considered secondary to the reduced body weight, as there is no effect on these organ weights relative to body weight. All other organ weights and ratios are considered to be within the normal range of variation and unaffected by treatment with thiamethoxam.

Table: Treatment-related organ weight changes

Sex	Parameter	0ppm	100ppm	1000ppm	2500ppm	10000ppm
Males	Body weight [g]	314.2	314.7	316.4	303.6	250.1*-
	Liver abs. [g]	14.95	14.92	14.87	14.89	16.07
	rel. [‰]	47.54	47.47	47.00	49.02	64.28*+
	Kidneys abs. [g]	2.260	2.359	2.470	2.524	1.918
	rel. [‰]	7.209	7.464	7.812	8.306*+	7.670
	Adrenals abs. [mg]]	81.46	73.86	72.02	78.18	68.38*
	rel. [‰]	0.261	0.236	0.227	0.256	0.274
	Thymus abs. [mg]]	712.0	643.8	675.9	598.5	541.1*
	rel. [‰]	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 abs. [g]	8.476	9.285	9.695	8.969	10.41*
	rel. [‰]	42.42	43.77	44.87	42.39	56.37*
	Kidneys abs. [g]	1.668	1.810	1.984*+	1.826	1.757
	rel. [‰]	8.390	8.536	9.204*+	8.658	9.575*
	Adrenals abs. [mg]	85.46	88.72	93.60	90.96	93.22
	rel. [‰]	0.428	0.420	0.435	0.433	0.506
	Thymus abs. [mg]	448.2	484.1	469.5	508.0	426.6
	rel. [‰]	2.255	2.291	2.178	2.397	2.300

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

At necropsy, dilatation of the renal pelvis occurred in 2 treated males, but no other treatment-related gross lesions were evident. Treatment-related microscopic changes were detected in males treated at ≥1000ppm and females at ≥ 2500ppm (Table below). The organs affected were liver, kidneys, adrenal glands and thyroid glands. Minimal to marked hypertrophy of centrilobular hepatocytes occurred in both sexes at 2500 and 10000ppm. In some animals at 10000ppm this was accompanied by depletion of glycogen deposits and/or cholangiofibrosis of bile ducts. In the kidneys, minimal to moderate hyaline change to the tubular epithelium occurred in males at 1000 or 2500ppm and hyperplasia of the pelvic epithelium occurred in one animal of each sex at 10000ppm. Many of the affected male animals also showed one or more further kidney lesions, focal calcification, pelvic dilatation, renal cyst, basophilic proliferation, lymphohistiocytic infiltration or an acute tubular lesion. In the adrenal cortex, the effect at 10000ppm was characterised by minimal to moderate fatty change mainly of the zona fasciculata in both males and females. In the thyroid gland, hypertrophy of the follicular epithelium occurred in some males and females, but the severity was greater in males. No other treatment-related microscopic lesions occurred. There was no effect on replicative DNA synthesis in hepatocytes of male animals at dose levels up to 10000ppm (report no. CB94/47, February 27, 1995 – see section 5.8.2.1).

The pattern of changes noted in the kidneys of males closely resembles the early stage of $\alpha_{2\mu}$-globulin nephropathy (AGN) (for further investigation of these changes see section 5.8.2 Supplementary studies on the active ingredient). Since AGN is generally accepted as a phenomenon exclusively found in male rats, the no-observed-(adverse)-effect-level in this study is based on the conclusion that AGN is not relevant to human risk assessment.

Table: Treatment-related histopathological findings

Tissue/finding	Males					Females				
Dose level (ppm)	0	100	1000	2500	10000	0	100	1000	2500	10000
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:										
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

Conclusion: Thiamethoxam at 10000ppm in the diet provides daily dose levels that exceed the MTD, and the results are a suitable basis on which to establish dose levels for a 90-day study.

No-observed-effect-level (NOEL): 100ppm in both sexes, equivalent to dose levels of 8.0mg/kg bw/day (males) and 8.7mg/kg bw/day (females), based on the findings of increased kidney weights in both sexes and hyaline changes to the renal tubular epithelium in males, at 1000ppm.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2005
Materials and Methods	
Results and discussion	

Conclusion

Reliability

Acceptability

Remarks

98/8 Doc IIIA section No.	**6.4.1 / 01**	**Subchronic oral toxicity test**
91/414 Annex II Point addressed	**5.3.2 / 01**	Short-term toxicity - oral 90-day studies

1. Annex point(s)	**IIA, 5.3.2. Short-term toxicity - Oral 90-day studies - dogs**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.3.2 / 01
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - 3-Month subchronic dietary toxicity study in Beagle dogs. Syngenta Crop Protection AG, unpublished report No. 942107, October 15, 1996.
4. Testing facility	[redacted]
5. Dates of work	August 07, 1995 - November 09, 1995
6. Test substance	ISO common name: Thiamethoxam, [redacted]
7. Test method	OECD 409 ≅ EEC B.27 ≅ FIFRA § 82-1≅ JMAFF Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Groups of 4 male and 4 female pure-bred Beagle dogs were treated orally for 13 weeks with thiamethoxam ([redacted] administered in the diet at concentrations of 0, 50, 250, 1000 and 2500/2000[1] ppm. Mortality, clinical signs and food consumption were checked daily and body weight was recorded weekly. Ophthalmoscopic examinations were conducted pre-test and at the end of treatment. Haematology, clinical chemistry and urinalysis were performed on all animals pre-test and at weeks 7 and 13. The animals were necropsied, examined *post mortem*, major organs weighed and tissue samples examined histopathologically.

Findings: 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 range 91.4 - 97.8% of nominal concentrations. Mean achieved daily dose levels were 0, 1.58, 8.23, 32.0 and 54.8mg/kg bw/day (males) and 0, 1.80, 9.27, 33.9 and 50.5mg/ kg bw/day (females), respectively.

All animals survived the scheduled treatment period and there were no clinical signs of an adverse effect of treatment (Table 1). Seven animals at 2500ppm lost weight during the first 2 weeks necessitating a dose reduction to 2000ppm. Subsequently, 3 of these animals showed markedly depressed weight gain, whilst weight gain of the remaining animals was unaffected by treatment. No effect occurred on weight gain at dose levels up to 1000ppm. Food consumption was depressed at 2500ppm and to a lesser extent when the dose level was reduced to 2000ppm. Food consumption was also slightly depressed in females at 1000ppm. There was no evidence of ocular toxicity at any dose level.

[1] : 2500ppm days 1 - 14, 2000ppm days 15 - 18, no treatment days 19 - 25 and 2000ppm from day 26 until termination.

Table 1: Body weight and food consumption

Dose level [ppm]	Males 0	Males 50	Males 250	Males 1000	Males 2000	Females 0	Females 50	Females 250	Females 1000	Females 2000
Cumulative body weight gain [kg]										
Week 4	1.075	1.125	1.050	1.175	0.300	1.150	0.975	1125	1.075	-0.60*
Week 8	1.975	2.100	1.900	2.125	1.075	2.100	1.750	1.925	2.325	-0.12*
Week 13	2.950	3.275	2.750	2.925	1.875	2.900	2.525	2.800	3.075	0.475*
Food consumption [g/day]										
Week 1	344.6	350.0	347.1	350.0	338.9	339.6	322.9	350.0	285.0	171.1*
Week 4	349.6	350.0	350.0	350.0	327.5	350.0	333.9*	350.0	327.9	254.6*
Week 8	350.0	350.0	350.0	350.0	303.2	350.0	350.0	350.0	346.1	222.1*
Week 13	350.0	350.0	350.0	350.0	324.6	350.0	350.0	350.0	341.4	233.9*

* $p \leq 0.05$ (Wilcoxon)

Treatment-related haematology and blood chemistry effects occurred at 1000 and 2000ppm (Table 2). Slight anaemia, associated with a tendency to hypochromasia, anisochromasia and microcytosis, had developed by week 13 in females at 2000ppm. Reduced white cell counts (total, neutrophils, monocytes and lymphocytes) also occurred in females. Males at 2000ppm showed reduced monocyte counts and a tendency to hypochromasia and anisochromasia of red blood cells. Eosinophilia occurred in some animals at this dose level. Some animals at 1000 and 2000ppm had prolonged thromboplastin times.

Table 2: Treatment-related haematology findings

Parameter (unit)	Week	Dose level [ppm] 0	50	250	1000	2000
Males						
WBC (G/l)	7	10.77	10.91	13.20	9.352	7.985
	13	9.233	10.41	10.65	9.548	10.24
Neut (G/l)	7	5.940	6.095	8.334	4.950	3.418
	13	5.035	6.150	6.675*	5.998	6.138
Baso (G/l)	7	0.055	0.045	0.046	0.043	0.039
	13	0.068	0.043	0.045*	0.043	0.035*
Mono (G/l)	7	0.550	0.385	0.561	0.364	0.126*
	13	0.328	0.368	0.335	0.340	0.058*
RBC (T/l)	7	6.470	6.300	5.885	6.357	6.415
	13	6.625	6.163*	6.218	6.550	6.446
HB (mmol/l)	7	8.575	8.425	8.038	8.400	8.525
	13	8.975	8.400	8.575	8.650	8.450
Hct (l)	7	0.421	0.420	0.390*	0.413	0.416
	13	0.444	0.409	0.417	0.420	0.416
Thromboplastin time (sec)	7	33.64	33.68	32.11	39.43*	37.24
	13	36.18	35.82	36.63	41.61	41.16+
Females						
WBC (G/l)	7	9.155	9.895	10.99	11.24*	4.581*
	13	8.223	8.590	10.08*	9.905	4.156*
Neut (G/l)	7	5.138	5.413	6.040	6.125	1.701*
	13	4.958	4.808	6.235	5.745	1.631*
Mono (G/l)	7	0.375	0.408	0.445	0.445	0.084*
	13	0.183	0.198	0.225	0.303	0.093
RBC (T/l)	7	6.330	6.083	6.618	6.078	6.036
	13	6.688	6.348	6.573	6.703	5.971*
Hb (mmol/l)	7	8.875	8.200	8.825	8.100	7.963
	13	9.400	8.550	8.850	8.850	7.813*-
Hct (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 time (sec)	7	31.26	35.95*	34.66	39.33*	36.59
	13	35.37	37.88	38.29	44.26*+	41.92+

*: $p \leq 0.05$ by Dunnett's "t" Test (two-tailed); - / + :negative / positive trend (Jonckheere)

Lower plasma Ca^{++} concentration and ALAT activity occurred in both sexes at 1000 and 2000ppm (Table 3). The latter is not considered to be an adverse effect since hepatocellular changes were not evident microscopically. Minimally reduced plasma albumin levels in both sexes at 1000 and 2000ppm, and minimally lower cholesterol and phospholipid levels in males at 2000ppm are considered to be physiological adaptive responses rather than primary effects of thiamethoxam. There were no treatment-related effects on urine physiological parameters or cellular and chemical constituents of urine.

Table 3: Treatment-related clinical chemistry findings

Parameter (unit)	Week	Dose level [ppm]				
		0	50	250	1000	2000
Males						
ALAT (U/l)	7	35.50	41.13	29.85	25.00*	18.13*-
	13	81.03	49.00	35.08	25.40*-	13.10*-
Alb (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
Phos-Lip (mmol/l)	7	4.465	4.160	4.285	4.208	3.703*
	13	4.455	4.235	4.108	4.068	3.698*
Chol (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						
ALAT (U/l)	7	50.20	43.35	37.70*	26.63*-	17.41*-
	13	53.83	42.50	40.73	25.23*-	13.10*-
Alb (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*
Chol	7	3.698	3.523	3.225	3.150	3.650
	13	3.733	3.665	3.170	3.175	3.488
Phos-lip	7	4.223	4.193	3.875	3.775	4.028
	13	4.210	4.265	3.720*	3.678	3.815

*: $p \leq 0.05$ 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 2000ppm (Table 4) and histological correlates were identified. Slightly reduced heart, liver and kidney weights in females at 2000ppm and increased thyroid weights in males at 50, 1000 and 2000ppm were not associated with histological changes and are considered incidental to treatment with thiamethoxam.

Table 4: Treatment-related organ weight changes

	Males					Females				
Dose Level [ppm]	**0**	**50**	**250**	**1000**	**2000**	**0**	**50**	**250**	**1000**	**2000**
No. examined	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Carcass weight *[kg]*	11.28	11.65	11.38	11.58	10.60	10.43	9.98	10.25	10.65	7.75
Testes/Ovaries										
- absolute [g][a]	16.54	14.76	14.60	15.59	9.38*	0.835	0.663	0.696	0.712	0.543*
- relative [% body weight]	1.545	1.336	1.384	1.439	0.929*-	0.087	0.072	0.073	0.074	0.076

[a] rounded values - report gives 3 decimal places; * $p \leq 0.05$ (Dunnett's t-Test, two tailed)- negative trend (Jonckheere)

A minimal to marked reduction in spermatogenesis and an increased incidence of spermatic giant cells occurred in the testes of all males at 2000ppm (Table 5). One male also showed moderate tubular atrophy. An immature stage of ovarian development, consistent with retarded maturation, occurred in 3 females at 2000ppm. The maturity of the uterus in 2 of these animals reflected the immature stage of ovarian development. Since prolonged growth retardation, as noted at 2000ppm, is known to adversely affect sexual maturation, a direct effect of thiamethoxam on the gonads is unlikely.

