No. CGA 293343/1589. Submitted to WHO by Syngenta Crop Protection AG.
- Cantoreggi S (1998) CGA 322704 tech. (metabolite of CGA 293343)—Acute oral toxicity in the rat. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Unpublished report No. 982001, 28 April 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Chapin RE et al. (1992) Methods for assessing rat sperm motility. *Reproductive Toxicology*, 6:267–273.
- Deparade E (1998) CGA 322704 tech. (metabolite of CGA 293343)—*Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. Novartis Crop Protection AG, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 982002, 31 March 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Deparade E (1999) CGA 293343 technical—*Salmonella*/mammalian-microsome mutagenicity test. Novartis Crop Protection AG, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 992020, 21 October 1999. Submitted to WHO by Syngenta Crop Protection AG.
- Dobovetzky M (1998) CGA 293343 tech.: Rat dietary two-generation reproduction study, includes: Effects on sperm cell parameters. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Unpublished report Nos 942121 and 982015, 20 July 1998, amended (1) 22 October 1998, amended (2) 12 November 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Dobovetzky M (1999) Amendments Nos 4 and 5 to: CGA 293343 tech.: Rat dietary two-generation reproduction study, includes: Effects on sperm cell parameters. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Unpublished report Nos 942121 and 982015, 20 July 1998, amended (1) 22 October 1998, amended (2) 12 November 1998; amended (3) 7 January 1999, amended (4) 26 July 1999, amended (5) 25 August 1999. Submitted to WHO by Syngenta Crop Protection AG.
- Dobovetzky M (2000) Amendment No. 1 to: CGA 293343 tech.—Rat oral teratogenicity study. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Unpublished report No. 942118, 7 August 1996. Amendment of 2 March 2000. Submitted to WHO by Syngenta Crop Protection AG.
- Elema JD, Arends A (1975) Focal and segmental glomerular hyalinosis and sclerosis in the rat. *Laboratory Investigation*, 33:554–561.
- Federal Register (2003) Clothianidin; pesticide tolerance. *Federal Register*, 68(104):32390–32400.
- Gavrieli Y, Sherman Y, Ben Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *Journal of Cell Biology*, 119(3):493–501.
- Gerson RJ et al. (1989) Animal safety and toxicology of simvastatin and related hydroxyl-methylglutaryl-coenzyme A reductase inhibitors. *American Journal of Medicine*, 87:4A-28S–4A-38S.
- Gerspach R (1996) CGA 293343 tech.—28-day repeated dose dermal toxicity study in the rat. Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942116, 8 October 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Green T (2002) Thiamethoxam: Comparative metabolism in mice and rats in vivo, and in mouse, rat and human liver fractions in vitro. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield,

- Cheshire, England. Unpublished report No. CTL/024607/res/rept. Submitted to WHO by Syngenta Crop Protection AG.
- Green T (2003a) Thiamethoxam (CGA293343): Metabolism in mice and rats during dietary feeding studies. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Unpublished report No. CTL/024606/res/rept. Submitted to WHO by Syngenta Crop Protection AG.
- Green T (2003b) Thiamethoxam (CGA293343): Changes in plasma cholesterol levels during dietary feeding studies. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/024607/research/report. Submitted to WHO by Syngenta Crop Protection AG.
- Green T (2003c) Thiamethoxam (CGA293343): Comparative hepatotoxicity in weanling and adult mice. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/024607/research/report-003. Submitted to WHO by Syngenta Crop Protection AG.
- Green T (2003d) Thiamethoxam (CGA293343): Hepatotoxicity of metabolites. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/024607/research/report-004. Submitted to WHO by Syngenta Crop Protection AG.
- Green T (2003e) Thiamethoxam (CGA293343): The role of nitric oxide in the development of hepatotoxicity in mice. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/024607/research/report/001. Submitted to WHO by Syngenta Crop Protection AG.
- Hard GC, Seely JC (2005) Recommendations for the interpretation of renal tubule proliferative lesions occurring in rat kidneys with advanced chronic progressive nephropathy (CPN). *Toxicologic Pathology*, 33:641–649.
- Hard GC, Johnson KJ, Cohen SM (2009) A comparison of rat chronic progressive nephropathy with human renal disease—implications for human risk assessment. *Critical Reviews in Toxicology*, 39:332–346.
- Hertner T (1995a) CGA 293343 technical—*Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. Ciba-Geigy Ltd, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 952014, 2 November 1995. Submitted to WHO by Syngenta Crop Protection AG.
- Hertner T (1995b) CGA 293343 tech.—Micronucleus test, mouse (OECD conform). Ciba-Geigy Ltd, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 952018, 15 December 1995. Submitted to WHO by Syngenta Crop Protection AG.
- MacDonald JS et al. (1988). Preclinical evaluation of lovastatin. *American Journal of Cardiology*, 62:16J–27J.
- Mewes K (1998) The metabolism of [thiazol-2-¹⁴C] CGA 293343 after multiple oral administration to mice. Novartis Crop Protection, Basel, Switzerland. Unpublished report to Study No. 027AM09, 3 November 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Mewes KE (2000) Absorption, metabolism and excretion of [oxadiazin-4-¹⁴C] CGA 293343 after dietary administration of CGA 293343 at four dose levels in the mouse. Syngenta Crop Protection AG (formerly Novartis Crop Protection AG), Basel, Switzerland. Unpublished report No. 027AM10. Submitted to WHO by Syngenta Crop Protection AG.
- Minnema DJ (1997) Acute neurotoxicity study of orally administered CGA 293343 tech. in rats. Covance Laboratories Inc., Vienna, VA, USA. Unpublished report No. 6117-364, 23 September 1997. Submitted to WHO by Syngenta Crop Protection AG.
- Minnema DJ (1998) 13-week dietary subchronic neurotoxicity study with CGA 293343 tech. in rats. Covance Laboratories Inc., Vienna, VA, USA. Unpublished report No. 6117-363, 23 June 1998. Submitted to WHO by Syngenta Crop Protection AG.

