Table 3: Incidence of non-neoplastic lesions in liver at 35 and 79 weeks

	Males						Females					
Dose level [ppm]	**0**	**5**	**20**	**500**	**1250**	**2500**	**0**	**5**	**20**	**500**	**1250**	**2500**
Week 35 - number examined	10	0	0	0	0	10	10	0	0	0	0	10
Inflammatory cell infiltration	1	-	-	-	-	8	3	-	-	-	-	8
single cell necrosis	0	-	-	-	-	10	0	-	-	-	-	9
Kupffer cell pigmentation	0	-	-	-	-	8	0	-	-	-	-	4
hepatocyte hypertrophy	0	-	-	-	-	9	0	-	-	-	-	10
Week 79 - number examined	50	50	50	50	50	50	50	50	50	50	50	50
deposition of pigment	2	2	3	13	33	44	6	5	3	5	14	30
focus of alteration	7	4	4	11	22	32	2	2	2	2	14	37
Hepatocellular hypertrophy	8	11	6	41	40	45	3	2	3	19	39	45
increased mitotic activity	-	-	-	1	10	8	1	1	-	4	5	4
Inflammatory cell infiltration	13	9	13	33	41	43	18	20	20	24	33	45
Kupffer cell hyperplasia	-	-	1	-	-	10	2	1	-	-	1	2
single cell necrosis	5	3	5	40	40	46	3	2	5	18	36	46

Treatment-related, non-neoplastic effects in tissues other than liver were increased incidences and severity of splenic extramedullary hematopoiesis and gastric mucosal epithelial hyperplasia in both sexes at 2500ppm (Table 4). The latter lesion was frequently accompanied by mild inflammatory cell infiltration, and in males, could be correlated with grossly observable thickening of the stomach. The incidences of some degenerative and inflammatory lesions were decreased at 2500ppm and, occasionally at 500 and 1250 ppm. Some effects were reduced in incidence; adrenal cortical hyperplasia, cataract formation in the lens, chronic nephropathy in males, pancreatic islet cell hyperplasia in males, lymphocytic infiltration of the salivary gland in females, chronic inflammation and dilatation of the seminal vesicles (correlated with reduced incidence of enlarged seminal vesicles at necropsy), splenic white pulp hyperplasia (correlated in females with decreased incidence of enlarged spleen at necropsy), and testicular tubular atrophy. Particularly in males, reduced incidences are considered to be associated with depressed body weight gain and are not regarded as adverse effects.

Table 4: Incidence of non-neoplastic lesions at 79 weeks (excluding liver)

		Males						Females					
Dose Level [ppm]		**0**	**5**	**20**	**500**	**1250**	**2500**	**0**	**5**	**20**	**500**	**1250**	**2500**
Adrenal glands	**No. exam.**	49	50	50	50	50	50	50	50	50	50	50	50
cortical hyperplasia		20	15	11	19	11	7	-	1	-	-	-	1
Eye lenses	**No. exam.**	50	50	50	50	50	50	49	50	50	49	50	50
Cataract		9	9	-	3	-	4	9	9	15	4	5	3
Glandular stomach	**No. exam.**	50	50	50	50	49	50	50	50	50	50	50	50
epithelial hyperplasia		10	14	8	14	13	24	7	8	5	7	6	20
Kidneys	**No. exam.**	50	50	50	50	50	50	50	50	50	50	50	50
chronic nephropathy		26	34	30	26	19	11	13	9	13	16	14	16
Pancreas	**No. exam.**	50	50	50	50	50	50	50	50	49	50	50	50
islet cell hyperplasia		9	5	10	8	6	-	-	-	-	-	-	-
Salivary glands	**No. exam.**	50	50	50	50	50	50	50	50	50	50	50	50
lymphocytic infiltration		4	5	4	4	3	2	13	11	13	5	6	5
Seminal vesicles	**No. exam.**	50	50	50	50	50	50	-	-	-	-	-	-
chronic inflammation		21	28	23	24	16	14	-	-	-	-	-	-
dilatation		29	34	24	31	22	19	-	-	-	-	-	-
Spleen	**No. exam.**	50	50	50	50	49	50	50	50	50	50	50	50
extramedullary hematopoiesis		18	17	23	27	23	36	26	28	25	24	27	34
white pulp hyperplasia		14	10	8	17	7	5	14	10	10	12	16	8
Testes	**No. exam.**	50	50	50	50	50	50	-	-	-	-	-	-
tubular atrophy		18	22	18	14	13	12	-	-	-	-	-	-