Table 5: Treatment-related histopathological findings

	Males					Females				
Dose [ppm]	**0**	**50**	**250**	**1000**	**2000**	**0**	**50**	**250**	**1000**	**2000**
Organ and finding /Animals in study	**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

Conclusion: A dose level of 2500/2000ppm is clearly toxic to the dog, based on marked weight loss. No-observed-effect-level (NOEL): 250ppm in both sexes, equivalent to dose levels of 8.23mg/kg bw/day (males) and 9.27mg/kg bw/day (females), based on prolonged thromboplastin times, slightly reduced plasma Ca^{2+} and minimal adaptive changes in blood chemistry at ≥1000ppm.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date March 2005

Materials and Methods

Results and discussion

	haematology and blood chemistry parameters at 2500/2000 ppm, and lower
Conclusion	
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No.	**6.4.1 / 02**	**Subchronic oral toxicity test**
91/414 Annex II Point addressed	**5.3.2 / 03**	Short-term toxicity - oral 90-day studies

1. Annex point(s)	**IIA, 5.3.2 Short-term toxicity - Oral 90-day studies - rats**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.3.2 / 03
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - 3-month oral toxicity study in rats (administration in food). Syngenta Crop Protection AG, unpublished report No. 942089, January 23, 1996. Amended November 04, 1998.
4. Testing facility	[redacted]
5. Dates of work	December 27, 1994 - March 30, 1995
6. Test substance	ISO common name: Thiamethoxam; [redacted]
7. Test method	OECD 408 ≅ EEC B.26 ≅ FIFRA § 82-1 ≅ JMAFF Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Groups of 10 male and 10 female Sprague-Dawley-derived rats (Tif:RAIf strain) were treated orally for 90 days with thiamethoxam [redacted] in the diet at concentrations of 0, 25, 250, 1250, 2500 and 5000ppm. Clinical signs, body weight, food and water consumption were monitored throughout the study. Ophthalmoscopic examinations were performed before dosing commenced and towards the end of treatment. Haematology, clinical chemistry and urinalysis were performed at the end of the study. All animals were killed, subjected to necropsy and *post mortem* examination, major organs were weighed and tissue samples were examined histopathologically.

Findings: 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 range 94.2 - 104.6% of nominal concentrations. Mean achieved daily dose levels were 0, 1.74, 17.6, 84.9, 168 and 329mg/kg bw/day (males) and 0, 1.88, 19.2, 92.5, 182 and 359mg/kg bw/day (females), respectively.

There were no treatment-related deaths or clinical signs of an adverse reaction to treatment, although one female at 1250ppm died on day 57 with no histopathological treatment-related findings. Ophthalmoscopic examinations revealed no evidence of ocular toxicity. Body weight gains were significantly retarded and food consumption was reduced in male groups at ≥1250ppm (Table 1). Weight gains and food consumption of all female groups and the male groups at up to 250ppm were unaffected by treatment. Water consumption in both sexes at 5000ppm was slightly increased.

Table 1: Body weight, body weight gain and food consumption

Dose level [ppm]	Males						Females					
	0	25	250	1250	2500	5000	0	25	250	1250	2500	5000
Body weight [g]:												
- week -1	145.4	151.2	147.0	149.8	157.6	151.4	134.7	138.4	135.6	134.9	139.9	135.0
- week 13	528.7	491.0	507.7	448.8-	467.6-	429.5*-	263.6	279.5	269.4	267.9	271.2	256.5
Body weight gain [g]	383.3	339.8	360.7	299.0	310.0	278.1	128.9	141.1	133.8	133.0	131.3	121.5
% gain (relative to control)		-11.3	-5.9	-22.0	-19.1	-27.4		9.5	3.8	3.2	1.9	-5.7
Food consumption [g]:												
- overall week-1-13	2551.7	2446.7	2515.9	2280.9	2375.0	2183.4	1641.3	1679.9	1691.7	1636.4	1695.5	1626.5
% (relative to control)		-4.1	-1.4	-10.6	-6.9	-14.4		2.4	3.1	-0.3	3.3	-0.9

* $p \leq 0.05$ (Wilcoxon); - negative trend (Jonckheere)

The only haematological effect was a minimal increase in the number of circulating platelets in males at 5000ppm (Table 2). Minimal differences between control and high dose haematological values that were statistically significant or showed a positive trend are considered to reflect physiological variation since individual values were within reference ranges. Minor treatment-related effects on clinical chemistry occurred in males at ≥1250ppm and in females at ≥2500ppm. The effects were increased urea levels in both sexes, and reduced glucose levels and increased creatinine levels in males only. Cholesterol levels were also raised in both sexes but at 5000ppm only. Minimal changes in the electrolyte balance and phosphate of both sexes are considered to be of no toxicological consequence. There were no treatment-related effects on urine physiological parameters or cellular and chemical constituents of urine.

Table 2: Haematology and clinical chemistry changes

Dose level [ppm]		0	25	250	1250	2500	5000
Males							
Plt	[G/l]	996.0	987.5	957.8	953.0	989.4	1117
Glucose	[mmol/l]	8.244	8.787	7.949	7.274*-	7.530-	7.205-
Urea	[mmol/l]	5.422	5.515	5.301	6.141	6.346	6.726+
Creatinine	[µmol/l]	52.88	55.77	54.98	60.65+	59.03+	64.28*+
Cholesterol	[mmol/l]	2.094	2.096	2.092	2.005	2.375	2.549+
Na+	[mmol/l]	142.8	142.2	142.4	142.0	141.4*-	142.1-
Cl^-	[mmol/l]	100.6	101.1	100.4	98.96-	98.24-	98.73-
$PO4^-$	[mmol/l]	1.656	1.5351	1.579	1.733	1.747+	1.810+
Females							
Plt	[G/l]	956.9	936.2	971.8	1006	1001	999.4
Glucose	[mmol/l]	7.320	7.582	7.554	7.154	7.246	7.152
Urea	[mmol/l]	7.319	6.828	7.500	6.814	8.340	7.943
Creatinine	[µmol/l]	56.23	53.37	59.63	56.19	59.81	57.16
Cholesterol	[mmol/l]	2.027	2.249	2.062	2.026	2.256	2.513
Na+	[mmol/l]	142.7	141.9	141.9	140.9	140.8-	141.1-
Cl^-	[mmol/l]	103.0	102.5	101.9	101.7	101.7-	100.6-
$PO4^-$	[mmol/l]	1.171	1.13	1.200	1.233	1.161	1.372

* $p \leq 0.05$ by Dunnett's "t" Test (two-tailed); - / + negative / positive trend (Jonckheere)

Post mortem examination at necropsy revealed no treatment-related effects on any tissue or organ examined. The absolute and/or relative weights of the adrenals, liver, kidneys, heart and spleen were increased in males at 5000ppm. Relative liver and kidney weights were also marginally increased in males at 2500ppm. There was no histopathological correlation to the increased heart weight of males at 5000ppm. Treatment-related effects in females were confined to a minimal reduction in thymus weights at 5000ppm (Table 3).

Table 3: Treatment-related organ weight findings

Organ	Dose level [ppm]	Male		Female	
		Absolute weight	Relative weight[a]	Absolute weight	Relative weight[a]
Liver	0	20.60	40.82	9.615	38.29
	25	19.30	40.85	9.792	36.71
	250	20.16	41.45	9.683	37.7
	1250	17.58	40.62	9.344	36.83
	2500	19.39	43.36	9.426	36.63
	5000	20.52	49.32*+	9.932	40.34
Kidney	0	3.224	6.434	1.947	7.769
	25	3.133	6.625	2.060	7.719
	250	3.171	6.535	2.070	8.057
	1250	2.921	6.764	1.960	7.708
	2500	3.182	7.115	1.942	7.555
	5000	3.260	7.857*+	2.005	8.128
Heart	0	1.427	2.837	0.919	3.661
	25	1.363	2.883	0.940	3.526
	250	1.356	2.794	0.902	3.516
	1250	1.373	3.156	0.923	3.634
	2500	1.343	3.023+	0.914	3.565
	5000	1.355	3.261*+	0.845-	3.475
Adrenals (both)	0	73.60	0.148	84.93	0.336
	25	65.81	0.139	88.15	0.330
	250	71.54	0.149	99.87	0.392
	1250	64.15	0.149	84.04	0.330
	2500	65.65	0.148	82.88	0.325
	5000	88.68	0.213*+	88.14	0.359
Spleen	0	0.764	1.509	0.466	1.863
	25	0.756	1.601	0.513	1.927
	250	0.778	1.600	0.429	1.671
	1250	0.761	1.752	0.508	2.002
	2500	0.753	1.688	0.519	2.019
	5000	0.799	1.916*+	0.504	2.047
Thymus	0	598.2	1.184	341.6	1.368
	25	575.2	1.217	338.8	1.266
	250	596.6	1.232	306.4	1.190
	1250	487.4	1.127	301.3	1.193
	2500	527.8	1.183	315.9	1.226
	5000	529.0	1.272	282.7	1.145

[a]: % of body weight, *: $p \leq 0.05$ by Dunnett's "t" Test (two-tailed); - / + :negative / positive trend (Jonckheere)

The liver, kidney, spleen and adrenal glands were identified as target organs for thiamethoxam (Table 4). Changes in liver morphology at 2500 and 5000ppm 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 5000ppm also showed minimal pigmentation of Kupffer cells. Changes in kidney morphology occurred at ≥250ppm in males and ≥2500ppm in females. Lesions detected 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. Increased incidence of pelvic dilatation and epithelial hyperplasia, and tubular cast formation were also evident. The pattern of effects in male rat kidneys at ≥250ppm is consistent with α-2μ-globulin nephropathy (AGN) described in the literature (e.g. Alison, et. al., 1994; Alden, 1986). AGN is generally accepted to be unique to male rats and not relevant to human risk assessment. Renal lesions in females were confined to an increased incidence of chronic tubular lesions and an increase in the severity of nephrocalcinosis. There was an increased incidence of fatty change in the adrenal cortex in males at ≥1250ppm and in females at ≥2500ppm. In the spleen, there was an increased incidence and severity of extramedullary hemopoiesis and hemosiderosis in males at 5000ppm and an increase in the severity of hemosiderosis in females at 2500 and 5000ppm.

Table 4: Histopathological findings

	Dose level [ppm]	0	25	250	1250	2500	5000
Males	**No. of animals**	**10**	**10**	**10**	**10**	**10**	**10**
Liver							
- Hepatocellular hypertrophy	incidence	0	0	0	0	6	10
- Lymphohistiocyt. Infiltration	incidence	3	4	6	5	4	9
- Cholangiofibr.	incidence	2	4	2	4	5	6
- Pigmentation Kupffer Cells	incidence	0	0	0	0	0	0
Kidneys							
- Hyaline change	incidence	1	0	4	8	10	10
	weighted grade	*1.0*	*0*	*1.3*	*1.5*	*1.7*	*2.3*
- Chronic tubular lesion	incidence	0	1	3	6	10	9
- Acute tubular lesion	incidence	0	0	0	3	8	9
- Lymphohistiocyt. Infiltration	incidence	2	2	1	3	6	3
- Bas. Proliferation	incidence	2	1	2	4	6	10
- Dilatation renal pelvis	incidence	1	0	2	1	5	1
- Hyperplasia pelv. epithel.	incidence	0	0	0	1	3	0
- Cast Formation	incidence	1	3	2	3	3	5
- Calcification renal cortex	incidence	0	0	0	0	0	1
- Nephrocalcinos.	incidence	0	0	0	0	0	0
Spleen							
- Hemosiderosis	incidence	7	8	9	7	9	10
	weighted grade	*1.0*	*1.9*	*1.4*	*1.6*	*1.7*	*2.0*
- Extramedullary hematopoiesis.	incidence	3	2	3	5	2	5
	weighted grade	*1.0*	*1.0*	*1.0*	*1.0*	*1.0*	*1.8*
Adrenal gland							
- Fatty change	incidence	4	5	5	7	7	8
Females	**No. of animals**	**10**	**10**	**10**	**10**	**10**	**10**
Liver							
- Hepatocellular hypertrophy	incidence	0	0	0	0	0	8
- Lymphohistiocyt. Infiltration	incidence	4	5	6	7	9	10
- Cholangiofibr.	incidence	2	1	2	1	3	1
- Pigmentation Kupffer Cells	incidence	0	0	0	0	0	6
Kidneys							
- Hyaline change	incidence	0	0	0	0	0	0
- Chronic tubular lesion	incidence	4	5	7	7	9	10
- Acute tubular lesion	incidence	0	0	0	0	0	0
- Lymphohistiocyt. Infiltration	incidence	0	1	0	0	0	0
- Bas. Proliferation	incidence	2	1	1	2	0	1
- Dilatation renal pelvis	incidence	2	0	1	1	0	0
- Hyperplasia pelv. epithel.	incidence	0	0	0	0	0	0
- Cast Formation	incidence	0	1	0	0	2	0
- Calcification renal cortex	incidence	0	0	0	0	0	1
- Nephrocalcinos.	incidence	10	10	10	10	10	10
	weighted grade	*1.6*	*1.8*	*1.9*	*1.7*	*2.4*	*2.5*
Spleen							
- Hemosiderosis	incidence	9	10	10	10	10	10
	weighted grade	*1.7*	*2.3*	*2.1*	*1.9*	*2.6*	*2.7*
- Extramedullary Hematopoiesis.	incidence	4	6	6	7	2	3
Adrenal gland							
- Fatty change	incidence	0	0	2	1	4	6

Conclusion: No-observed-adverse-effect-level (NOAEL): 250ppm (males) and 1250ppm (females), equivalent to dose levels of 17.6mg/kg bw/day (males) and 92.5 mg/kg bw/day (females), based on reduced body weight gain and histological findings in the adrenals in males at 1250ppm and histological changes in the kidneys, adrenals and spleen in females at 2500ppm. The NOEL established for males, 25ppm, is not relevant to human risk assessment since it is based on the occurrence of $\alpha_{2\mu}$-globulin nephropathy, a pathological condition unique to the male rat.