- Moxon ME (2004) Thiamethoxam: Two generation reproduction study in rats. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/RR0941/regulatory/report, 19 February 2004. Submitted to WHO by Syngenta Crop Protection AG.
- Müller T, Stampf P (1996) Absorption, distribution, and excretion of [thiazol-2-¹⁴C] and [oxadiazin-4-¹⁴C] CGA 293'343 in the rat. Ciba-Geigy Ltd, Basel, Switzerland. Unpublished report No. 027AM01, 15 August 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Newman TB, Hulley SB (1996) Carcinogenicity of lipid-lowering drugs. *Journal of the American Medical Association*, 275:55–60.
- Noakes JP (2003a) CGA 293343 (thiamethoxam), CGA 322704 and CGA 265307: Comparative toxicity in the liver of male Tif:MAGf and CD-1 mice. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/XM7081/research/report. Submitted to WHO by Syngenta Crop Protection AG.
- Noakes JP (2003b) CGA 293343 (thiamethoxam): Assessment of hepatic cell proliferation and apoptosis in female rats upon treatment for up to fifty weeks. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/XR7068/regulatory/report. Submitted to WHO by Syngenta Crop Protection AG.
- Oda S (1996a) An acute oral toxicity study of CGA 293343 tech. in rats. Bozo Research Center Inc., Tokyo, Japan. Unpublished report No. B-3120, CG 942111. Submitted to WHO by Syngenta Crop Protection AG.
- Oda S (1996b) An acute oral toxicity study of CGA 293343 tech. in mice. Bozo Research Center Inc., Tokyo, Japan. Unpublished report No. B-3122, CG 952058, 23 May 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Oda S (1996c) An acute dermal toxicity study of CGA 293343 tech. in rats. Bozo Research Center Inc., Tokyo, Japan. Unpublished report No. B-3121, CG 942112, 23 May 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Ogorek B (1996a) CGA 293343 tech.—Gene mutation test with Chinese hamster cells V79. Ciba-Geigy Ltd, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 952015, 12 January 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Ogorek B (1996b) CGA 293343 tech.—Autoradiographic DNA repair test on rat hepatocytes (OECD conform) in vitro; Ciba-Geigy Ltd, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 952017, 29 January 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Ogorek B (2000) CGA 293343 tech.—Autoradiographic DNA repair test on mouse hepatocytes (OECD conform) in vitro. Novartis Crop Protection AG, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 992066, 14 April 2000. Submitted to WHO by Syngenta Crop Protection AG.
- Persohn E (1995) CGA 293343 tech.—Assessment of replicative DNA synthesis in the course of a 28-day oral (feeding) toxicity study in male rats. Ciba-Geigy Ltd, Basel, Switzerland. Unpublished report No. CB94/47, 27 February 1995. Submitted to WHO by Syngenta Crop Protection AG.
- Rao GN (2002) Diet and kidney diseases in rats. *Toxicologic Pathology*, 30:651–656.
- Rümbeli R (1998) The metabolism of [thiazol-2-¹⁴C] and [oxadiazin-4-¹⁴C] CGA 293'343 in the rat. Determination of metabolites L14, MU3 and R6 in urine and faeces. Novartis Crop Protection AG, Switzerland. Amendment 1 to unpublished report No. 027AM02, 5 November 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Shibata R (1996a) A primary skin irritation study of CGA 293343 tech. in rabbits. Bozo Research Center Inc., Tokyo, Japan. Unpublished report No. B-3124, CG 942113, 31 May 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Shibata R (1996b) A primary eye irritation study of CGA-293343 tech. in rabbits. Bozo Research Center Inc., Tokyo, Japan. Unpublished report No. B-3123, CG 942114, 31 May 1996. Submitted to WHO by Syngenta Crop Protection AG.