At 79 weeks, the incidence of hepatocellular adenoma was significantly greater than concurrent and historical control levels in both sexes at ≥500ppm. The incidence of hepatocellular adenocarcinoma was also significantly greater than concurrent and historical control levels in females at 1250 ppm and in both sexes at 2500 ppm (Table 5). The only other treatment-related neoplastic alterations were decreased incidences of systemic malignant lymphomas in females (consistent with significantly lower spleen weights) and harderian gland adenomas in males, neither of which are regarded as adverse effects (Table 6).

Table 5: Incidence of neoplastic lesions in liver at 79 weeks

Dose Level [ppm]	Malignant or benign	Males						Females					
		0	5	20	500	1250	2500	0	5	20	500	1250	2500
Liver - no. examined		50	50	50	50	50	50	50	50	50	50	50	50
hepatocellular adenocarcinoma	m	3	3	2	4	4	16	-	-	-	-	2	3
1st hepatocellular adenoma	b	9	5	8	17	21	39	-	-	-	5	8	28
2nd hepatocellular adenoma	b	3	2	3	3	12	31	-	-	-	-	5	14
multiple hepatocellular adenomas	b	-	1	-	-	6	14	-	-	-	-	2	7
metastatic carcinoma	m	-	-	-	-	1	-	1	-	-	-	1	-
hemangioma	b	-	1	1	-	-	-	2	1	-	-	-	-
1st hemangiosarcoma	m	1	-	-	-	1	-	1	-	-	-	1	-
2nd hemangiosarcoma	m	1	-	-	-	1	-	-	-	-	-	-	-
hepatoblastoma	m	-	-	-	-	1	-	-	-	-	-	-	-
metastatic osteosarcoma	m	-	-	-	-	-	-	-	-	1	-	-	-
any hepatic neoplastic lesion		**11**	**7**	**10**	**19**	**22**	**44**	**-**	**-**	**-**	**5**	**9**	**29**

Table 6: Incidence of neoplastic lesions at 79 weeks (excluding liver)

Dose Level [ppm]	Malignant or benign	Males						Females					
		0	5	20	500	1250	2500	0	5	20	500	1250	2500
Abdominal cavity - no. examined		4	2	4	0	5	0	3	5	4	4	4	3
fibroma	b	-	-	-	-	1	-	-	-	-	-	-	-
sarcoma nos	m	-	-	1	-	-	-	-	-	-	-	-	-
Adrenal glands- no. examined		49	50	50	50	50	50	50	50	50	50	50	50
cortical adenoma	b	-	1	2	-	-	-	-	-	-	-	-	-
subscapular adenoma	b	-	2	-	-	1	-	-	-	-	-	-	-
Adrenal medullas- no. examined		49	50	50	50	50	50	50	50	50	50	50	50
malignant medullary tumour	m	-	1	-	-	-	-	-	-	-	-	-	-
Brain - no. examined		50	50	50	50	50	50	50	50	50	50	50	50
metastatic carcinoma	m	-	-	-	-	-	-	-	-	-	-	-	1
Forestomach- no. examined		50	50	50	50	49	50	50	50	50	50	50	50
squamous carcinoma	m	-	-	-	-	1	-	-	-	-	-	-	-
Harderian glands- no. examined		50	50	50	50	50	50	50	50	50	50	50	50
adenocarcinoma	m	-	-	-	-	3	-	-	-	-	-	-	-
adenoma	b	8	12	8	8	7	5	1	1	2	1	1	1
Large intestine - no. examined		50	50	50	50	49	50	50	50	50	50	50	50
adenocarcinoma	m	4	-	-	1	-	-	1	1	2	-	-	-
Lung - no. examined		50	50	50	50	50	50	50	50	50	50	50	50
1st bronchial-alveolar adenoma	b	5	7	9	6	8	9	3	5	4	1	3	5
2nd bronchial-alveolar adenoma	b	1	-	-	-	1	2	-	-	-	-	-	-
bronchial-alveolar carcinoma	m	5	2	3	6	3	4	3	2	3	2	2	1
metastatic carcinoma	m	-	-	-	-	-	2	-	-	-	-	-	-
metastatic fibrosarcoma	m	-	-	-	-	-	-	-	-	1	-	-	-
Mammary gland - no. examined		0	0	0	0	0	0	50	50	50	50	50	50
malignant adenoacanthoma	m	-	-	-	-	-	-	-	-	-	2	-	1
adenocarcinoma	m	-	-	-	-	-	-	-	1	-	2	1	-
Ovaries - no. examined		0	0	0	0	0	0	50	50	49	50	50	49
fibroma	b	-	-	-	-	-	-	-	-	-	1	1	-
Pancreas - no. examined		50	50	50	50	50	50	50	49	50	50	50	50
acinar adenocarcinoma	m	-	-	1	-	-	-	-	-	-	-	-	-
ductal adenocarcinoma	m	-	-	-	-	-	-	1	-	-	-	1	-
benign islet cell tumour	b	-	-	-	-	-	-	-	-	-	-	-	1
Parathyroid gland - no. examined		0	0	0	0	0	0	42	38	28	40	36	37
adenoma	b	-	-	-	-	-	-	-	1	-	-	-	-