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98/8 Doc IIIA section No.	**6.4.1 / 03**	**Subchronic oral toxicity test**
91/414 Annex II Point addressed	**5.3.2 / 04**	Short-term toxicity - oral 90-day studies

1. Annex point(s)	**IIA, 5.3.2. Short-term toxicity - Oral 90-day studies - mice**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.3.2 / 04
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - 3-month range finding toxicity study in mice (administration in food). Syngenta Crop Protection AG, unpublished report No. 942105, August 13, 1996.
4. Testing facility	[redacted]
5. Dates of work	February 06, 1995 - May 10, 1995
6. Test substance	ISO common name: Thiamethoxam; [redacted]
7. Test method	OECD 408 Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Materials and methods: Groups of 10 male and 10 female mice (Tif:MAGf, SPF strain) were treated orally for 13 weeks with thiamethoxam ([redacted] by admixture in the diet at concentrations of 0, 10, 100, 1250, 3500 and 7000ppm. Mortality and clinical signs were checked daily, food/water consumption and body weight were recorded weekly. Haematology was performed on all animals at the end of the treatment period. The animals were necropsied, examined *post mortem*, major organs weighed and tissue samples examined histopathologically.

Findings: 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. Mean achieved daily dose levels were 0, 1.41, 14.3, 176, 543 and 1335mg/kg bw/day (males) and 0, 2.01, 19.2, 231, 626 and 1163mg/kg bw/day (females), respectively.

No treatment-related deaths occurred during the study (Table 1), but one male at 7000ppm died during blood sampling. Clinical signs of an adverse effect of treatment were restricted to transient respiratory sounds, without dyspnoea, at 3500 and 7000ppm. Body weight gain was markedly reduced in males and to a lesser extent in females at 7000ppm and slightly also in males at 3500ppm. The food consumption of females at 7000ppm was reduced and to a lesser extent in females at 3500ppm. It was impossible to evaluate any effect on food consumption in the males due to spillage.

Table 1: Body weight, body weight gain and food consumption

Dose level [ppm]	Males						Females					
	0	10	100	1250	3500	7000	0	10	100	1250	3500	7000
Body weight [g]												
- week 0	31.05	30.88	30.96	32.38	32.42	31.92	25.73	24.98	25.72	25.53	25.60	25.32
- week 13	49.62	49.60	48.14	51.10	47.61	34.60*	31.84	30.42	30.57	31.10	30.48	29.16
Body weight gain [g]	18.57	18.72	17.18	18.72	15.19	2.68	6.11	5.44	4.85	5.57	4.88	3.84
% difference (relative to control)		+0.8	-7.5	+0.8	-18.2	-85.6		-11.0	-20.6	-8.8	-20.1	-37.2
Food consumption [g]												
- overall week 1-13	617.13	589.67	635.44	628.13	649.33	625.66	611.09	549.53	574.87	535.84	514.91	467.11
% (relative to control)		-4.4	+3.0	+1.8	+5.2	+1.4		-10.1	-5.9	-12.3	-15.7-	-23.6

* p≤ 0.01, Lepage

Anaemia associated with a tendency to hyperchromasia and macrocytosis of red cells occurred in all males and one female at 7000ppm (Table 2). The only other haematological effect was a minimal elevation in platelets numbers in females at 1250, 3500 and 7000ppm.

Table 2: Treatment-related haematology findings

Dose level [ppm]	Males						Females					
	0	10	100	1250	3500	7000	0	10	100	1250	3500	7000
RBC (T/l)	10.54	10.56	10.52	10.17	10.07	8.852-	10.66	10.92	10.88	10.84	10.67	9.734[a]
Hb (mmol/l)	8.810	9.740	9.730	9.660	9.622	8.611-	10.06	10.26	10.03	10.27	9.885	8.960-
Hct (1)	0.477	0.488	0.480	0.475	0.472	0.437-	0.502	0.496	0.498	0.507	0.489	0.450
Platelets (G/l)	1512	1483	1399	1416	1429	1334	1146	1193	1185	1337*+	1338*+	1326+

*: p ≤ 0.01; (Lepage); - / + negative / positive trend (Jonckheere) [a] mean value reduced due to very low value (2.220T/l) in one animal

No treatment-related macroscopic changes were evident at necropsy. Absolute and relative liver weights of females at 3500 and 7000ppm, and the relative liver weight of males at 7000ppm were increased. Ovary and spleen weights of females were reduced at 3500 and 7000ppm (Table 5.3.2.3-3). A marked depression of carcass weight in both sexes at 7000ppm is considered to have affected the absolute and/or relative weights of the heart, kidneys, adrenal glands, thymus and spleen (males only) and thyroid gland, since there were no histological correlates for these changes. Histological correlates were identified for liver and ovaries.

Table 3: Treatment-related changes in organ weights

Organ weight		Male						Female					
Dose level (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)	Abs	2.774	2.636	2.502	2.641	2.738	2.138	1.787	1.698	1.732	2.005	2.108+	1.822+
	Rel	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)	Abs	--	--	--	--	--	--	48.10	50.07	41.26	43.21	38.71	31.64*-
	Rel	--	--	--	--	--	--	1.598	1.632	1.354	1.354	1.288-	1.235-
Spleen (g)	Abs	0.093	0.090	0.087	0.092	0.88	0.097	0.097	0.098	0.104	0.092	0.087	0.085-
	Rel	1.961	1.886	1.868	1.896	1.936	3.105	3.216	3.180	3.398	2.904	2.874	3.406

*: p < 0.01 (Lepage); - / + negative / positive trend (Jonckheere)

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 7000ppm (Table 4). At 7000ppm, minimal to moderate necrosis of single hepatocytes was also present. Both sexes at 1250ppm also showed minimal centrilobular hypertrophy. Males at 100ppm 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 7000ppm 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.

Table 4: Incidence of treatment-related histopathological findings

Organ/finding	Male						Female					
Dose level (ppm):	0	10	100	1250	3500	7000	0	10	100	1250	3500	7000
No. examined:	10	10	10	10	10	10	10	10	10	10	10	10
Liver:												
lymphocytic infiltr.	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
hypertrophy	0	0	8	9	10	10	0	0	0	2	10	10
Ovaries:												
atrophy	--	--	--	--	--	--	0	0	1	1	5	10

Conclusion: No-observed-adverse-effect-level (NOAEL): 100ppm, equivalent to a dose level of 14.3mg/kg bw/day (males) and 19.2mg/kg bw/day (females), based on the finding of liver hypertrophy at ≥1250ppm in males and liver hypertrophy and raised platelet counts at ≥1250ppm in females. The absolute NOEL in males is 10ppm, equivalent to a dose level of 1.4mg/kg bw/day. The effect at 100ppm was a minimal hepatocyte hypertrophy in males only, seen in the absence of any other hepatic changes. Hepatocyte hypertrophy is an adaptive effect, which is generally not considered as a toxic effect and is reversible following withdrawal of the chemical[2]. Such effects can be seen with inducers of xenobiotic metabolising enzymes; indeed, thiamethoxam has been shown to be a moderate inducer in mice. This dose level of 100ppm is therefore considered to be a No Observable **Adverse** Effect Level (NOAEL). The effects at this dose level are not toxicologically significant and are thus not considered relevant to human risk assessment.

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[2] WM Haschek and CG Rousseaux. Fundamentals of Pathology. Academic Press 1998, pp 130-145

Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted].
Remarks	[redacted]

98/8 Doc IIIA section No. 6.4.2 / 01	Subchronic dermal toxicity test
91/414 Annex II Point addressed 5.3.3 / 01	Short-term toxicity - other routes

1. Annex point(s)	**IIA, 5.3.3. Short-term toxicity - Other routes**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.3.3/01
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - 28-Day repeated dose dermal toxicity study in the rat. Syngenta Crop Protection AG, unpublished report No. 942116, October 08, 1996.
4. Testing facility	[redacted]
5. Dates of work	Feruary 06, 1996- March 07, 1996
6. Test substance	ISO common name: Thiamethoxam; [redacted]
7. Test method	OECD 410 ≅ EEC B.9 ≅ FIFRA § 82-2 Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Groups of 5 male and 5 female Sprague-Dawley-derived rats (Tif: RAIf, SPF strain) were exposed dermally to thiamethoxam [redacted] at dose levels of 0, 20, 60, 250 and 1000mg/kg bw/day, by topical application under occlusive dressing for 6 hours/day, 5 days/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 500mg/ml in order of increasing dose level. Mortality and clinical signs were checked daily, individual body weights and food and water consumption were recorded weekly. Haematological and clinical chemistry investigations were performed on all animals towards the end of the treatment period. All animals were subjected to necropsy and *post mortem* examination, major organs were weighed and selected tissues examined histopathologically.

Findings: Representative analyses of the formulations on 4 occasions demonstrated achieved concentrations in the range 109.9 - 116.0%.

No deaths occurred, there were no clinical signs of an adverse reaction to treatment, and no local irritation at the application site occurred at any dose level. Weight gain of males at 1000mg/kg was retarded during the first 2 weeks of treatment but was unaffected by treatment at other dose levels and in females at 1000mg/kg. Food consumption was unaffected by treatment at all dose levels.

Haematological profiles were unaffected by treatment at all dose levels. All statistically significant values either showed no dose-relationship or recorded values were within historical ranges. Females at 250 and 1000mg/kg had slightly raised plasma glucose levels and minimally raised alkaline phosphatase activities (Table 1). Triglyceride levels were also slightly raised in females at 1000mg/kg. Significantly elevated plasma K^+ levels in all male groups are considered not to have been influenced by thiamethoxam since 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 (inorganic phosphate levels in males at 1000mg/kg) or their occurrence showed no relationship to dose level.

Table 1: Treatment-related clinical chemistry findings

Parameter	Male					Female				
Dose level (mg/kg)	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^{*+}
Alk. 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^{*+}

* $p < 0.05$ (Wilcoxon); $^{+}$ positive trend ($p < 0.01$, Jonckheere)

No macroscopic anomalies were detected at necropsy and all organ weights and ratios were unaffected by treatment. Morphological changes in the skin of 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 the administration of thiamethoxam. The liver, kidneys and adrenal glands were identified as target organs on the basis of histological findings (Table 2). An increased incidence of minimal inflammatory cell infiltration and necrosis of single hepatocytes occurred in the liver of females at 250 and 1000mg/kg. The elevated incidence of this lesion at 60mg/kg is 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 1000mg/kg. 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 1000mg/kg. No other groups were affected.

Table 2: Incidences of histopathological findings

Organ/finding						Female				
Dose level (mg/kg)	0	20	60	250	1000	0	20	60	250	1000
No. examined:	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
intrahep. bile duct cholang/fibrosis	1	0	0	1	1	0	0	0	0	0
extramedullary hematopoiesis	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

Conclusion: No-observed-effect-level (NOEL): 250mg/kg (males) and 60mg/kg (females) based on the occurrence of renal tubular hyaline change and minimal growth retardation at 1000mg/kg (males) and histological changes in the liver at 250mg/kg (females). In view of the low order of toxicity by the percutaneous route, a 90-day dermal study is not required.

Evaluation by Competent Authorities	
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98/8 Doc IIIA section No.	**6.5 / 01**	**Chronic oral toxicity test**
91/414 Annex II Point addressed	**5.3.2 / 02**	Long-term toxicity - oral 1-year studies

1. Annex point(s)	**IIA, 5.3.2. Long-term toxicity - Oral 1-year study - dogs**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.3.2 / 02
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - 12-month chronic dietary toxicity study in Beagle Dogs. Syngenta Crop Protection AG, unpublished report No. 942108, July 22, 1998.
4. Testing facility	[redacted]
5. Dates of work	August 19, 1996 - August 19, 1997
6. Test substance	ISO common name: Thiamethoxam, [redacted]
7. Test method	OECD 452 ≅ FIFRA § 83-1 ≅ JMAFF Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Groups of 4 male and 4 female dogs (pure-bred beagles, weight range 7.5 - 13.0kg, [redacted]) were treated orally for 52 weeks with thiamethoxam ([redacted] by admixture in the diet, at concentrations of 0, 25, 150, 750 and 1500ppm. The animals were observed twice daily for mortality and daily for clinical signs, body weights were recorded weekly and food consumption was recorded daily. Eye examinations were conducted pre-test and at the end of treatment. Haematology, clinical chemistry and urinalysis were performed pre-test and in weeks 13, 26 and 52. The animals were sacrificed after 52 weeks treatment and subjected to necropsy and *post mortem* examination. Organ weights were recorded, samples of organs and tissues preserved and prepared for histological evaluation and then examined microscopically.

Findings: 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 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 1433ppm in order of increasing nominal concentration. Mean achieved dose levels, based on analytically determined values, were 0.70, 4.05, 21.0 and 42.0mg/kg bw/day (males) and 0.79, 4.49, 24.6 and 45.1 mg/kg bw/day (females).

No deaths or treatment-related clinical signs occurred during the study. The overall body weight gain of males at 1500ppm was reduced by 26% during the study. Other male groups were unaffected by treatment (Table 1). Transient body weight loss occurred in females at 1500ppm at study start, but body weight subsequently increased and overall weight gain was comparable to the controls. Mean weight gain in 750ppm females was depressed due to two animals that lost weight during the treatment period in spite of normal food intake. Since weight gain was not substantially affected at 1500ppm, this finding is considered incidental to treatment. Mean food consumption and conversion ratios were unaffected by treatment in male groups but were slightly reduced in females at 750 and 1500ppm at the start of treatment. No ocular toxicity was evident at week 52.

Table 1: Mean body weights at start and cumulative body weight gains (kg)

Dose level [ppm]	Male					Female				
	0	25	150	750	1500	0	25	150	750	1500
Weight week -1	11.30	11.28	11.63	11.18	11.68	9.675	9.400	9.875	9.925	9.800
Gain at week 13	0.350	0.225	0.175	0.175	0.150	0.900	0.825	0.800	0.050	0.625
Gain at week 26	0.575	0.575	0.400	0.575	0.325	0.950	1.150	0.875	0.075	0.925
Gain at week 39	0.975	1.050	0.950	0.875	0.300	1.450	1.625	1.500	0.375	1.475
Gain at week 52	0.950	1.125	1.125	1.000	0.700	1.625	1.750	1.450	0.550	1.750

There were no treatment-related changes in haematological profiles at any dose level. Although prothrombin times in both sexes at 1500ppm were lower than the controls throughout treatment, the values were not appreciably different from their respective pre-test values, and are not attributed to treatment with thiamethoxam (Table 2).