- Shutoh Y (1996) CGA 293343 tech.: Acute inhalation toxicity study in rats. The Institute of Environmental Toxicology, Tokyo, Japan. Unpublished report No. IET 95-0120, CG 942122, 14 August 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Soames T (2003) Thiamethoxam (CGA 293343 tech.): Sublobular assessment of hepatic cell proliferation after 40 weeks. Syngenta Ltd, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Syngenta unpublished report No. CTL/03R026/research/report. Submitted to WHO by Syngenta Crop Protection AG.
- Sonich-Mullin C et al. (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. *Regulatory Toxicology and Pharmacology*, 34:146–152.
- Tanaka A et al. (1995) Acceleration of renal dysfunction with ageing by the use of androgen in Wistar/Tw rats. *In Vivo*, 9:495–502.
- Thanei P, Rümble R (1998) The metabolism of [thiazol-2-¹⁴C] and [oxadiazin-4-¹⁴C] CGA 293'343 in the rat. Novartis Crop Protection AG, Switzerland. Unpublished report No. 027AM02, 18 September 1998 and 5 November 1998 (Amendment 1 to the report). Submitted to WHO by Syngenta Crop Protection AG.
- Trendelenburg C (1998) CGA 293343 tech.—Effects on biochemical parameters in the liver following administration to male and female mice. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB98/11, 15 September 1998. Submitted to WHO by Syngenta Crop Protection AG.
- von Keutz E, Schluter G (1998) Preclinical safety evaluation of cerivastatin, a novel HMGCoA reductase inhibitor. *American Journal of Cardiology*, 82:11J–17J.
- Waechter F (2003) CGA 293343 tech.: Effects on selected biochemical parameters in the liver following dietary administration to female rats for 1 and 10 weeks. Health Assessment/Cell Biology, Syngenta Crop Protection AG, Basel, Switzerland. Syngenta unpublished report No. CB 02/34, 30 January 2003. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (1998) CGA 293343 tech.—Assessment of hepatic cell proliferation in mice. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB98/12, 24 September 1998. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (1999a) CGA 293343 tech.—Histopathologic evaluation of the liver in male mice upon treatment with a single high dose of CGA 293343 tech. (thiamethoxam). Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB99/60, 17 December 1999. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (1999b) Histochemical assessment of hepatic apoptosis upon treatment of male mice with CGA 293'343 tech. (thiamethoxam) for up to 9 months. Novartis Crop Protection AG, Toxicology/Cell Biology, Basel, Switzerland. Unpublished report No. CB 99/57, 6 December 1999. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (2000a) Immunohistochemical assessment of α_{2u} -globulin in the rat kidney upon administration of CGA 293343 for 28 days. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB 00/16, 3 July 2000. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (2000b) Immunohistochemical assessment of α_{2u} -globulin in the rat kidney upon administration of CGA 293343 for 3 months. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB 99/55, 3 July 2000. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (2000c) Immunohistochemical assessment of α_{2u} -globulin in the rat kidney upon administration of CGA 293343 for 12 months. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB 00/14, 3 July 2000. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (2000d) Immunohistochemical assessment of α_{2u} -globulin in the rat kidney upon administration of CGA 293343 for 24 months. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB 00/15, 3 July 2000. Submitted to WHO by Syngenta Crop Protection AG.

- Weber E (2000e) Morphometric assessment of thymic atrophy in F₁ females of a two-generation reproduction study in the rat with CGA 293343 tech. Novartis Crop Protection AG, Basel, Switzerland. Unpublished report No. CB 00/18, 25 February 2000. Submitted to WHO by Syngenta Crop Protection AG.
- Weber E (2003) Assessment of hepatic cell proliferation and apoptosis in male mice upon treatment with CGA 293343 technical for up to fifty weeks. Syngenta Crop Protection AG, Testing Facility, Basel, Switzerland. Syngenta unpublished report No. CB 00/12. Submitted to WHO by Syngenta Crop Protection AG.
- Winkler G (1995) CGA 293343 tech.—Skin sensitisation test in the guinea pig—maximization test. Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942115, 21 December 1995. Submitted to WHO by Syngenta Crop Protection AG.
- Winkler G (1996a) CGA 293343 tech.—Rat oral teratogenicity study. Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942118, 7 August 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Winkler G (1996b) CGA 293343 tech.—Rabbit oral teratogenicity. Ciba-Geigy Ltd, Short/Long-term Toxicology, Stein, Switzerland. Unpublished report No. 942119, 13 August 1996. Submitted to WHO by Syngenta Crop Protection AG.
- Zeugin S (1996) CGA 293343 tech.—Cytogenetic test on Chinese hamster cells in vitro. Ciba-Geigy Ltd, Genetic Toxicology, Basel, Switzerland. Unpublished report No. 952016, 18 June 1996. Submitted to WHO by Syngenta Crop Protection AG.