- zero incidence

Table 6: Continued

	Malignant or benign	Males						Females					
Dose Level [ppm]		**0**	**5**	**20**	**500**	**1250**	**2500**	**0**	**5**	**20**	**500**	**1250**	**2500**
Peripheral nerve - no. examined		0	0	0	0	0	0	50	50	50	50	50	50
benign Schwannoma	b	-	-	-	-	-	-	-	-	-	-	-	1
Pituitary gland - no. examined		48	50	50	48	50	50	50	50	50	50	49	50
adenocarcinoma pars distalis	m	-	-	-	-	-	-	-	-	-	-	-	1
adenoma pars distalis	b	1	1	-	-	-	-	7	8	7	6	9	7
Prostate gland - no. examined		50	50	50	50	50	50	0	0	0	0	0	0
adenocarcinoma	m	-	-	1	-	-	-	-	-	-	-	-	-
Seminal vesicles - no. examined		50	50	50	50	50	50	0	0	0	0	0	0
leiomyosarcoma	m	-	-	-	1	-	-	-	-	-	-	-	-
Skeletal muscle - no. examined		50	50	50	50	50	50	50	50	50	50	50	50
rhabdomyosacrcoma	m	1	-	-	-	-	-	-	-	-	-	-	-
Skin/subcutis - no. examined		50	50	50	50	50	50	50	50	50	50	50	50
metastatic carcinoma	m	-	-	-	-	-	1	-	-	-	-	-	-
squamous carcinoma	m	-	-	1	-	-	-	-	-	-	-	-	-
fibrosarcoma	m	1	-	-	-	-	-	-	-	1	1	-	1
hemangioma	b	-	-	1	-	-	-	-	-	-	-	-	-
benign fibrous histiocytoma	b	-	-	-	-	1	-	-	-	-	-	-	-
benign Schwannoma	b	-	-	-	-	1	-	-	-	-	-	-	-
Systemic neoplasias - no. exam[d]		50	50	50	50	50	50	50	50	50	50	50	50
myeloid leukaemia	m	-	-	-	-	-	-	-	-	1	-	-	-
malignant lymphoma	m	4	3	6	1	5	6	11	9	8	9	8	4
hystiocytic sarcoma	m	-	-	-	-	-	-	-	-	-	-	-	1
Urinary bladder - no. examined		50	50	50	50	50	50	50	48	50	49	50	49
hemangioma	b	-	-	-	1	-	-	-	-	-	-	-	-
Uterus - no. examined		0	0	0	0	0	0	50	50	50	50	50	50
adenocarcinoma	m	-	-	-	-	-	-	-	-	1	-	-	1
hemangioma	b	-	-	-	-	-	-	1	-	-	-	-	-
leiomyoma	b	-	-	-	-	-	-	1	1	2	-	1	2
leiomyosarcoma	m	-	-	-	-	-	-	1	-	-	-	-	-
Vagina - no. examined		0	0	0	0	0	0	50	50	50	50	50	50
leiomyoma	b	-	-	-	-	-	-	1	-	1	-	-	1
Total no. with one neoplasm		22	13	23	18	20	9	17	25	16	22	25	17
Total no. with multiple neoplasms		11	12	10	14	18	36	8	2	7	4	7	22
Total no. tumour-bearers		33	25	33	32	38	45	25	27	23	26	32	39
Total no. with liver neoplasm(s)		11	7	10	19	22	44	0	0	0	5	9	29
Total no. with one or more neoplasms excluding liver		22	18	23	13	16	1	25	27	23	21	23	10

- zero incidence

Conclusion: The occurrence of increased liver weight and increased incidences of non-neoplastic alterations in the liver, hypertrophy, pigment deposition, mitotic activity, Kupffer cell hyperplasia and single cell necrosis, indicate the MTD was reached/exceeded at dose levels of ≥500ppm.