Table 2: Haematological findings

	Dose level [ppm]	0	25	150	750	1500
Males	**Week**					
Prothrombin time [rel. 1]	-1	1.01	0.92	0.96	0.99	0.93
	13	1.06	0.98	0.96*	0.98*	0.88*-
	26	1.03	0.95	0.93	0.93	0.85*-
	52	0.98	0.94	0.90	0.92	0.83*-
Females						
Prothrombin time [rel. 1]	-1	0.96	0.95	0.85	0.90	0.89
	13	1.00	0.99	0.95	0.91-	0.89*-
	26	0.99	1.00	0.94	0.89	0.88-
	52	0.98	0.98	0.93	0.92	0.88-

* $p \leq 0.04$, Wilcoxon; - negative trend ($p \leq 0.01$, Jonckheere)

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 1500ppm (Table 3). A slight to moderate decrease in alanine aminotransferase activity, relative to pre-test values, occurred in males at 750 and 1500ppm 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 effect. Females at 1500ppm 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 urine composition at any dose level.

Table 3: Clinical Chemistry

	Week	Dose level [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 (umol/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*+
Alanine aminotransferase (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 (umol/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

* $p \leq 0.04$, Wilcoxon; +/- positive / negative trend ($p \leq 0.01$, Jonckheere)

Analysis of absolute and relative organ weights (Table 4) indicated that there was a minimal decrease in absolute and relative testis weights in 1500ppm animals, due mainly to low testis weights from two animals. Higher heart and thyroid weights in 1500ppm males and lower ovary and spleen weights in females at 750 and 1500ppm did not have histopathological correlates and are therefore considered incidental.

Table 4: Organ weight changes

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

- : negative trend ($p \leq 0.01$, Jonckheere) [a]: (g); [b] : % body weight x 10

At necropsy, 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 which commonly occur in control animals from this source and are considered incidental to treatment. Histopathological examination of tissues/organs revealed a slight increase in the incidence of tubular atrophy in the testes at 750 and 1500ppm (Table 5). At 1500ppm, this correlated with reduced testis weights in two animals. Other microscopic alterations observed, particularly non-specific 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.

Table 5: Incidence of histopathology findings in selected tissues

		Males					Females				
Dose Level [ppm]		0	25	150	750	1500	0	25	150	750	1500
Adrenal glands	**No. exam.**	4	4	4	4	4	4	4	4	4	4
cortical fatty change		-	-	-	1	-	1	3	3	3	3
Lung	**No. exam.**	4	4	4	4	4	4	4	4	4	4
fibrosis		1	-	1	-	1	3	2	1	1	2
bronchial-alveolar hyperplasia		-	-	1	-	-	1	1	-	-	-
chronic inflammation		3	2	2	3	4	4	4	4	4	2
osseous metaplasia		-	-	-	-	-	-	-	-	1	-
Testes	**No. exam.**	4	4	4	4	4	-	-	-	-	-
tubular atrophy		1	1	1	2	2	-	-	-	-	-
spermatid giant cells		2	1	1	-	1	-	-	-	-	-
inflammatory cell infiltration		-	-	1	-	-	-	-	-	-	-
Thymus	**No. exam.**	4	4	4	4	4	4	4	4	4	4
atrophy		3	4	3	3	4	3	2	2	4	3
developmental cyst		2	3	1	2	1	-	3	2	3	1
granuloma		-	-	-	-	-	-	-	-	1	-
phagocytic cells		-	-	-	-	-	1	-	-	-	-

Conclusion: No-observed-effect-level (NOEL): 150ppm (males and females), equivalent to dose levels of 4.05mg/kg bw/day (males) and 4.49mg/kg bw/day (females), based on the observation of increased plasma creatinine and urea levels in both sexes, increased incidence of tubular atrophy in testes, and reduced food consumption in females at 750ppm. Reduced weight gain, increased plasma creatinine and urea levels in both sexes,

increased incidence and severity of tubular atrophy in the testis, and reduced food consumption in females occurred at 1500ppm.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2005
Materials and Methods	
Results and discussion	
Conclusion	Thiamethoxam administered to Beagle dogs produced toxic effects at 750 and
Reliability	
Acceptability	
Remarks	

98/8 Doc IIIA section No. 6.6.1 / 01	**In-vitro gene mutation study in bacteria**
91/414 Annex II Point addressed 5.4.1 / 01	Genotoxicity Studies - *In vitro* testing

1. Annex point(s)	**IIA, 5.4.1 Genotoxicity testing - In vitro studies**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.4.1 / 01
3. Authors (year) Title Owner, Date	Th. Hertner (1995a) CGA 293'343 technical - Salmonella and Escherichia/mammalian-microsome mutagenicity test. Syngenta Crop Protection AG, unpublished report No. 952014, November 02, 1995.
4. Testing facility	Ciba-Geigy Ltd., GeneticToxicology, Basel, Switzerland
5. Dates of work	September 08, 1995 - September 26, 1995
6. Test substance	ISO common name: Thiamethoxam,
7. Test method	OECD 471 ≅ EEC B.14 ≅ EPA-TSCA § 798.5265 ≅ JMHW Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: The highest dose level used was determined in a previous range-finding test in which six concentrations of thiamethoxam, from 20.6 to 5000µg/plate, were tested with *S. typhimurium* TA 100 and *E. coli* WP2 uvrA.

Thiamethoxam in dimethylsulphoxide (DMSO) solvent was tested on five histidine-auxotrophic strains (TA 98, TA 100, TA 102, TA 1535 and TA 1537) of *Salmonella typhimurium* and on the tryptophan-auxotrophic strain WP2 uvrA of *Escherichia coli*, by plate incorporation, at concentrations of 0 (solvent control), 312.5, 625, 1250, 2500 and 5000µg/plate. Two independent assays were performed, both experiments with and without S9 metabolic activation. S9 fraction was obtained from male RAI strain rats previously treated i/p with 500mg/kg Aroclor 1254. After preparation, the plates were inverted and incubated for about 48 hours at 37±1.5°C and evaluated by colony counting and determining the background lawn. Concurrent strain-specific mutagens were applied to all strains as positive controls in both experiments. (Table 1):

Table 1: Positive controls

Without metabolic activation			With metabolic activation	
Strain	**Positive control**	**Concentration**	**Positive control**	**Concentration**
TA 100	Sodium azide	2.0 µg/plate	2-Aminoanthracene	1.5 µg plate
TA 102	Mitomycin-C	0.5 µg/plate	2-Aminoanthracene	5.0 µg plate
TA 1535	Sodium azide	2.0 µg/plate	Cyclophosphamide	200.0 µg/plate
WP2 uvrA	4-Nitroquinoline	2.0 µg/plate	2-Aminoanthracene	20.0 µg/plate
TA 98	2-Nitrofluorene	5.0 µg/plate	2-Aminoanthracene	1.5 µg/plate
TA 1537	9-Aminoacridine	80.0 µg/plate	2-Aminoanthracene	1.5 µg/plate

Findings: In the range finding test normal background growth occurred with both strains, with and without metabolic activation and no appreciable cytotoxicity was observed at 5000 µg/plate, the highest concentration evaluated. HPLC analysis of the lowest concentration of thiamethoxam, a serial dilution of the highest concentration, demonstrated actual concentration to be 100.4% and 98.6% of nominal concentration.

Thiamethoxam did not increase the number of revertants in any of the strains used, with and without metabolic activation, in either experiment when compared to the vehicle controls (Table 2). There was no evidence of toxicity to the bacteria at any concentration, in either experiment. The positive controls produced a marked increase of the number of revertant colonies.

Table 2: Mean mutant colony counts with S. typhimurium and E. coli

Strain	TA 100		TA 102		TA 1535		TA 98		TA 1537		WP2 uvrA	
S9-mix	-	+	-	+	-	+	-	+	-	+	-	+
Experiment 1												
Solvent control	136.67	158.00	257.67	234.33	26.00	22.00	27.33	44.00	9.00	16.00	26.33	44.33
Thiamethoxam:												
312.5 µg/plate	147.00	167.33	271.67	221.67	18.67	22.67	24.33	46.67	13.00	12.67	33.00	38.00
625.0 µg/plate	147.33	157.67	242.00	237.33	21.33	19.00	26.00	51.00	10.00	18.33	22.33	38.33
1250.0 µg/plate	141.33	163.00	254.33	220.33	17.33	20.67	29.67	40.67	13.00	13.67	19.67	35.67
2500.0 µg/plate	146.67	168.67	281.00	214.33	16.33	22.00	29.33	37.67	16.33	17.00	24.00	37.33
5000.0 µg/plate	151.67	173.33	241.67	206.33	18.00	19.33	29.67	39.67	10.67	15.33	20.00	35.67
Positive control[a]	898.67	2006.33	1085.00	1356.67	729.33	264.67	801.33	1916.33	677.33	347.33	790.00	944.00
Experiment 2												
Solvent control	120.67	129.67	283.67	274.00	19.00	15.00	20.67	37.67	6.33	11.00	21.67	23.00
Thiamethoxam:												
312.5 µg/plate	129.97	142.33	300.00	299.00	15.67	12.33	17.33	29.67	9.67	8.67	22.00	20.33
625.0 µg/plate	127.00	145.33	277.00	296.00	13.67	19.00	23.33	29.67	11.33	12.00	20.67	18.33
1250.0 µg/plate	126.33	132.67	281.00	294.33	13.00	14.00	20.00	26.67	6.00	7.00	17.67	21.33
2500.0 µg/plate	113.00	142.33	318.33	297.67	16.00	15.67	19.67	24.33	8.00	11.33	18.67	22.00
5000.0 µg/plate	126.67	141.00	290.67	294.33	13.00	14.33	21.00	29.67	6.67	8.00	18.00	18.00
Positive control[a]	1066.67	1055.00	1055.00	1216.00	699.33	256.00	838.67	1852.00	1043.33	374.67	1046.67	865.33

a see table 5.4.1.1-1 for agents used as positive controls

Conclusion: Thiamethoxam and its metabolites did not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used in the study.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date March 2005

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

98/8 Doc IIIA section No.	**6.6.1 / 02**	**In-vitro gene mutation study in bacteria**
91/414 Annex Point addressed	**II 5.4.1 / 01**	Genotoxicity Studies - *In vitro* testing

1.	**Annex Point(s)**	**IIA, 5.4.1 Genotoxicity testing - In vitro studies**
2.	**Reference point (location) in dossier**	Volume 7, Section 3, Annex IIA, point 5.4.1
3.	**Authors (year)** **Title** **Owner, Date**	Deparade, E. (1999) CGA 293343 tech. – Salmonella/Mammalian-Microsome Mutagenicity Test Syngenta Crop Protection AG, unpublished report No 992020. Syngenta File No. CGA 293343/1127, 21.10.1999
4.	**Testing facility**	Novartis Crop Protection AG, GeneticToxicology, Basel, Switzerland
5.	**Dates of work**	06.05.1999 to 14.07.1999
6.	**Test substance**	Thiamethoxam
7.	**Test method**	OECD 471 ≅ EEC B.14 ≅ EPA-OPPTS 870.5100 ≅ MITI Deviations – S-9 mix from mice either untreated, treated with a single oral dose of Aroclor 1254, or treated with CGA 293343 tech. for 14 days at dietary concentrations of 50 ppm, 500 ppm, or 2500 ppm
8.	**GLP**	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Thiamethoxam (batch no. %) in dimethylsulphoxide (DMSO) solvent was tested on five histidine-auxotrophic strains (TA 98, TA 100, TA 102, TA 1535 and TA 1537) of *Salmonella typhimurium* by plate incorporation, at concentrations of 0 (solvent control), 312.5, 625, 1250, 2500 and 5000µg/plate. The standard S9 fraction was obtained from male Tif1bm:MAG strain mice previously treated orally with Aroclor 1254. Further S9 fractions were obtained from mice treated with thiamethoxam for 14 days at dietary concentrations of 50, 500, or 2500 ppm. In order to confirm the results, the experiment with S9 from mice fed the highest concentration of thiamethoxam was repeated at double the S9 concentration (20%).

After preparation, the plates were inverted and incubated for about 48 hours at 37±1.5°C and evaluated by colony counting and determining the background lawn. Concurrent strain-specific mutagens were applied to all strains as positive controls in both experiments as outlined in the Table 1.

Table 1: Positive controls

Strains	Positive control	Concentration	Remark
TA 100	Benzo(a)pyrene	1.0 µg/plate	experiment with Aroclor 1254-induced mouse liver
TA 100	N-Nitrosodimethylamine	750.0 µg/plate	
TA 1535	Cyclophosphamide	200.0 µg/plate	solvent was bidistilled water
TA 102	2-Aminoanthracene	4.0 µg/plate	
TA 98	2-Aminoanthracene	1.0 µg/plate	
TA 1537	2-Aminoanthracene	1.5 µg/plate	

Findings:

No increases in the incidence of revertant mutant colonies were noted in the strains tested in any of the experiments (Table 2).