Appendix 1: Application of the IPCS conceptual framework for cancer risk assessment

(IPCS framework for analysing the relevance of a cancer mode of action for humans)

A1. Hepatocarcinogenesis in mice

A1.1 Introduction

All information relating to a mode of action has been summarized in this document, so reference will be made to the relevant paragraphs rather than redescribing the data in this section. The analytical approach applied to the postulated mode of action is based on the methods developed by the International Programme on Chemical Safety (IPCS) (Sonich-Mullin et al., 2001; Boobis et al., 2006). The incidences of any hepatic tumours in mice after 18 months of exposure to thiamethoxam at 0, 5, 20, 500, 1250 or 2500 ppm were, in males, 11, 7, 10, 19, 22 and 44 in groups of 50 and, in females, 0, 0, 0, 5, 9 and 29 in groups of 50 (Bachmann, 1998b). Most were hepatocellular tumours. The incidences of primary hepatocellular adenomas in these same dose groups of 50 mice of each sex were, respectively, for males, 9, 5, 8, 17, 21 and 39; and, for females, 0, 0, 0, 5, 8 and 28. In addition, hepatocellular carcinomas were significantly increased in males of the highest-dose group, with incidences of 3, 3, 2, 4, 4 and 16, whereas in females, there were a few hepatocellular carcinomas in the two highest dose groups only: 0, 0, 0, 0, 2 and 3. Thus, any explanation for the induction of these tumours should account for their occurrence in both male and female Tif:MAGf(SPF) mice (but particularly in male mice) from a dietary exposure concentration of 500 ppm. Some other neoplasms were also occasionally observed, but they tended to show a random distribution with dose. At the same dose level and higher, there were also a number of non-neoplastic lesions in the liver that were statistically significantly increased. These included pigment deposition (lipofuscin and haemosiderin), foci of cellular alteration, hepatocellular hypertrophy, single-cell necrosis and inflammatory cell infiltration.

There was no evidence for hepatocellular adenoma or carcinoma induction in rats receiving thiamethoxam in their diets at 0, 10, 30, 500 or 1500 ppm (males) or 0, 10, 30, 1000 or 3000 ppm

(females) for 2 years (Bachmann, 1998a). The numbers of hepatocellular adenomas in these groups (of 49 or 50 of each sex) were, for males, 0, 1, 0, 1 and 1; and, for females, 2, 0, 2 and 2. The numbers of hepatocellular adenocarcinomas were also sporadic, being, for males, 0, 0, 1, 1 and 0; and, for females, 0, 0, 1, 0 and 0. Thus, any mode of action should also account for the species specificity of the response. There were, furthermore, no treatment-related tumour findings in any other organ, and, in liver, there was no dose-related increase in non-neoplastic lesions in rats, except for an increase in clear cell foci, which are probably indicative of excess glycogen accumulation, in females, and only at a dose level of 3000 ppm.

The genetic toxicity database for thiamethoxam indicates that it is not a genetic toxin; therefore, modes of action were investigated with, most probably, a dose below which tumours would not be expected to occur (i.e. a “threshold”).

A1.2 Postulated mode of action (theory of the case)

Thiamethoxam is metabolized to a metabolite (CGA 330050) in mice that is hepatotoxic, and its action is exacerbated by a subsequent metabolite (CGA 265307) that is a competitive inhibitor in the activity of nitric oxide synthase, which plays a role in ameliorating the effects of hepatotoxic substances (Green, 2003e). This pathway occurs, but is of much less importance in rats, in which CGA 265307 is generated via CGA 322704. This second pathway also occurs in mice. The induced hepatotoxicity is followed by regenerative hepatocellular hypertrophy and proliferation. Neoplasia develops from foci of these altered cells.

A1.3 Key events

1. Thiamethoxam is metabolized via two major pathways, one of which occurs in both mice and rats ($\rightarrow$ CGA 322704 $\rightarrow$ CGA 265307), whereas the other appears less likely to occur at expected exposure levels, on kinetic grounds, except in mice ($\rightarrow$ CGA 330050 $\rightarrow$ CGA 265307). The relative rate of metabolism of thiamethoxam in mice versus rats is about 54:1 for the first pathway and about 87:1 for the second pathway.
2. The actively toxic metabolite, CGA 330050, induces the same changes in mouse liver as thiamethoxam itself. CGA 265307 does not seem to be toxic, but it is a competitive inhibitor with arginine, the natural substrate of nitric oxide synthase. Nitric oxide modulates the adverse effects of TNF α released from endothelial cells during chemical-induced hepatotoxicity. Thus, hepatotoxicity is enhanced by CGA 265307. In the absence of hepatotoxicity, there would be no effect of this metabolite. The expression of hepatotoxicity during weeks 1–10 includes general perturbation of hepatocyte metabolism, such as lowering of plasma cholesterol, induction of cytochrome P450, decreased protein synthesis, and glycogen and lipid accumulation.
3. During weeks 8–50, there is development of hepatocellular hypertrophy, lymphocytic infiltration and pigmentation of hepatocytes and Kupffer cells, single-cell necrosis and apoptosis, and blood chemistry changes.
4. In the latter part of this period (weeks 20–50), there is a sustained increase in cell replication rates among centrilobular hepatocytes.
5. Hepatocellular neoplasms begin to develop (even in controls) in weeks 40–50, but higher incidences in treated groups are observed only at the end of the 18-month experiment.