No-observed-effect-level (toxicity): 20ppm, equivalent to mean dose levels of 2.63 and 3.68mg/kg bw/day in males and females, respectively, based on increased liver weight and non-neoplastic hepatic pathology at dose levels of ≥ 500ppm.

No-observed-effect-level (carcinogenicity): 20ppm, equivalent to mean dose levels of 2.63 and 3.68mg/kg bw/day in males and females, respectively, based on an increased incidence of hepatic tumours at dose levels of ≥500ppm.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]

[redacted]

Conclusion

Reliability

Acceptability

Remarks

98/8 Doc IIIA section No.	**6.7 / 02** **Carcinogenicity study**
91/414 Annex II Point addressed	**5.5.2 / 01** Long-term Toxicity and Carcinogenicity

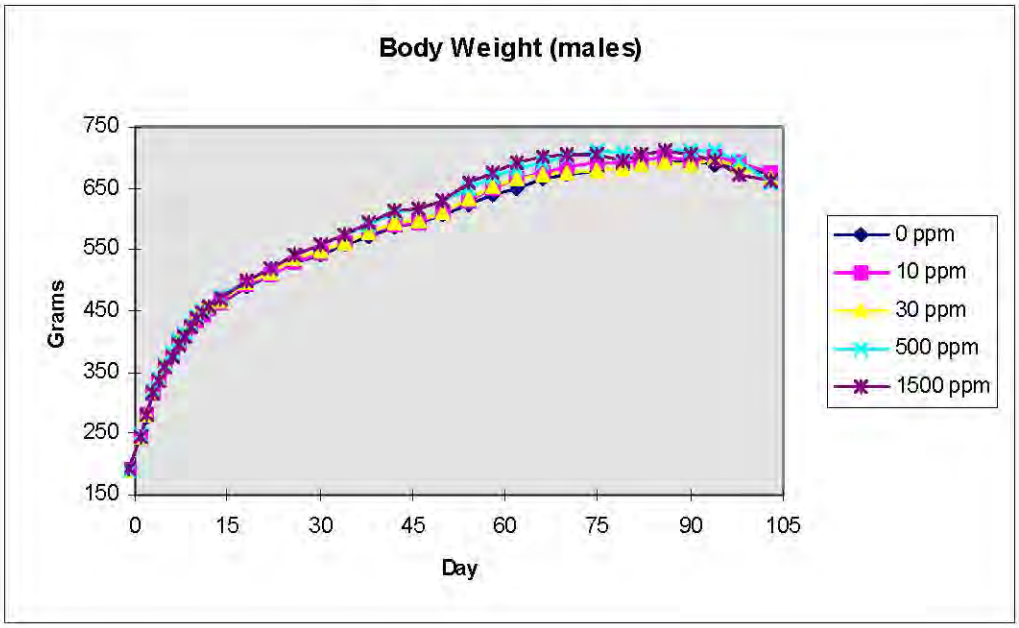
1. Annex point(s)	**IIA, 5.5.2 Long term toxicity and carcinogenicity - rats**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.5.2/01
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - 24-Month carcinogenicity and chronic toxicity study in rats. Syngenta Crop Protection AG, unpublished report No. 942110, July 27, 1998.
4. Testing facility	[redacted]
5. Dates of work	August 07, 1995 - August 21, 1997
6. Test substance	ISO common name: Thiamethoxam, [redacted]
7. Test method	OECD 453 ≡ FIFRA § 83-5 ≡ JMAFF Deviations - none
8. GLP	Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