Table 2: Mean mutant colony counts with S. typhimurium

Experiment 1 (non induced mouse liver) and Experiment 2 (Aroclor induced mouse liver)										
Strain	TA 100		TA 98		TA 1535		TA 102		TA 1537	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Solvent control	*105.0*	*114.0*	*37.3*	*40.3*	*16.0*	*15.3*	*235.7*	*205.3*	*8.3*	*9.3*
Thiamethoxam:										
312.5 µg/plate	106.7	120.0	36.3	38.7	17.7	14.7	234.7	204.0	7.7	15.0
625.0 µg/plate	83.7	121.7	29.3	36.0	15.0	17.3	218.7	220.0	5.3	13.0
1250.0 µg/plate	82.0	121.0	38.7	41.0	13.0	17.0	215.7	210.0	9.3	13.0
2500.0 µg/plate	79.7	121.0	35.7	38.7	14.3	14.3	217.7	206.3	9.7	12.3
5000.0 µg/plate	80.3	121.0	25.0	32.3	14.0	14.7	177.7	157.0	9.3	9.0
Positive control[a]	*239.3*	*258.7*		*577.7*	*274.0*		*662.7*		*100.7*	
Experiment 3 (Mouse liver induced with 50 ppm thiamethoxam in the diet)										
Solvent control		*123.7*		*29.0*		*19.0*		*295.7*		*11.0*
Thiamethoxam:										
312.5 µg/plate		121.3		32.3		16.3		249.7		13.7
625.0 µg/plate		114.3		31.0		19.7		278.3		13.0
1250.0 µg/plate		111.7		31.7		21.0		291.3		13.0
2500.0 µg/plate		109.0		33.7		12.7		264.7		12.7
5000.0 µg/plate		115.7		19.3		16.7		258.0		12.7
Positive control[a]		*332.3*		*607.0*						
Experiment 4 (Mouse liver induced with 500 ppm thiamethoxam in the diet)										
Solvent control		*112.0*		*26.0*		*13.0*		*302.7*		*16.3*
Thiamethoxam:										
312.5 µg/plate		114.0		25.3		17.7		277.3		12.7
625.0 µg/plate		117.0		29.0		13.7		234.7		7.7
1250.0 µg/plate		117.7		31.0		13.3		212.0		10.0
2500.0 µg/plate		111.0		29.3		15.0		182.7		6.7
5000.0 µg/plate		109.0		25.7		14.0		152.0		16.0
Positive control[a]		*332.3*		*607.0*						
Experiments 5 and 6 (Mouse liver induced with 2500 ppm thiamethoxam in the diet)										
	5	**6**	**5**	**6**	**5**	**6**	**5**	**6**	**5**	**6**
Solvent control	*104.3*	*84.0*	*40.7*	*45.3*	*16.3*	*19.7*	*261.0*	*221.7*	*10.0*	*15.0*
Thiamethoxam:										
312.5 µg/plate	109.0	90.3	40.0	52.0	17.0	14.7	230.0	238.3	12.7	10.0
625.0 µg/plate	107.3	91.3	42.3	40.7	20.3	19.3	245.7	233.3	10.3	10.3
1250.0 µg/plate	102.3	86.7	21.0	40.3	20.3	17.0	237.3	218.3	9.0	14.0
2500.0 µg/plate	93.0	93.3	28.3	44.3	21.0	19.3	249.0	194.7	11.0	10.0
5000.0 µg/plate	84.0	85.3	23.3	39.3	15.3	20.0	210.7	209.3	11.0	14.7
Positive control[a]	*598.3*	*314.0*	*454.7*	*403.0*						

a see table 5.4.1.2-1 for agents and the concentrations used as positive controls

Conclusion: It is concluded that thiamethoxam did not induce gene mutations in strains TA100, TA 102, TA 1535, TA 98, and TA 1537 under the various experimental conditions applied.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date March 2005

Materials and Methods

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

98/8 Doc IIIA section No.	**6.6.1 / 03**	**In-vitro gene mutation study in bacteria**
91/414 Annex II Point addressed	**5.8.2 / 03**	Toxicity of metabolites

1. Annex point(s)	**IIA, 5.8.1 Other toxicological studies - Toxicity studies of metabolites as referred to in the introduction point (vii)**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.1 / 03
3. Authors (year) **Title** **Owner, Date**	E. Deparade (1998a) CGA 322'704 tech. (Metabolite of CGA 293'343) - Salmonella and Escherichia/mammalian - microsome mutagenicity test. Syngenta Crop Protection AG, unpublished report No. 982002, March 31, 1998
4. Testing facility	Novartis Crop Protection AG, Genetic Toxicology, Basel, Switzerland
5. Dates of work	February 05, 1998 - February 20, 1998
6. Test substance	CGA 322'704 tech., [redacted].
7. Test method	OECD 471 ≅ EEC B.14 ≅ EPA-TSCA § 798.5265 ≅ JMAFF Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Department des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: The highest dose level used was determined in a previous range-finding test in which six concentrations of CGA 322'704 tech., from 20.6 to 5000µg/plate, were tested with *S. typhimurium* TA 100 and *E. coli* WP2 uvrA .

CGA 322'704 tech. ([redacted] in dimethylsulphoxide (DMSO) solvent was tested on five histidine-auxotrophic strains (TA 98, TA 100, TA 102, TA 1535 and TA 1537) of *Salmonella typhimurium* and on the tryptophan-auxotrophic strain WP2 uvrA of *Escherichia coli*, by plate incorporation, at concentrations of 0 (solvent control), 312.5, 625, 1250, 2500 and 5000µg/plate. Two independent assays were performed, both experiments with and without S9 metabolic activation. The original experiment with and without metabolic activation and the confirmatory experiment without activation were carried out as standard plate incorporation assay. The confirmatory experiment with metabolic activation was performed as a preincubation assay. S9 fraction was obtained from male Tif:RAI/SPF strain rats previously treated i/p with 500mg/kg Aroclor 1254. After preparation, the plates were inverted and incubated for about 48 hours at 37±1.5°C and evaluated by colony counting and determining the background lawn. Concurrent strain-specific mutagens were applied to all strains as positive controls in both experiments (Table 1).

Table 1: Positive controls

	Without metabolic activation		With metabolic activation	
Strain	**Positive control**	**Concentration**	**Positive control**	**Concentration**
TA 100	Sodium azide	2.0 µg/plate	2-Aminoanthracene	1.5 µg/plate
TA 102	Mitomycin-C	0.5 µg/plate	2-Aminoanthracene	4.0 µg/plate
TA 1535	Sodium azide	2.0 µg/plate	Cyclophosphamide	200.0 µg/plate
WP2 uvrA	4-Nitroquinoline	2.0 µg/plate	2-Aminoanthracene	20.0 µg/plate
TA 98	2-Nitrofluorene	5.0 µg/plate	2-Aminoanthracene	1.5 µg/plate
TA 1537	9-Aminoacridine	80.0 µg/plate	2-Aminoanthracene	1.5 µg/plate

Evaluation criteria: The test substance is considered positive in the test system if one or both of the following conditions are met:

- At least a reproducible doubling of the mean revertants per plate above that of the negative control at any concentration for one or more of strains TA 98, TA 1535, TA 1537 or WP2 uvrA.
- A reproducible increase of the mean number of revertants per plate at any concentration above that of the negative control by at least a factor 1.5 for strains TA 100 or TA 102.

Generally, a concentration-related effect should be demonstrable.

Findings: In the range finding test normal background growth occurred with both strains, with and without metabolic activation and no appreciable cytotoxicity was observed at 5000µg/plate, the highest concentration evaluated, except for the *E.coli* strain which revealed reduced numbers of revertant colonies at the upper concentrations due to a growth inhibiting effect. HPLC analysis of concentrations of CGA 322'704 tech. in the range of the test samples, demonstrated actual concentration to be 91.1% and 94.0% of nominal concentration.

CGA 322'704 tech. did not increase the number of revertants in any of the strains used, with and without metabolic activation, in either experiment, when compared to the vehicle controls. Normal background growth was observed with all strains at all concentrations. Due to a growth inhibiting effect in the experiments with metabolic activation, the numbers of revertant colonies were reduced on strains E. coli (2500 and 5000µg/plate) and TA 102 (5000µg/plate). A similar effect was visible with strain E. coli in the experiments without metabolic activation at the concentration of 5000µg/plate (Table 2). The positive controls produced a marked increase of the number of revertant colonies.

Table 2: Mean revertant colony counts with S. typhimurium and E. coli

Experiment 1												
Strain	TA 100		TA 102		TA 1535		TA 98		TA 1537		WP2 uvrA	
S9-mix	-	+	-	+	-	+	-	+	-	+	-	+
Solvent control	124.67	119.67	300.67	268.67	18.67	20.00	20.33	40.67	17.00	15.67	24.67	16.67
CGA 322'704 tech.:												
312.5 µg/plate	128.67	112.33	251.33	266.00	20.67	19.33	25.67	35.00	15.67	11.67	24.33	17.00
625.0 µg/plate	125.67	121.33	277.00	254.67	22.33	16.67	20.00	31.00	11.33	10.67	21.67	17.00
1250.0 µg/plate	129.00	118.33	256.00	194.67	26.33	14.00	19.33	33.00	13.00	12.00	19.00	17.33
2500.0 µg/plate	144.67	119.00	223.00	165.00	23.33	15.00	22.67	35.67	13.33	13.67	18.33	13.67
5000.0 µg/plate	141.33	120.67	185.00	99.67	22.33	14.00	24.33	33.00	14.33	11.67	10.67	8.00
Positive control[a]	1196.67	1989.33	1836.67	1579.33	707.33	150.00	251.67	1459.67	1619.00	259.00	752.00	1260.67
Experiment 2												
Solvent control	107.00	77.67	278.33	296.33	19.33	16.00	20.00	27.67	11.33	14.67	31.67	30.00
CGA 322'704 tech.:												
312.5 µg/plate	99.00	72.33	264.00	288.67	20.00	17.00	20.00	35.00	13.00	14.00	21.67	24.33
625.0 µg/plate	100.33	77.33	252.00	269.33	19.67	16.33	15.67	26.00	12.67	13.00	16.67	25.00
1250.0 µg/plate	96.67	66.00	218.00	257.00	17.33	20.33	16.00	27.33	9.67	13.67	17.00	15.33
2500.0 µg/plate	105.33	65.67	209.67	190.00	20.00	18.33	19.67	19.33	13.00	16.00	17.67	14.67
5000.0 µg/plate	103.33	52.00	152.00	133.33	20.67	18.00	17.00	26.33	10.33	10.67	3.67	9.33
Positive control[a]	1145.33	558.67	1687.33	1272.33	701.00	222.67	738.00	1373.67	1264.67	226.67	853.00	469.33

a) see table 5.8.1.1-1 for agents used as positive controls

Conclusion: CGA 322'704 tech. and its metabolites did not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used in the study.

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98/8 Doc IIIA section No.	**6.6.1 / 04**	**In-vitro gene mutation study in bacteria**
91/414 Annex II Point addressed	**5.8.2 / 04**	Toxicity of metabolites

1. Annex point(s)	**IIA, 5.8.1 Other toxicological studies - Toxicity studies of metabolites as referred to in the introduction point (vii)**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.1 / 04
3. Authors (year) **Title** **Owner, Date**	E. Deparade (1998b) NOA 407'475 tech. (Metabolite of CGA 293'343) - Salmonella and Escherichia/mammalian - microsome mutagenicity test. Syngenta Crop Protection AG, unpublished report No. 982014, June 09, 1998
4. Testing facility	Novartis Crop Protection AG, Genetic Toxicology, Basel, Switzerland
5. Dates of work	February 24, 1998 - May 26, 1998
6. Test substance	NOA 407'475 tech.,
7. Test method	OECD 471 ≅ EEC B.14 ≅ EPA-TSCA § 798.5265 ≅ MITI Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Department des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: The highest dose level used was determined in a previous range-finding test in which six concentrations of NOA 407'475 tech., from 20.6 to 5000µg/plate, were tested with *S. typhimurium* TA 100 and *E. coli* WP2 uvrA

NOA 407'475 tech. in bidistilled water was tested on five histidine-auxotrophic strains (TA 98, TA 100, TA 102, TA 1535 and TA 1537) of *Salmonella typhimurium* and on the tryptophan-auxotrophic strain WP2 uvrA of *Escherichia coli*, by plate incorporation, at concentrations of 0 (solvent control), 312.5, 625, 1250, 2500 and 5000µg/plate. Two independent assays were performed, both experiments with and without S9 metabolic activation. A second confirmatory experiment on strain TA 100 with metabolic activation was conducted at the same concentration range. All experiments with and without metabolic activation were carried out as standard plate incorporation assays. S9 fraction was obtained from male Tif:RAI/SPF strain rats previously treated i/p with 500mg/kg Aroclor 1254. After preparation, the plates were inverted and incubated for about 48 hours at 37±1.5°C and evaluated by colony counting and determining the background lawn. Concurrent strain-specific mutagens were applied to all strains as positive controls in all experiments. (Table 1):

Table 1: Positive controls

	Without metabolic activation		With metabolic activation	
Strain	**Positive control**	**Concentration**	**Positive control**	**Concentration**
TA 100	Sodium azide	2.0 µg/plate	2-Aminoanthracene	1.5 µg/plate
TA 102	Mitomycin-C	0.5 µg/plate	2-Aminoanthracene	4.0 µg/plate
TA 1535	Sodium azide	2.0 µg/plate	Cyclophosphamide	200.0 µg/plate
WP2 uvrA	4-Nitroquinoline	2.0 µg/plate	2-Aminoanthracene	20.0 µg/plate
TA 98	2-Nitrofluorene	5.0 µg/plate	2-Aminoanthracene	1.5 µg/plate
TA 1537	9-Aminoacridine	80.0 µg/plate	2-Aminoanthracene	1.5 µg/plate

Evaluation criteria: The test substance is considered positive in the test system if one or both of the following conditions are met:

- At least a reproducible doubling of the mean revertants per plate above that of the negative control at any concentration for one or more of strains TA 98, TA 1535, TA 1537 or WP2 uvrA.
- A reproducible increase of the mean revertants per plate at any concentration above that of the negative control by at least a factor 1.5 for strains TA 100 or TA 102.

Generally, a concentration-related effect should be demonstrable.

Findings: In the range finding test normal background growth occurred with both strains, with and without metabolic activation and no appreciable cytotoxicity was observed at 5000µg/plate, the highest concentration evaluated. HPLC analysis of concentrations of CGA 407’475 tech. in the range of the test samples, demonstrated actual concentration to be 95.0% and 94.2% of nominal concentration.