A1.4 Concordance of dose–response relationships

All of the features considered to contribute to the key events occurred in dose–response relationships; however, some of the studies contributing this information included multiple sampling

times, and therefore it is difficult to separate concentration and time effects. What is critical to the proposed mode of action, however, is that the key events should be observed at 500 ppm or lower.

After 14 days of treatment with a 2500 ppm diet, there were small, non-significant increases in absolute and relative liver weights of male and female mice in one study (Trendelenburg, 1998). In contrast, in another study (Weber, 1998), there were no statistically significant liver weight increases at 2500 ppm after 7, 13, 27 or 59 days in male mice, whereas there were statistically significant increases after 7 and 27 days in female mice at 2500 ppm, but not lower doses. In the 18-month study, relative (to body weight) liver weights were insignificantly increased after 35 weeks only in females at 2500 ppm, but by week 79, the relative liver weights were significantly increased in females from 500 ppm and in males from 1250 ppm. Absolute liver weights were significantly increased in both male and female mice at 79 weeks from 1250 ppm (Bachmann, 1998b).

Trendelenburg (1998) also found that after 14 days of treatment with a 2500 ppm diet, there were small, non-significant increases in hepatic cytochrome P450 in male and female mice at 2500 ppm, but not at 500 ppm. 7-Ethoxyresorufin *O*-deethylase (EROD), PROD and BROD activities were all significantly increased in female mice at 2500 and 500 ppm, and PROD activity was also significantly increased in female mice at 100 ppm. In male mice, however, PROD and BROD activities were significantly increased only at 2500 ppm. Other activities of hepatic enzymes were significantly altered only at 2500 ppm (e.g. testosterone hydroxylations or oxidations, particularly in females, and, in males and females, uridine diphosphate-glucuronosyltransferase, glutathione *S*-transferase and epoxide hydrolase activities) (Trendelenburg, 1998).

In a study of BrdU labelling in the livers of mice, the labelling indices were increased in male mice at 2500 ppm after 3, 7 and 59 days and at 500 ppm at day 13. There was no effect in males at 100 ppm. In females, there were significant increases in labelling index at days 7 and 59 (but not at day 3 or 27) in the 2500 ppm group, but there was no effect at 500 ppm at any time (Weber, 1998). BrdU labelling of liver cells was also measured after treatment of mice with 0, 50, 200, 500, 1250, 2500 or 5000 ppm for up to 50 weeks (Weber, 2003). In this study, the lowest dose at which BrdU labelling was significantly increased was 1250 ppm after 40 weeks, but not at shorter times or at 50 weeks. However, labelling was measured over the entire liver lobules in this study. When the observations were restricted to those regions in which necrosis and apoptosis were concentrated, a significant increase in cell replication rates was also observed at 500 ppm (Soames, 2003).

Apoptotic activity was demonstrated using the TUNEL staining method. TUNEL-positive objects were significantly increased at all doses from 50 ppm at 40 weeks and from 500 ppm at 30 weeks. The increase was dose related from 500 ppm (Weber, 2003).

In the same 59-day study mentioned above (Weber, 1998), there were clearly dose-related increases in the incidence of enlarged centrilobular and midzonal hepatocytes with increased amounts of glycogen, lipids and, to a lesser extent, smooth endoplasmic reticulum. These changes were observed in males across all doses and with an increased severity at day 59, and, in females, there was an increase in incidence and severity at 2500 ppm. Pigment granules were observed in centrilobular hepatocytes and in Kupffer cells in male and female mice at 2500 ppm after 59 days. Dose-related increases in incidence and severity of fatty change were observed in the later multiple sampling time study with doses up to 5000 ppm (Weber, 2003; see above); however, the hepatocytic vacuolation and glycogen deposition were not observed.

Following 7 days of treatment, plasma cholesterol concentrations were significantly reduced in adult male mice at doses of 500 ppm (the lowest tested) and higher (Green, 2003c) (in contrast, they tended to be raised in rats of 1- and 3-month studies and were unaffected in dogs). Serial blood chemistry measurements in male mice over 50 weeks indicated increased AST and ALT activities at 2500 and 5000 ppm at specific sampling times from 10 to 50 weeks, while combining all sampling times resulted in increases of both liver toxicity indicator enzymes from 1250 ppm. Microscopic changes (mainly fatty change) occurred from 500 ppm in this experiment (Weber, 2003).