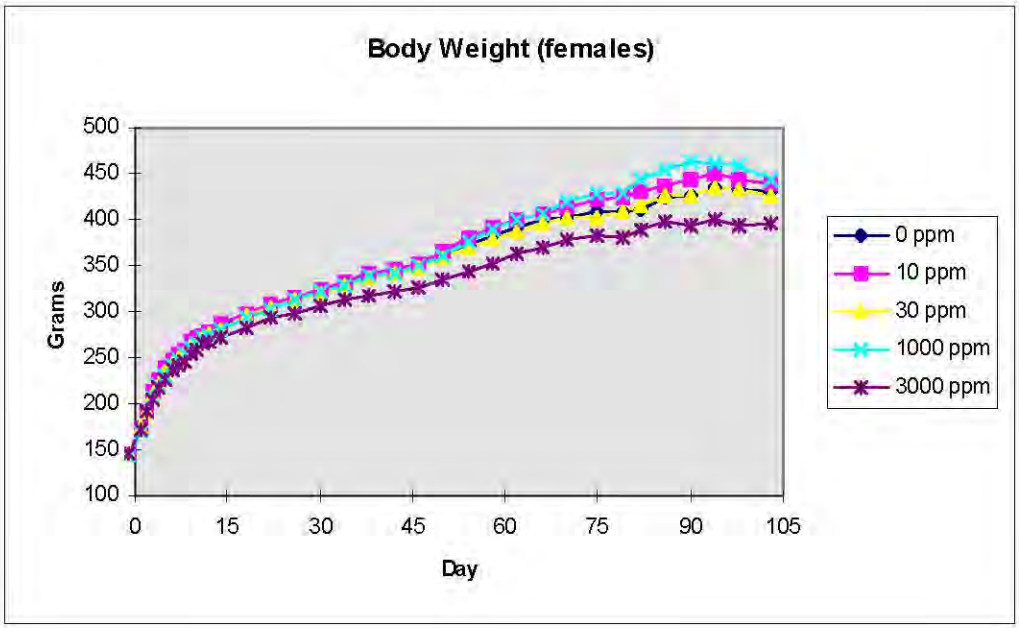
Material and methods: Groups of 70 male and 70 female Sprague-Dawley rats (strain Tif: RAIf, SPF, body weight range 114.2 - 224.7g, source [redacted]) were administered thiamethoxam (batch no. [redacted] orally for 104 weeks, by admixture in the diet, at concentrations of 0, 10, 30, 500 or 1500ppm (males) and 0, 10, 30, 1000 or 3000ppm (females). Twenty animals/sex/group were designated for clinical laboratory investigations and 50 animals/sex/group for the oncogenicity study. Additional groups of 10 animals/sex/group were similarly treated with thiamethoxam for 52 weeks and sacrificed for interim evaluation. Clinical observations were made daily, body weights and food consumption recorded weekly for 13 weeks and monthly thereafter, and water consumption recorded monthly. The eyes of all animals in the carcinogenicity subgroup were examined pre-test and at 104 weeks. Control and high-dose animals were also examined at weeks 26, 52 and 77. Laboratory investigations were performed at weeks 13, 27, 53, 78 and pre-terminally on 20 animals/sex/group for haematology and on 10 animals/sex/group for clinical chemistry and urinalysis. All animals were subjected to detailed necropsy and *post mortem* examination. Organ weights of all animals which survived until terminal sacrifice were recorded. Tissue/organ samples from all animals sacrificed at 52 weeks, those which died or were killed during the study and all animals killed at the end of the study, were preserved. Microscopic examination of tissues was performed on all animals, of all treatment and control groups, with the exception of the 20 animals/sex/group designated for laboratory investigations. In life and organ weight data were statistically analysed by a univariate technique, using non-parametric methods where appropriate. Survival analysis was by Cox's regression model. Neoplastic lesions were analysed using Peto's mortality prevalence test, and non-neoplastic lesions by the Cochran-Armitage linear trend test.

Findings: Diet analyses demonstrated thiamethoxam to be stable in feed for at least 7 weeks at room temperature, whereas fresh diets were prepared monthly. Analysis of diet samples 13 times demonstrated a homogeneous distribution of thiamethoxam and achieved concentrations in the range 89.3 - 107.6% nominal during the 4 week use period. The overall mean analytically determined concentrations were 10, 31, 507, 1011, 1502 and 3033ppm in order of increasing nominal concentration. Mean dose levels, based on analytically determined concentrations, were calculated to be 0.41, 1.29, 21.0 and 63.0mg/kg bw/day (males) and 0.48, 1.56, 50.3 and 155mg/kg bw/day (females).