NOA 407’475 tech. did not increase the number of revertants in any of the strains used, with and without metabolic activation, in either experiment, when compared to the vehicle controls. Since the results obtained with strain TA 100 in both experiments with metabolic activation were considered equivocal, a second confirmatory experiment with metabolic activation (experiment 3) was performed at the same concentration range. TA 100 was found to be not mutagenic in this second confirmatory experiment conducted with metabolic activation. Due to a growth inhibiting effect of the test substance, a reduction in the growth of the bacterial background lawn occurred in the original experiment without activation on all strains and in the first confirmatory experiment without activation on strains TA 98, TA 1537 and TA 102 at the concentration of 5000µg/plate. The number of revertants was reduced in the original experiment (TA 100) and first confirmatory experiment (TA 102 and TA 98) without metabolic activation at the concentration of 5000µg/plate (Table 2). The positive controls produced a marked increase of the number of revertant colonies.

Table 2: Mean colony counts with S. typhimurium and E. coli

Experiment 1												
Strain	TA 100		TA 102		TA 1535		TA 98		TA 1537		WP2 uvrA	
S9-mix	-	+	-	+	-	+	-	+	-	+	-	+
Solvent control	106.67	82.33	318.67	327.33	20.67	15.67	22.67	37.33	16.67	15.67	27.67	20.33
NOA 407'475 tech.:												
312.5 µg/plate	125.67	80.00	338.67	317.33	22.33	17.33	26.33	34.00	15.33	12.67	33.67	24.00
625.0 µg/plate	105.33	100.67	333.00	291.00	18.67	20.33	28.67	36.00	16.33	18.00	33.67	25.00
1250.0 µg/plate	121.67	93.33	322.67	306.67	20.33	16.33	26.00	31.33	15.00	14.33	34.33	26.67
2500.0 µg/plate	98.00	87.67	337.33	308.00	22.33	13.67	27.00	37.00	15.00	14.33	29.67	20.67
5000.0 µg/plate	21.33	146.00	195.00	327.33	16.67	18.33	12.33	34.33	12.00	17.67	21.33	25.67
Positive control[a)]	1090.33	1710.00	1719.00	1730.67	639.00	222.00	256.67	1438.00	1264.33	303.67	659.33	1256.33
Experiment 2												
Solvent control	106.00	99.33	345.00	333.33	17.00	19.00	27.33	41.33	13.00	16.67	23.33	21.00
NOA 407'475 tech.:												
312.5 µg/plate	97.33	101.33	345.00	326.67	22.33	18.33	24.00	34.67	9.33	12.33	20.00	15.00
625.0 µg/plate	105.00	96.67	349.00	311.67	20.33	19.67	22.33	37.33	13.33	14.00	21.00	18.00
1250.0 µg/plate	112.00	87.67	353.33	294.67	20.00	23.00	26.00	38.67	15.67	15.00	20.67	13.33
2500.0 µg/plate	113.33	90.33	357.67	319.00	24.00	22.67	26.00	40.00	14.00	15.33	21.33	26.00
5000.0 µg/plate	62.00	143.67	133.00	327.00	19.33	32.00	6.33	32.00	6.67	20.33	17.00	23.00
Positive control[a)]	1073.33	1615.67	1712.33	1561.33	646.00	256.33	313.00	1399.67	1171.33	308.67	613.67	1159.67
Experiment 3												
Solvent control		100.67										
NOA 407'475 tech.:												
312.5 µg/plate		101.67										
625.0 µg/plate		95.67										
1250.0 µg/plate		96.00										
2500.0 µg/plate		87.33										
5000.0 µg/plate		101.00										
Positive control[a)]		1567.67										

a) see table 5.8.1.2-1 for agents used as positive controls

Conclusion: Based on the results of these experiments and on standard evaluation criteria, it is concluded that NOA 407'475 tech. (metabolite of CGA 293'343) and its metabolites did not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used in the study.

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98/8 Doc IIIA section No.	**6.6.2 / 01**	**In-vitro cytogenicity study in mammalian cells**
91/414 Annex II Point addressed	**5.4.1 / 04**	Genotoxicity Studies - *In vitro* testing

1. Annex point(s)	**IIA, 5.4.1 Genotoxicity studies - In vitro studies**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.4.1 / 04
3. Authors (year) Title Owner, Date	S. Zeugin (1996) CGA 293'343 tech. - Cytogenetic test on Chinese hamster cells in vitro. Syngenta Crop Protection AG, unpublished report No. 952016, June 18, 1996.
4. Testing facility	Ciba-Geigy Ltd., Genetic Toxicology, Basel, Switzerland
5. Dates of work	February 28, 1996 - June 03, 1995
6. Test substance	ISO common name: Thiamethoxam,
7. Test method	OECD 473 ≅ EEC B.10 ≅ EPA-TSCA § 798.5375 ≅ JMAFF Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Thiamethoxam (in dimethylsulphoxide (DMSO) solvent was tested on Chinese hamster ovary cells *in vitro*, by incorporation in the culture medium, in quadruplicate, for 21 hours without metabolic activation and for 3 hours (with 18 hours recovery) with metabolic activation. Concentrations of 0 (solvent control), 283.75, 567.5 and 1135µg/ml without S9 metabolic activation, and 0 (solvent control), 1135, 2270 and 4540µg/ml with metabolic activation were used in the first experiment. A second, confirmatory experiment was performed at concentrations of 0, 1135, 1702.5 and 2270µg/ml without activation and 0, 2270, 3405 and 4540µg/ml with activation. A further experiment was performed exposing cells for 45 hours, without activation, to 0, 851.25, 1135 and 1702.5µg/ml and for 3 hours with activation, and a 42 hour recovery period to 0, 2270, 3405 and 4540µg/ml. 0.2µg/ml Mitomycin C (MMC) was the positive control for experiments without activation and 20µg/ml cyclophosphamide for experiments with activation. S9 fraction was obtained from male RAI strain rats previously treated i/p with 500mg/kg Aroclor 1254.

Two hours prior to harvesting, the cultures were treated with 0.4µg/ml colcemid to arrest cells in metaphase. The cells were spread on glass slides, air dried and stained. A cytotoxicity test was performed as an integral part of the study by determination of the percentage mitotic suppression in at least 2000 cells from one slide/group. Whenever possible two hundred well spread metaphase figures with 19 to 21 centromeres from two cultures (100 metaphases per replicate culture) in each group were scored. At least fifty metaphases were scored in the positive controls (25 per replicate culture). The slides were examined blind for specific and non-specific structural aberrations.

The influence of thiamethoxam on the CHO cell cycle was evaluated at the experimental concentrations. The DNA distribution was determined by flow cytometry and compared with the profile of the respective control culture.

Findings: The rationale for dose selection, based on mitotic indices, is shown in Table 1. In the absence and in the presence of metabolic activation, a shift in the DNA distribution profile was not detected and no evidence of cell cycle disturbance was apparent.

Analytical determination of thiamethoxam at the lowest concentration, prepared by serial dilution, revealed the test article was stable in the vehicle used. The actual concentrations were 125.2 and 114.0% of nominal. The

concentration values indicated in the report are nominal, and not corrected according to analytically determined concentrations.

The incidence of specific chromosomal aberrations in the thiamethoxam-treated groups, at all concentrations at the 21 hour sampling interval, both with and without metabolic activation, were not significantly different from negative control values (Table 2). Incidentally, at the 45 hour sampling interval, with metabolic activation, 0% metaphases with specific chromosomal aberrations was scored in the negative control cultures. Therefore, a statistically significant increase in the incidence of cells with specific chromosomal aberrations (1.5%) occurred at a concentration of 3405.0µg/ml. However, a value of 1.5% is within the historical control range and does not fulfil the criteria for a positive response.

Non-specific chromosomal aberrations (chromatid gaps) were encountered in all experiments within the generally observed frequency.

Treatment of CHO cells with the positive control materials produced a high incidence of specific chromosomal aberrations in both experiments, demonstrating the sensitivity of the test system.

Table 1: Mitotic Index (% of control) of CHO cells

Original study			Confirmatory study					
Experiment	**1**	**2**	**Experiment**	**1**	**3**	**Experiment**	**2**	**4**
S-9	-	+	-			+		
Treatment (h)	21	3	Treatment (h)	21	45	Treatment (h)	3	3
Recovery (h)	0	18	Recovery (h)	0	0	Recovery (h)	18	42
Thiamethoxam (µg/ml)			Thiamethoxam (µg/ml)			Thiamethoxam (µg/ml)		
4540.0	0.00	9.20	2270.0	4.95		4540.0	10.40	11.50
2270.0	1.50	11.90	1702.5	7.00		3405.0	11.15	14.15
1135.0	9.10	13.05	1135.0	8.40	2.40	2270.05	11.90	17.15
567.5	10.80	14.40	851.25	9.90	9.30	1702.5	b	b
283.75	11.30	a	567.5	10.25	10.35	1135.0	b	b
141.88	a	a	425.63	a	11.25	851.25	b	b
70.94	a	a	283.75	a	11.90	567.5	b	b
35.47	a	a	212.81	a	a	425.63	14.10	18.40

Concentrations with underlined mitotic index were used for chromosome analysis

a When three subsequent concentrations with a frequency of 70% mitosis or more in relation to the solvent control are found, the evaluation of the lower concentration is omitted.

b When the three highest concentrations show a frequency of 70% mitosis or more in relation to the solvent control, the evaluation of the lower concentrations, with exception of the lowest, is omitted.

Table 2: Incidence (%) of specific chromosome aberrations in CHO cells

	Original study		Confirmatory study			
Experiment	**1**	**2**	**1**	**2**	**3**	**4**
S-9	-	+	-	+	-	+
Treatment (h)	21	3	21	3	45	3
Recovery (h)	-	18	-	18	-	42
Negative control	**1.5**	**3.0**	**0.5**	**1.5**	**1.0**	**0**
4540.0 µg/ml		4.5		1.5		0
3405.0 µg/ml				1.0		1.5*
2270.0 µg/ml		2.5	1.0	0.5		1.0
1702.5 µg/ml			1.0			
1135.0 µg/ml	1.5	3.0	0.5			
851.25 µg/ml					1.5	
567.5 µg/ml	3.0				0.5	
425.63µg/ml					0.5	
283.75 µg/ml	0.5					
Positive control						
Cyclophosphamide		54.0***		64.0***		-
Mitomycin-C	66.0***		60.0***		-	

- No positive control; * $p \leq 0.05$; *** $p \leq 0.001$ (Chi-square test)

Conclusion: No evidence was found of a clastogenic effect of thiamethoxam in *in vitro* cultures of Chinese hamster ovary cells.

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98/8 Doc IIIA section No.	6.6.3 / 01	In-vitro gene mutation assay in mammalian cells
91/414 Annex II Point addressed	5.4.1 / 02	Genotoxicity Studies - *In vitro* testing

1. **Annex point(s)**	**IIA, 5.4.1 Genotoxicity testing - In vitro studies**
2. **Reference point (location) in dossier**	Volume 7, Section 3, Annex IIA, point 5.4.1 / 02
3. **Authors (year) Title Owner, Date**	B. Ogorek (1996a) CGA 293'343 tech. - Gene mutation test with Chinese hamster cells V79. Novartis Crop Protection AG, unpublished report No. 952015, January 12, 1996.
4. **Testing facility**	Ciba-Geigy Ltd., Genetic Toxicology, Basel, Switzerland
5. **Dates of work**	October 31, 1995 - December 13, 1995
6. **Test substance**	ISO common name: Thiamethoxam,
7. **Test method**	OECD 476 ≅ EEC B.17 ≅ EPA-TSCA § 798.5300 Deviations - none
8. **GLP**	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: The highest concentrations used in the experiments were determined in an initial cytotoxicity range-finding test in which 12 concentrations of thiamethoxam, from 1.63 to 3333.3µg/ml (limit of solubility), were tested with and without S9 metabolic activation.

Thiamethoxam in dimethylsulphoxide (DMSO) solvent was tested on Chinese hamster V79 cells by incorporation in the growth medium, at concentrations of 0 (solvent control), 123.3, 370.0, 1110 and 3333µg/ml with S9 metabolic activation, and 0 (solvent control), 61.67, 185.0, 555.0 and 1665µg/ml without metabolic activation. Two independent assays were performed. In the second experiment, concentrations of 0, 416.25, 832.5, 1665 and 3330µg/ml with S9 metabolic activation, and 0, 277.5, 555.0, 1110 and 2220µg/ml without metabolic activation were used. S9 fraction was obtained from male RAI strain rats previously treated i/p with 500mg/kg Aroclor 1254. Duplicate cultures were exposed to thiamethoxam, solvent alone or positive control mutagens for 5 hours with S9 (positive control - 1.0µl/ml N-nitroso-dimethylamine = DMN) and for 21 hours without S9 (positive control - 0.3µl/ml ethylmethansulphonate = EMS). After exposure, the cultures were incubated at 37°C for 7 - 8 days for expression during exponential growth. At the end of the expression period the cultures were counted with a haemocytometer or Coulter counter. Mutant selection was by exposure to 6-thio-guanine followed by incubation at 37°C for 7 - 8 days, after which the cultures were fixed and stained and the mutated clones counted by eye.

Cytotoxicity was estimated from the cloning efficiency immediately after exposure for determination of survival values. Viability at the end of expression was estimated from the cloning efficiency.

Findings: In the preliminary cytotoxicity test, the highest concentration with metabolic activation produced 49% growth inhibition whereas complete growth inhibition occurred without metabolic activation. At the next lower concentration 67% growth inhibition occurred.

Analysis of test article concentration in the stock solution and the lowest concentration prepared by serial dilution, showed thiamethoxam to be stable in the vehicle used. The actual concentrations were 86.2 and 93.1% of the nominal concentration of the stock solution and the lowest concentration, respectively.

In both experiments, with and without metabolic activation, no significant increase in the mutation frequencies was observed following exposure to thiamethoxam. In contrast, both DMN and EMS produced highly statistically significant increases in mutation frequencies. (Table 1).