Combining all sample time points showed a dose-related increase in apoptosis in the liver from 500 ppm, whereas the BrdU labelling index for all time points combined increased from 1250 ppm (Weber, 2003).

In the 18-month study, relative (to body weight) liver weights were insignificantly increased after 35 weeks only in females at 2500 ppm, but by week 79, the relative liver weights were significantly increased in females from 500 ppm and in males from 1250 ppm. Absolute liver weights were significantly increased in both male and female mice at 79 weeks from 1250 ppm. Increased microscopically observable hepatotoxicity was recorded in males and females of the 2500 ppm group at week 35 and from 500 ppm at week 79. Hepatocellular neoplasms were increased in a dose-related manner in male and female mice at week 79 from 500 ppm.

A1.5 Temporal association

In the mouse strain and metabolite comparison study with observations in which Tif:MAGf and CD-1 mice were fed thiamethoxam (2500 ppm), CGA 322704 (2000 ppm) or CGA 265307 (500 ppm) for 1, 10 and 20 weeks (Green, 2003d; Noakes, 2003a), few effects were noted after 1 week, whereas they did occur after 10 and/or 20 weeks. Thiamethoxam-associated hepatic effects observed early in treatment of both Tif:MAGf and CD-1 mice were increases in:

- hepatocellular hypertrophy incidence and/or severity after 10 and 20 weeks;
- hepatocellular necrosis incidence and/or severity after 10 and 20 weeks;
- inflammatory cell infiltration incidence and/or severity after 20 weeks (but not after 10 weeks);
- hepatocellular apoptosis incidence and/or severity after 10 and 20 weeks in CD-1 mice, but only after 10 weeks in TifMAGf mice;
- pigmentation incidence and/or severity after 10 and 20 weeks, but only in CD-1 mice;
- cell proliferation (BrdU immunohistochemistry) in TifMAGf mice at week 20 and in CD-1 mice at weeks 10 and 20.

Apoptosis was not observed in either strain in the Noakes (2003a) study, and none of these effects was observed following parallel treatment with either CGA 322704 or CGA 265307. The use of two strains demonstrated that the effects were not strain specific, but that CD-1 mice seemed to be more susceptible than the TifMAGf mice that were used in the carcinogenicity study with thiamethoxam.

Sustained increases in cell proliferation (240%), measured specifically in centrilobular hepatocytes (the region of the liver in which single-cell necrosis and apoptosis were concentrated), were observed in male mice at 500 ppm at 40 weeks.

A1.6 Strength, consistency and specificity of association of tumour response with key events

The relative rates of thiamethoxam metabolism in mice versus rats *in vitro* are about 54:1 for thiamethoxam → CGA 322704 → CGA 265307 and about 87:1 for thiamethoxam → CGA 330050 → CGA 265307. CGA 322704 (clothianidin) is not carcinogenic, and, although CGA 330050 has not been tested for carcinogenicity, it is known to be as hepatotoxic as thiamethoxam itself. Thus, the kinetics favours the generation of much larger quantities of the toxic metabolite in mice than in rats, consistent with the species difference in the hepatotoxicity and carcinogenicity of thiamethoxam.

A comparison of the hepatotoxicity of thiamethoxam metabolites CGA 322704, CGA 265307 and CGA 330050 administered in the diet for up to 20 weeks to TifMAGf mice (Green, 2003d) demonstrated that only CGA 330050 induced the same changes as thiamethoxam; no changes were observed following CGA 322704 or CGA 265307 administration at any time. Plasma cholesterol was

reduced after 1 and 10 weeks of CGA 330050 treatment at 500 and 1000 ppm; hepatocellular hypertrophy, single-cell necrosis/apoptosis and cell replication rates were increased after 10 weeks, and plasma AST and ALT activities were increased at weeks 10 and 20. In contrast, feeding Tif:RAIf rats with CGA 330050 at 500 or 1000 ppm for 1 week reduced plasma cholesterol levels and the plasma aminotransferase activities.

A1.7 Biological plausibility and coherence

The mode of action proposed for the induction of liver tumours in mice but not rats is largely supported by the available data, but there appear to be some inconsistencies in the details of the dose–response relationships. Thus, evidence of hepatotoxicity involving all dose levels at which tumour incidences were significantly increased was a relatively late-occurring observation, whereas it was frequently necessary to combine all sample time results in order to demonstrate a significant effect considered to be key to the proposed mode of action.