Survival incidence was not affected by treatment, there were no clinical signs of an adverse effect of treatment and the incidences of single and multiple palpable masses were unaffected by treatment at all dose levels. Body weight development in all male groups was unaffected by treatment, but females at 3000ppm had slightly depressed weight gain from week 3 until termination, at which time cumulative weight gain was depressed by 12.6% (Figure 1). Food consumption was unaffected by treatment in both sexes at all dose levels, but water consumption of males at 1500ppm was raised by 13%. No effect on water consumption occurred in other groups. Ophthalmological examinations revealed no evidence of ocular toxicity at any time point.

Figure 1: Body weight development





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

There were no treatment-related effects on organ weights at any dose level, at either 52 or 104 weeks. (Table 1). At 104 weeks, mean carcass weight in high dose males and females was reduced by 4 and 8%, respectively. There were no significant differences from control values in absolute and relative organ weights among the males, with the exception of a minor negative trend in the relative thyroid weight of males at 1500ppm. The trend was considered not to be of toxicological significance because the mean value was similar to historical control values and no related histological changes were evident. Mean absolute adrenal weights appeared elevated in males at 10 and 1500ppm, but this difference was no longer apparent when animals with extremely high values were not included. Female mean relative thyroid weights were higher than control values in all treated groups, but remained within the historical control range. In the absence of corroborative clinical chemistry and/or histological changes, the differences were judged to be without toxicological significance. The differences in adrenal weights between control and 10 and 1000ppm females did not persist when animals with extreme values were not included.

Table 1: Organ weights

Organ	Males					Females				
		Week 53		Week 105			Week 53		Week 105	
	Dose [ppm]	Absolute weight [g]	Relative weight [% of bw]	Absolute weight [g]	Relative weight [% of bw]	Dose [ppm]	Absolute weight [g]	Relative weight [% of bw]	Absolute weight [g]	Relative weight [% of bw]
Body weight (g)	0	589.7		630.3		0	345.9		402.7	
	10	572.8		629.4		10	318.3		410.8	
	30	553.1		619.5		30	353.4		398.5	
	500	623.4		617.7		1000	323.0		411.2	
	1500	554.2		606.7		3000	324.6		370.7	
Liver (g)	0	21.23	35.89	21.98	35.02	0	11.69	33.78	15.55	38.75
	10	18.16	31.25	22.27	35.53	10	10.54	33.25	15.74	38.46
	30	18.28	33.08	22.04	36.06	30	11.67	33.09	15.58	39.31
	500	20.62	33.10	22.74	37.61	1000	10.63	32.95	16.24	39.85
	1500	19.72	35.63	22.80	37.88	3000	11.25	34.66	15.38	41.72+
Adrenals (mg)	0	70.17	0.12	112.7	0.18	0	69.65	0.20	87.3	0.22
	10	65.19	0.12	322.7	0.56	10	73.84	0.23	107.0	0.27*+
	30	66.53	0.12	105.1	0.18	30	73.99	0.21	95.8	0.25
	500	70.27	0.11	169.1	0.32	1000	73.33	0.23	105.6*+	0.26*+
	1500	73.71	0.14	251.8	0.48	3000	75.44	0.24	88.5	0.25*
Thyroids (mg)	0	30.20	0.05	111.3	0.17	0	37.30	0.11	45.59	0.11
	10	32.34	0.06	73.8	0.12	10	33.99	0.11	49.50+	0.12
	30	33.17	0.06	63.4	0.10	30	35.97	0.10	48.67	0.12+
	500	30.67	0.05	69.4	0.12	1000	33.32	0.11	60.80*+	0.15*+
	1500	34.22	0.06	61.7	0.10+	3000	36.32	0.11	49.82	0.14*+

* $p \leq 0.01$, Le Page test; +/- $p \leq 0.01$ (Jonckheere)

Post mortem examination of animals sacrificed after 52 and 104 weeks, and animals that died spontaneously between 52 and 104 weeks, revealed no treatment-related lesions. Most gross lesions occurred at comparable frequencies in the control and treated groups. Morphological correlates of gross lesions showing inter-group differences in incidence did not indicate an effect of treatment. The nature of the lesions was similar to those occurring spontaneously in this strain of rat.