Table 1: Mutation frequencies in Chinese hamster V79 cells

First experiment			Second experiment		
Treatment	**Mean mutant frequency [x10⁶]**	**Mean mutant factor**	**Treatment**	**Mean mutant frequency [x10⁶]**	**Mean mutant factor**
With metabolic activation					
EMS	120.76**	17.29	EMS	122.87**	6.53
0	6.8		0	3.36	
3330.0 µg/ml	6.97	1.00	5000.0 µg/ml	4.20	1.25
1110.0 µg/ml	6.94	0.99	1666.7 µg/ml	3.41	1.01
370.0 µg/ml	7.53	1.08	555.6 µg/ml	3.28	0.98
123.3 µg/ml	8.11	1.16	185.2 µg/ml	3.05	0.91
Without metabolic activation					
DMN	1514.49**	189.19	DMN	1748.34**	436.98
0	8.01		0	4.00	
1665.0 µg/ml	7.73	0.97	5000.0 µg/ml	-	-
555.0 µg/ml	7.90	0.99	1666.7 µg/ml	3.39	0.85
185.0 µg/ml	8.29	1.04	555.6 µg/ml	3.84	0.96
61.7 µg/ml	6.13	0.77	185.2 µg/ml	3.94	0.98

** $p < 0.001$

Conclusion: Thiamethoxam or its metabolites did not induce gene mutations in cultured V79 Chinese hamster cells either in the presence or absence of metabolic activation.

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98/8 Doc IIIA section No.	**6.6.3 / 02**	**In-vitro gene mutation assay in mammalian cells**
91/414 Annex II Point addressed	**5.4.1 / 03**	Genotoxicity Studies - *In vitro* testing

1. Annex point(s)	**IIA, 5.4.1 Genotoxicity testing - In vitro studies**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.4.1 / 03
3. Authors (year) Title **Owner, Date**	B. Ogorek (1996b) CGA 293'343 tech. - Autoradiographic DNA repair test on rat hepatocytes (OECD conform) *in vitro.* Syngenta Crop Protection AG, unpublished report No. 952017, January 29, 1996.
4. Testing facility	Ciba-Geigy Ltd., Genetic Toxicology, Basel, Switzerland
5. Dates of work	November 13, 1995 - December 20, 1995
6. Test substance	ISO common name: Thiamethoxam,
7. Test method	OECD 482 ≅ EEC B.10 ≅ EPA-TSCA § 798.5550 Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: A preliminary cytotoxicity test on thiamethoxam. in culture medium at 10 concentrations between 3.26 and 1665µg/ml (highest soluble concentration) was performed. Induction of unscheduled DNA synthesis was investigated using primary hepatocytes derived by *in situ* collagenase perfusion from adult male Tif:RAIf rats weighing 205 - 325 g. Freshly isolated, washed hepatocytes, attached to coverslips, were exposed to thiamethoxam in DMSO at concentrations of 0 (solvent control), 13.01, 52.04, 208.13, 416.25, 832.5 and 1665µg/ml for 16 - 18 hours. Two independent experiments were performed, each treatment on quadruplicate cultures. A positive control (2-acetaminofluorene, 2-AAF, 45 µM) was also performed. Autoradiography was used to determine the uptake of tritiated-thymidine. Following swelling of the nuclei, the cells were fixed and the coverslips mounted on microscope slides for autoradiography. The autoradiographs were developed after 4 days storage at 4°C and stained in haematoxylin solution and counterstained in eosin. The net nuclear grain counts were determined, blind, for three slides (50 cells/slide) per treatment and control group. Silver grains over the nuclei and cytoplasm of the hepatocytes were counted. The incorporation of radioactive material in the cytoplasm was determined by counting the silver grains in three cytoplasmic regions adjacent to the nucleus. Net nuclear grain values were then calculated. Cells in DNA synthesis phase, showing more than 120 grains/nucleus, were excluded.

Findings: In both independent experiments, none of the concentrations of thiamethoxam induced a significant increase in the mean gross or net nuclear grain counts when compared to the solvent control group, despite a reduction of 80% in hepatocyte viability at the highest concentration in the first experiment. The positive control group, 2-AAF, induced 100% of cells to go into repair in both experiments. Group mean net grain counts are shown in Table 1. The percentage distribution of gross and net nuclear grain counts was not substantially shifted to higher values.

Table 1: Group mean net grain counts

Experiment no.	Treatment	Net nuclear grain count		Net nuclear grain count of cells in repair		Percent cells in repair (Net nuclear grains ≥ 2)	
	(µg/ml)	Mean	SD	Mean	SD	Mean	SD
1	0 (DMSO)	0.5	0.4	3.6	0.5	22.7	8.3
	10 (2AAF)	16.7	4.9	16.7	4.9	100.0	0.0
	1665	0.3	0.4	3.3	0.5	22.7	8.3
	832.5	0.5	0.7	3.3	0.5	22.7	13.3
	416.25	0.5	0.4	3.9	0.1	24.7	9.2
	208.13	0.6	0.4	3.7	0.6	25.3	6.4
	52.04	0.3	0.4	3.4	0.3	23.3	2.3
	13.01	1.0	0.7	4.1	0.5	29.3	9.5
2	0 (DMSO)	0.5	0.1	3.2	0.2	26.0	5.3
	10 (2AAF)	15.1	2.7	15.1	2.7	100.0	0.0
	1665	0.8	0.4	3.4	0.3	25.3	5.0
	832.5	0.6	0.3	3.4	0.6	24.0	8.0
	416.25	0.7	0.2	4.0	0.4	24.0	4.0
	208.13	0.7	0.3	3.8	0.5	20.7	6.1
	52.04	0.7	0.4	3.4	0.2	20.7	8.3
	13.01	0.8	0.6	3.2	0.6	29.3	12.1

Conclusion: There was no evidence of induction of DNA damage by thiamethoxam or its metabolites, indicating an absence of genotoxic potential in this *in vitro* test system.

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	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted].
Remarks	[redacted]

98/8 Doc IIIA section No.	6.6.3 / 03	In-vitro gene mutation assay in mammalian cells
91/414 Annex II Point addressed	5.4.1 / 03	Genotoxicity Studies - *In vitro* testing

1.	**Annex Point(s)**	**IIA, 5.4.1 Genotoxicity testing - In vitro studies**
2.	**Reference point (location) in dossier**	Volume 7, Section 3, Annex IIA, point 5.4.1 / 02
3.	**Authors (year)** **Title** **Owner, Date**	Ogorek, B. (2000) CGA 293343 tech. - Autoradiographic DNA repair test on mouse hepatocytes (OECD conform) *in vitro* Syngenta Crop Protection AG, Basel, Switzerland, Study report No. 992066, Syngenta file No. CGA 293343/1195, 14.04.2000
4.	**Testing facility**	Novartis Crop Protection AG, GeneticToxicology, Basel, Switzerland
5.	**Dates of work**	18.01.2000, to 21.02.2000
6.	**Test substance**	Thiamethoxam
7.	**Test method**	OECD 482, EPA OPPTS 870.5550, 87/302/EEC B.18. Deviations – none
8.	**GLP**	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Test material: CGA 293343 tech.; Primary hepatocytes were isolated from adult male Tif:MAG mice weighing 25 to 32 g by *in situ* collagenase perfusion through the heart. The freshly isolated mouse hepatocytes were allowed to attach to the coverslips during an attachment period of 1.5-2 hours. Unattached cells were then removed. The mutagenicity assay was initiated immediately after having removed the unattached cells, by supplementing the medium (Williams Medium E containing 10% foetal bovine serum) in the compartments with the test material and the vehicle control. Six pre-selected concentrations[3] of the test substance, one positive control (2-acetylaminofluorene, 2-AAF, 45 µM) and a negative control containing the vehicle (DMSO) were run and two independent sets of experiments were performed. Each treatment was performed on four cultures. Immediately after addition of the test substance, ^{3}H-thymidine was added to the medium in each compartment. The treatment period was about 16-18 hours (over night). At the end of the treatment the cells were washed twice. The nuclei were swollen by treatment with 1% sodium citrate for ten minutes and then the cells were fixed with ethanol: acetic acid (3:1 % v:v]). The coverslips were mounted on microscope slides and prepared for autoradiography. The autoradiographs were developed after 4 days of storage at 4 °C. The developed autoradiographs were stained in haematoxylin solution, rinsed in tap water and counterstained in eosin. Prior to scoring slides were coded. Silver grains over the nuclei and cytoplasm of the hepatocytes were counted. Three slides (50 cells/slide) from each of the treatment groups and from the positive and the negative controls were scored. The incorporation of radioactive material in the cytoplasm was determined by counting the silver grains in three cytoplasmic regions adjacent to the nucleus, each with an area equivalent to that of the nucleus. The net values were calculated by subtracting the average grain count over the cytoplasm from the total over the nuclei. Cells which were in the DNA synthesis phase showed more than 120 silver grains/nucleus. These cells were excluded from the determination of the silver grain/nucleus count.
CGA 293343 tech. was dissolved in DMSO at room temperature. The highest concentration used for the assay was

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3 Cytotoxicity determination was performed as an integral part of the first (original) DNA repair assay. Eleven concentrations ranging from 3.67 to 3750 µg/ml spaced by a factor of 2 were tested. The highest concentration to be scored was determined using the following criteria:

- A sufficiently large number of cells must adhere to the coverslips.
- At least 25% of the cells must show viability upon examination by means of the vital-staining technique.
- A sufficient number of viable cells must be in good condition upon morphological examination.

375 mg/ml. Lower concentrations of the test substance were obtained by appropriate dilution of the stock solution with bidistilled water. The respective solutions were added 1:100 to the cell culture medium.

Findings: In the cytotoxicity test 11 concentrations ranging from 3.67 µg/ml to 3750.00 µg/ml spaced by a factor of two were tested. At the four highest concentrations cellular viability was reduced by more than 85% compared with the viability of the solvent control. The seven lower concentrations decreased viability in a dose-dependent manner by about 50 to 20%. Accordingly, concentrations between 7.33 and 234.38 µg/ml were selected for analysis in the original DNA repair assay.

CGA 293343 tech. was tested at the concentrations of 7.33, 14.65, 29.30, 58.60, 117.19, and 234.38 µg/ml (original DNA repair experiment) and 7.35, 14.69, 29.37, 58.75, 117.50, and 235.00 µg/ml (confirmatory experiment). Higher concentrations were not tested, due to strong toxicity. 2-acetylaminofluorene (45 µmol/l) was used as a positive control.

In both experiments performed, after treatment with CGA 293343 tech., the mean nuclear grain counts as well as the net nuclear grain counts (i.e. mean number of silver grains per nucleus minus mean number of silver grains per nucleus-equivalent area of cytoplasm) revealed no relevant difference from the respective vehicle control. Group mean net grain counts are shown in Table 1. There was no biologically relevant increase of the percentages of cell in repair (net grain count ≥2.0).

Analytical control: The test material in solution was analysed by HPLC with UV detection to confirm the intended concentrations to be used in the DNA repair experiments and the stability of the test substance in the vehicle used. This determination was performed with the lowest concentration of the stock solutions used in the two DNA repair experiments. The values found of the two samples analysed were 100% for the original experiment and 101% for the confirmatory experiment of the nominal concentrations, also demonstrating sufficient stability of the test substance in the vehicle used.

Conclusion: The measurement of unscheduled DNA repair in mouse hepatocytes *in vitro* did not reveal any indication for DNA damage by thiamethoxam or its metabolites.

Table 1: Group mean net grain counts

Experiment no.	Treatment	Net nuclear grain count		Net nuclear grain count of cells in repair		Percent cells in repair (Net nuclear grains ≥ 2)	
	(µg/ml)	Mean	SD	Mean	SD	Mean	SD
1	0 (DMSO)	-0.0	0.2	2.8	0.2	11.3	1.2
	10 (2-AAF)	9.4	1.6	9.4	1.6	100.0	0.0
	234.38	0.2	0.4	3.2	0.3	18.7	6.4
	117.19	-0.0	0.2	2.9	0.5	16.0	2.0
	58.60	0.2	0.3	2.9	0.1	10.0	5.3
	29.30	-0.3	0.2	2.4	0.2	4.7	1.2
	14.65	-0.1	0.5	2.3	0.1	15.3	2.3
	7.33	-0.5	0.6	2.9	0.3	7.3	5.0
2	0 (DMSO)	-0.1	0.3	3.2	0.2	7.3	2.3
	10 (2-AAF)	8.5	1.3	8.7	1.2	98.0	2.0
	235.00	0.1	0.1	3.4	0.2	16.0	9.2
	117.50	-0.1	0.1	2.8	0.3	14.0	3.5
	58.75	0.1	0.4	2.8	0.3	12.7	8.1
	29.37	0.0	0.2	2.6	0.1	18.7	2.3
	14.69	0.8	0.5	3.5	0.5	30.7	11.0
	7.35	0.0	0.1	3.0	0.2	10.0	2.0

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98/8 Doc IIIA section No. 6.6.4 / 01	**If positive in 6.6.1, 6.6.2 or 6.6.3, then an in-vivo mutagenicity study will be required (bone marrow assay for chromosomal damage or a micronucleus test)**
91/414 Annex II Point addressed 5.4.2 / 01	Genotoxicity Studies - *In vivo* testing, somatic cells

1. Annex point(s)	**IIA, 5.4.2 Genotoxicity testing - In vivo studies in somatic cells**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.4.2/01
3. Authors (year) Title Owner, Date	███ (1995b) CGA 293'343 tech. - Micronucleus test, mouse (OECD conform). Syngenta Crop Protection AG, unpublished report No. 952018, December 15, 1995.
4. Testing facility	███
5. Dates of work	September 26, 1995 - November 22, 1995
6. Test substance	ISO common name: Thiamethoxam, ███
7. Test method	OECD 474 ≅ EEC B.12 ≅ EPA-TSCA § 798.5395≅ MITI JAPAN Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and Methods: A preliminary MTD study was performed in mice by the fixed dose procedure at dose levels of thiamethoxam up to 2000mg/kg, to determine the highest dose level producing significant toxicity but not death.