A1.8 Other modes of action

A number of properties of thiamethoxam have been identified, some of which appear to be time- and dose-related key events that characterize the emergence of liver cancer in thiamethoxam-treated mice in a manner that resembles that of the cholesterol-lowering drugs, statins, which also induce liver tumours in mice. Thus, it might be postulated that thiamethoxam has a statin-like mode of action; however, the studies conducted so far provide no evidence for a statin-like inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, this being the rate-limiting enzyme in cholesterol biosynthesis. Thus, the cholesterol-lowering effect seen in a number of thiamethoxam studies in mice might have no mechanistic significance for neoplasia.

Furthermore, the phenobarbital-like inductions noted in liver phase I and phase II enzymes may be misleading, as the effects on these enzymes are small and probably insufficient to account for the emergence of liver tumours (Trendelenburg, 1998).

Finally, there is no evidence for any genotoxic or mutagenic action of thiamethoxam or its metabolites; therefore, there is no basis for proposing a genotoxic mode of action.

A1.9 Uncertainties, inconsistencies and data gaps

The weight of the evidence suggests that the mode of action for thiamethoxam-induced liver tumours in mice is based on the hepatotoxicity of its metabolite CGA 330050, the effects of which are increased by CGA 265307. The other major metabolic pathway produces the non-toxic CGA 322704, from which CGA 265307 is produced. This reasoning seems persuasive, but there are some weaknesses in the dose–response relationships for hepatotoxicity. They are not as strong as they could be, given that the increase in tumour incidence is observed at 78 weeks from a dose level of 500 ppm. Certain key events occur rather late on the time scale, raising the question as to whether they are truly part of the neoplastic process. It is also questionable whether the procedure of combining data from all sampling times is a legitimate way of demonstrating that there are dose-related responses during preneoplasia consistent with the neoplastic outcome.

A1.10 Assessment of postulated mode of action

A case has been proposed to explain the induction of hepatocellular neoplasms in mice exposed to thiamethoxam. Some weaknesses have been described above, among them being the

rather unusual statistical technique of combining data from all observation times in order to demonstrate a significant effect in properties and the late appearance of others (at all relevant dose levels) considered to be key events in the mice. The temporal and dose-related occurrence of key events in mice is summarized in [Figure A1](#) and [Table A1](#).

Human relevance analysis

1. Is the weight of evidence sufficient to establish a mode of action in animals?

There are many elements in the toxicology of thiamethoxam that are consistent with a mode of action based on large quantitative differences in metabolism between mice and rats and the hepatotoxicity of a particular metabolite produced in mice. If it is accepted that hepatotoxicity is the basis for the neoplasia, then a reasonable case has been made for this mode of action in rodents.

2. Can human relevance of the mode of action be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

Limited studies (Green, 2002) comparing the metabolism of thiamethoxam in vitro by liver microsomal preparations indicate that human microsomes are capable of producing both CGA 330050 and CGA 265307. Therefore, the human relevance of the proposed mode of action cannot be ruled out on qualitative differences in the key events.

3. Can human relevance of the mode of action be reasonably excluded on the basis of fundamental, quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

Limited human in vitro metabolism studies (Green, 2002) suggest that humans are more similar to rats or even less capable than rats, compared with mice, in producing the active metabolite CGA 330050. Thus, the relative rates of thiamethoxam metabolism in mice versus rats in vitro (based on maximum rate $[V_{\max}]/K_m$ ratios) are about 54:1 for thiamethoxam $\rightarrow$ CGA 322704 $\rightarrow$ CGA 265307 and about 87:1 for thiamethoxam $\rightarrow$ CGA 330050 $\rightarrow$ CGA 265307. Similar comparisons of mouse versus human show that the overall relative metabolic rate ratios for these pathways are 371:1 and 238:1, respectively. The relative rate ratios for the critical conversion of thiamethoxam to CGA 330050 are mouse:rat 10.6:1 and mouse:human 25.6:1. Rats do not develop tumours following treatment with thiamethoxam. Thus, mice appear to be particularly sensitive to this mode of action. Because of the threshold nature of the mode of action and the unique sensitivity of mice, it is concluded that humans are unlikely to be at risk for developing tumours following exposures to thiamethoxam.

4. Statement of confidence, analysis and implications

There are, as described above, some weaknesses in the proposed mode of action, in that there may be perceived to be differences in the dose levels at which key events are observed and those at which tumours emerge; however, the differences are not substantial, and it may be that they are no more than manifestations of the differences occurring between experiments conducted at different times and under different conditions. The analysis suggests that there is not any absolute difference between mice on one hand and rats and humans on the other. The crucial difference is quantitative, and the dose levels required to produce liver toxicity and cancer by thiamethoxam exposure would not be reached at human dietary levels of exposure, indeed, in any of these species, but particularly in humans. Furthermore, the absence of any evidence for genotoxicity of thiamethoxam indicates that a non-linear dose–response model for tumours applies to mice.