52 week sacrifice: No treatment-related neoplastic lesions occurred in animals sacrificed at 52 weeks or that died during the first 52 weeks. Only 3 malignant and 7 benign neoplasias were identified. All were of the type that spontaneously occur in this strain of rat, and the distribution between the control and treated groups did not indicate

an effect of treatment (Table 2). Non-neoplastic, treatment-related lesions at 52 weeks were increased incidences of renal tubular regenerative changes, chronic tubular lesion and tubular basophilic proliferation, in males at 500 and 1500ppm. Minimally increased incidences of renal tubular and pelvic lymphocytic infiltration also occurred at 1500ppm, but without tubular hyaline change. These alterations are considered to represent the sequelae of alpha-2µ -globulin mediated nephropathy. The kidneys of females at all dose levels were unaffected. There was a minimal increase in the severity of splenic haemosiderosis in females at 3000ppm (Table 3). In the liver, there were increased incidences of cholangiofibrosis in males at 10ppm (5/10), 500ppm (6/10) and 1500ppm (4/10) compared to the controls (2/10), and increased incidence of inflammatory cell infiltration in males at 10ppm (4/10), 30ppm (3/10) and 500ppm (5/10) relative to controls (1/10). These findings, which were not apparent after 104 weeks treatment, are considered incidental to treatment because of the lack of a dose relationship. Other non-neoplastic lesions occurred in animals that died during the first year or that were sacrificed at 52 weeks, but all commonly occur in this strain of rat, and the incidence, distribution, and/or morphology did not indicate an effect of treatment.

Table 2: Incidence of neoplastic lesions up to 52 weeks

	Males					**Females**				
Dose level (ppm)	**0**	**10**	**30**	**500**	**1500**	**0**	**10**	**30**	**1000**	**3000**
No. examined	10	10	10	10	10	10	10	10	10	10
lymphoma (m)		1								
oligodendroglioma (m)		1								
mammary adenocarcinoma (m)									1	
subcutaneous fibroma (b)	1									
mammary fibroadenoma (b)				1						
pituitary adenoma (b)					1	1			1	
prostate adenoma (b)					1					
lymph node hemangioma (b)					1					

b = benign, m = malignant

Table 3: Incidence of treatment-related non-neoplastic lesions at 52 weeks

		Males					Females				
Dose Level [ppm]		**0**	**10**	**30**	**500**	**1500**	**0**	**10**	**30**	**1000**	**3000**
5.5.1.1.1.2 KI DNEYS	No. exam.	10	10	10	10	10	10	10	10	10	10
chronic tubular lesion		2	2	2	4	6	1	1	1	2	-
tubular basophilic proliferation		-	1	-	2	1	-	-	2	-	-
total regenerative tubular lesions		**2**	**3**	**2**	**6**	**7**	**1**	**1**	**3**	**2**	-
lymphocytic infiltration		1	1	-	2	3	-	2	-	-	-
Renal Pelves	No. exam.	10	10	10	10	10	10	10	10	10	10
lymphocytic infiltration		1	-	-	1	3	-	1	-	-	-
Spleen	No. exam.	10	10	10	10	10	10	10	10	10	10
hemosiderosis		9	8	9	9	8	10	10	10	9	10
average grade		2.3	2.4	2.7	2.2	2.5	2.4	2.5	2.5	2.3	3.0

104 week sacrifice: Treatment-related, non-neoplastic changes in the kidneys and liver occurred in animals sacrificed at 104 weeks or that died between weeks 53 and 104. In the kidneys, a slightly increased incidence of slight/moderate chronic nephropathy occurred in males at 1500ppm (Table 4) accompanied by a minimal increase in incidence of lymphocytic infiltration of the renal cortex at 1500ppm. Two animals also showed tubular hyaline change. In the liver, a treatment-related increase occurred in the incidence of slight/moderate focal cellular alteration, generally of the clear cell subtype, in females at 3000ppm. All other non-neoplastic findings in the liver and kidneys were of a similar nature, severity and incidence in treated and control groups. In other tissues, some non-neoplastic lesions occurred at slightly higher incidences in treated groups than in controls, but in all instances these are considered unrelated to treatment with thiamethoxam since their occurrence is not dose-related, or the incidence is within the historical control range or known to occur spontaneously in aged rats of this strain.