Groups of 5 male and 5 female mice (Tif:MAGf strain, body weight range 24 - 38g) were treated once orally, by gavage, with thiamethoxam ███ as a suspension in bidistilled water, at dose levels of 0 (solvent control), 312.5, 625.0 and 1000mg/kg bw, and for females only, 1250mg/kg bw. Multiple groups of mice were used for the solvent control and high dose groups for bone marrow sampling at multiple time points after treatment. A further group of mice was treated once orally with 64mg/kg bw cyclophosphamide (CPA). Body weights were recorded pre-dose and signs of toxicity recorded after treatment and before sacrifice. Groups of 10 mice from all dose groups were sacrificed 24 hours after treatment. Additional groups of 10 mice from the vehicle control and high dose groups were sacrificed 16 and 48 hours after treatment. Femoral bone marrow samples were collected into foetal calf serum, smears were prepared, stained, coded and scored by light microscopy. The incidence of micronucleated polychromatic erythrocytes (MNPCE) in at least 2000 polychromatic erythrocytes (PCE) and the ratio of PCE to normochromatic erythrocytes (NCE) in at least 1000 erythrocytes were determined for each slide. Statistical significance was assessed by the Chi-squared-contingency-test, $p<0.05$.

Findings: Analysis of dose preparations revealed actual concentrations in the range 104.1 - 119.9% nominal concentration.

The dose level of 1250mg/kg bw caused death in 7/13 females. Treatment at 1000mg/kg bw produced signs of toxicity including lethargy, hunched posture and ataxia. Some animals treated at 625mg/kg bw also showed reduced locomotor activity.

At all sampling intervals (16, 24, and 48 hours), no significantly increased incidence of MNPCE occurred in thiamethoxam treated groups relative to the vehicle control. In contrast, the positive control group showed a significant increase in MNPCE in both sexes (Table 1). The ratios of

polychromatic to normochromatic erythrocytes after treatment with thiamethoxam indicated no effects on erythropoiesis at any dose level.

Table 1: Percentage of MNPCE (PCE/NCE ratio) at different preparation times

Test article and concentration	Sex	16 hours	24 hours	48 hours
Vehicle control (bidistilled water)	male female **mean**	0.03 (0.68) 0.03 (1.27) **0.03 (0.98)**	0.07 (0.63) 0.02 (1.20) **0.05 (0.92)**	0.04 (0.63) 0.08 (1.02) **0.06 (0.83)**
Thiamethoxam (312.5mg/kg)	male female **mean**		0.02 (0.84) 0.00 (0.86) **0.01 (0.85)**	
Thiamethoxam (625mg/kg)	male female **mean**		0.01 (0.68) 0.01 (0.88) **0.01 (0.78)**	
Thiamethoxam (1000[1] mg/kg)	male female **mean**	0.04 (0.74) 0.06 (0.91) **0.05 (0.83)**	0.09 (0.87)	0.05 (0.75)
Thiamethoxam (1250[2]mg/kg)	female		0.04 (0.81)	0.02 (1.16)
Positive control Cyclophosphamide (64mg/kg)	male female **mean**		3.28* (0.91) 1.10* (0.95) **2.19* (0.93)**	

* $p < 0.0001$ (Chi-Square Test)

1 Due to symptoms of toxicity the dose level was reduced to 1000mg/kg.

2 Females for 24 and 48 hours sampling were treated with 1250mg/kg, which proved highly toxic.

Conclusion: Treatment of mice with thiamethoxam at dose levels up to 1250mg/kg, a dose level producing severe toxicity, did not elicit a clastogenic or aneugenic effect.

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98/8 Doc IIIA section No.	**6.7 / 01**	**Carcinogenicity study**
91/414 Annex II Point addressed	**5.5.1 / 01**	Long-term Toxicity and Carcinogenicity

1. Annex point(s)	**IIA, 5.5.1 Long term toxicity and carcinogenicity - mice**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.5.1/01
3. Authors (year) Title Owner, Date	███ CGA 293'343 tech. - 18-month oncogenicity study in mice. Syngenta Crop Protection AG, unpublished report No. 942109, June 02, 1998.
4. Testing facility	███
5. Dates of work	September 25, 1995 - April 10, 1997
6. Test substance	ISO common name: Thiamethoxam, ███
7. Test method	OECD 453 ≅ FIFRA § 83-2 ≅ JMAFF Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: Groups of 60 male and 60 female albino mice (Tif:MAGf, SPF strain, body weight range 20.9 - 42.0g, source ███) were administered thiamethoxam (███ ███) orally for 78 weeks, by admixture in the diet, at concentrations of 0, 5, 20, 500, 1250 and 2500ppm. Ten animals/sex/group were designated for clinical laboratory investigations and 50 animals/sex/group for the oncogenicity study. Additional groups of 10 animals/sex in the control and high dose groups were similarly treated with thiamethoxam for 35 weeks and sacrificed for interim evaluation. Clinical observations were made daily, body weights and food consumption recorded weekly for 13 weeks and monthly thereafter. Haematological investigations were performed at weeks 53 and 78 on 10 animals/sex/group. All animals were subjected to detailed necropsy and *post mortem* examination. Organ weights of all animals which survived to scheduled sacrifice were recorded. Tissue/organ samples from all animals sacrificed at 35 weeks, those which died or were killed during the study and all animals killed after 78 weeks, were preserved. Microscopic examination of tissues was performed on all animals of the oncogenicity subgroup. The livers of the additional 10 animals/sex in the control and high dose groups were also examined microscopically under H&E staining and 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.

Findings: 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 2598ppm. Mean dose levels, calculated on the basis of analytically determined concentrations, were 0.65, 2.63, 63.8, 162 and 354mg/kg bw/day (males) and 0.89, 3.68, 87.6, 215 and 479mg/kg bw/day (females).

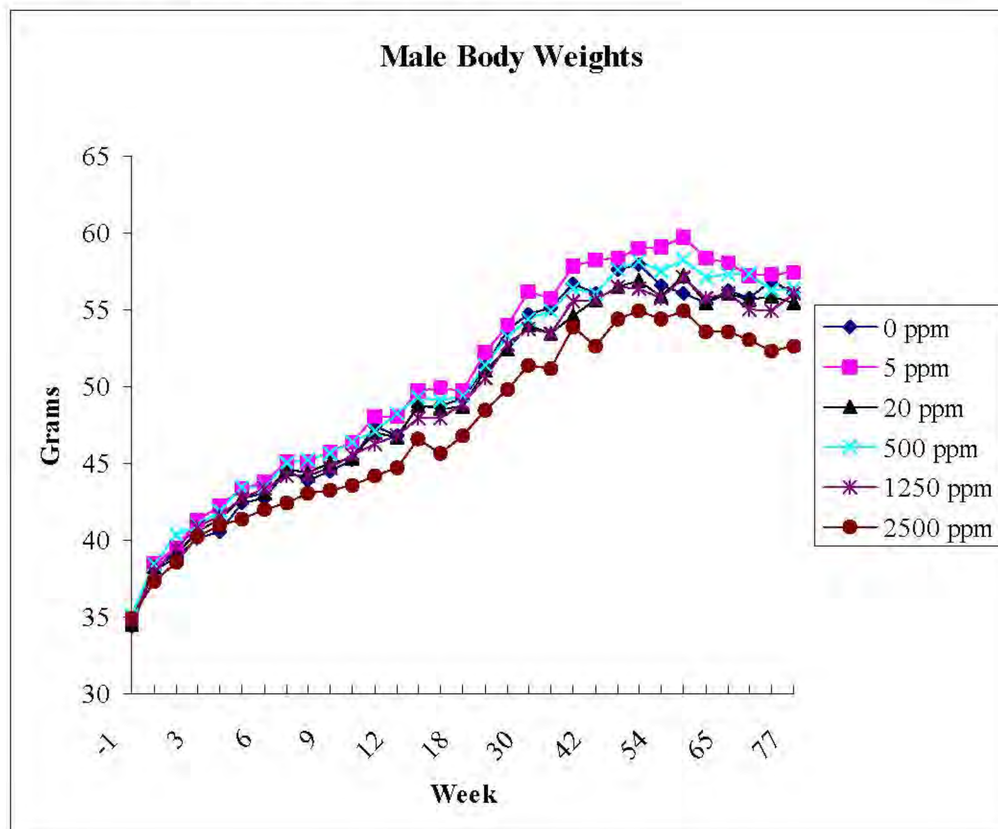
Survival incidence and the incidences of single/multiple palpable masses were not affected by treatment in either sex. Clinical signs were restricted to a slightly increased incidence of abdominal distension in females at 1250ppm and in males at 1250 and 2500ppm (Table 1). This finding correlated histologically, in most cases, with benign or malignant liver tumours. There was a slightly increased incidence of hypoactivity in females at 2500ppm, which correlated with perimortal findings in early deaths. At 2500ppm, body weight gain in males was retarded from week

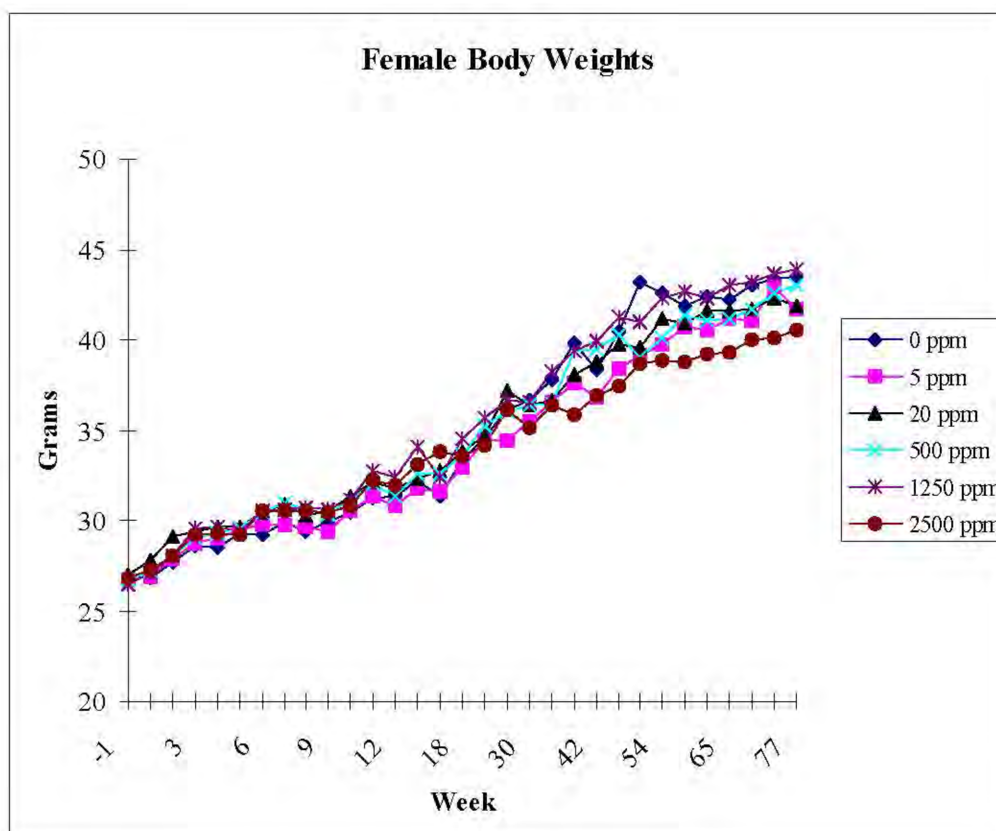
7 and in females from week 38, resulting in an overall depression of weight gains of 18 and 14%, respectively (Fig. 1). Food consumption was unaffected by treatment in both sexes at all dose levels.

Table 1: Incidence of treatment-related clinical observations

	Males						Females					
Dose level [ppm]	**0**	**5**	**20**	**500**	**1250**	**2500**	**0**	**5**	**20**	**500**	**1250**	**2500**
Number of animals examined	70	60	60	60	60	70	70	60	60	60	60	70
Abdomen distended n	2	0	1	1	5	6	3	4	3	1	8	2
first observed (week)	56	-	50	73	41	78	42	60	60	57	25	60
Hypoactivity n	3	1	1	2	2	1	1	1	2	2	1	5
first observed (week)	69	75	77	77	41	79	75	7	59	38	77	38

Figure 1: Mean body weight development





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 2500ppm at 53 and 78 weeks but since RBC, Hb and Hct values were unaffected by treatment, the differences were considered to have no toxicological significance. Occasional statistically significant differences from the controls occurred but are considered not to be toxicologically significant because there was no dose-relationship and/or the changes were too small to be of biological relevance. Lymphatic leukaemia occurred in two control females, one female at 5ppm, one female and two males at 500ppm, and one female at 1250ppm. Females with leukaemia contributed to higher mean WBC counts and unstained cell counts in control and 500ppm 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 2500ppm. At week 79, mean carcass weight was reduced in males at 2500ppm. Liver weights were increased dose-dependently in both sexes at 500, 1250 and 2500ppm (Table 2). Other significant differences at 2500ppm 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 weights).

Table 2: Treatment-related organ weight changes at 35 and 79 weeks

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

* $p \leq 0.01$ (LePage); +/- = $p \leq 0.01$ (Jonckheere); [a] % body weight x 10

There were no treatment-related gross lesions at necropsy in week 35. Macroscopic examination at week 79 revealed an increased incidence of masses and nodules in the liver at doses ≥500ppm, particularly in male groups. Thickening of the stomach in three males at 2500ppm, a decreased incidence of enlarged seminal vesicles at 1250 and 2500ppm, and a decreased incidence of enlarged spleen in females at 2500ppm are considered to be treatment-related since histological correlates were noted. A slight increase in the incidence of uterine masses at 2500ppm is not supported by microscopic findings and this is 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 2500ppm (Table 3). The severity ranged from minimal to moderate. At week 79, treatment-related, non-neoplastic lesions in the liver occurred at ≥500ppm, 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 hemosiderin), and hyperplasia of Kupffer cells. The lesions ranged in severity from minimal to marked and their incidences generally showed a positive dose-relationship.