Figure A1. The dose–response relationship for hepatocellular changes over 10–50 weeks (a–d) compared with the liver tumour incidence over 80 weeks (e)

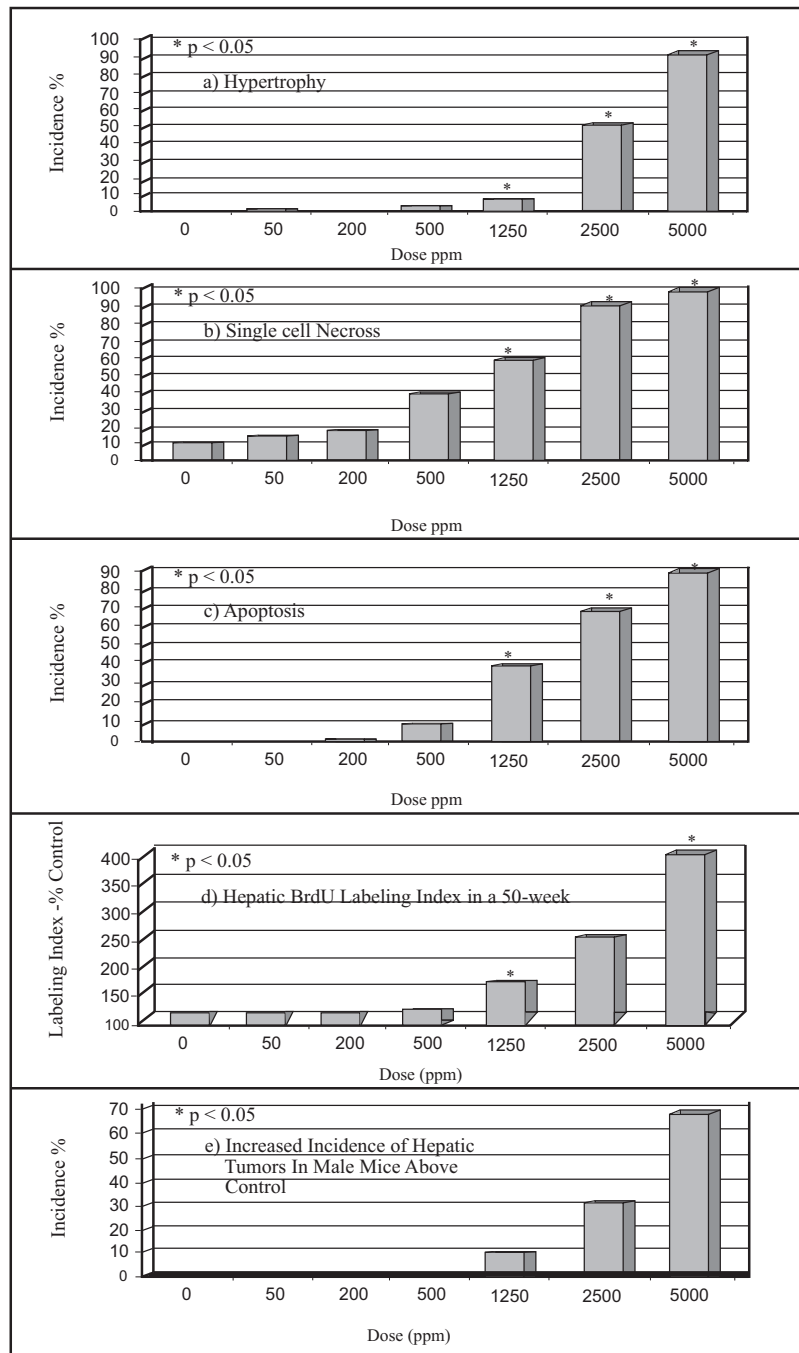


Table A1. Comparison of mode of action data relevant to hepatotoxicity and hepatocarcinogenicity in mice

	Dietary concentration (ppm)						
	0 (control)	5	20	100	500	1250	2500
Males							
Proliferation	—	—	—	None	Weak	—	Significant (3 days)
Enzyme induction	—	—	—	None	Very weak	—	Significant
Tumour incidence (hepatic neoplasia)	11/50 (22%)	7/50 (14%)	10/50 (20%)	—	19/50 (38%)	22/50 (44%)	44/50 (88%)
<i>Historical incidence: mean (range)</i>							
Hepatocellular adenoma	22.14% (10–34%)						
Hepatocellular adenocarcinoma	10.51% (0–16%)						
Females							
Proliferation	—	—	—	Very weak	Weak	—	Significant (7 days)
Enzyme induction	—	—	—	Very weak	Some	—	Significant
Tumour incidence (hepatic neoplasia)	0/50 (0%)	0/50 (0%)	0/50 (0%)	—	5/50 (10%)	9/50 (18%)	29/50 (58%)
<i>Historical incidence: mean (range)</i>							
Hepatocellular adenoma	3.2% (0–16%)						
Hepatocellular adenocarcinoma	0.38% (0–2%)						