Table 4: Non-neoplastic, treatment-related lesions at 104 weeks

		Males					Females				
Dose Level [ppm]		**0**	**10**	**30**	**500**	**1500**	**0**	**10**	**30**	**1000**	**3000**
Kidneys	No. exam.	50	50	49	50	50	50	50	49	50	50
lymphocytic infiltr.		10	10	7	14	17	2	3	4	2	2
average grade		1.6	1.7	1.4	1.9	1.7	1.5	2.0	1.5	1.5	2.0
chronic nephropathy		30	35	32	37	42	12	10	8	6	10
average grade		2.4	2.6	2.4	2.5	2.7	2.8	2.9	2.0	2.2	2.5
tubular hyaline change		0	1	1	0	2	2	1	0	0	0
Liver	No. exam.	50	50	49	50	50	50	49	49	50	50
focus of cellular alteration		20	21	15	21	20	10	21	12	15	26
average grade		2.2	2.3	1.6	2.0	2.0	1.9	1.6	1.5	1.4	2.1
subtype:											
amphophilic		3	3	1	5	1	3	2	1	1	3
basophilic		1	2	-	2	-	4	6	5	5	6
clear cell		13	10	11	11	14	3	5	2	5	15
eosinophilic		3	5	2	3	5	-	8	4	4	2
mixed		-	1	1	-	-	-	-	-	-	-

- zero incidence

All neoplastic findings, both malignant and benign, occurring at 104 weeks (Table 5) are considered incidental to treatment with thiamethoxam since the incidences in treated and control groups are similar, or the inter-group distribution shows no relationship to dose level, or the incidences are within historical control ranges and the lesions are known to occur spontaneously in aged rats.

Table 5: Incidence of neoplastic lesions at 104 weeks

		Males					Females				
Dose Level [ppm]	**m/b**	**0**	**10**	**30**	**500**	**1500**	**0**	**10**	**30**	**1000**	**3000**
Abdominal cavity - no. examin.		50	50	50	50	50	50	50	49	50	50
fibroma	b	-	-	-	-	1	-	-	-	-	-
leiomyosarcoma	m	-	-	-	-	-	-	-	-	-	1
malignant mesothelioma	m	1	-	-	-	-	-	-	-	-	-
sarcoma nos	m	-	-	-	-	1	-	-	-	-	-
metastatic carcinoma	m	-	-	-	-	-	1	-	-	-	-
Adrenal glands - no. examined		50	50	49	50	50	50	49	49	50	50
cortical adenocarcinoma	m	-	-	1	-	1	-	-	-	-	-
1st cortical adenoma	b	6	9	4	10	4	1	2	3	3	-
2nd cortical adenoma	b	-	-	-	2	-	-	-	-	-	-
myelolioma		1	-	-	-	-	-	-	-	-	-
metastatic mesothelioma	m	1	-	-	-	-	-	-	-	-	-
benign medullary tumour	b	1	3	2	1	4	-	1	-	-	-
malignant medullary tumour	m	-	5	1	1	1	-	-	-	-	-
Blood vessels - no. examined		0	0	1	0	0	0	0	1	0	0
metastatic carcinoma	m	-	-	1	-	-	-	-	1	-	-
Bone - no. examined		50	50	50	50	50	50	50	49	50	50
osteosarcoma	m	-	-	-	-	-	-	-	-	-	1
Brain - no. examined		50	50	50	50	50	50	50	49	50	50
malignant astrocytoma	m	-	-	-	1	2	-	-	1	-	-
benign granular tumour	b	2	2	-	1	3	1	1	2	-	2
metastatic carcinoma	m	-	-	-	-	-	-	-	1	-	-
Cartilage - no. examined		50	50	50	50	50					
chondrosarcoma	m	-	-	-	-	1	-	-	-	-	-
Ears - no. examined		1	1	1	1	2	2	0	1	1	0
localized infil. carcinoma	m	-	1	-	-	-	-	-	-	1	-
Epididymides - no. examined		50	50	50	50	50	-	-	-	-	-
metastatic mesothelioma	m	1	-	-	-	-	-	-	-	-	-
Oesophagus - no. examined		50	50	50	50	48	50	50	49	50	50
squamous cell carcinoma	m	-	-	-	-	-	-	-	1	-	-
Eyes - no. examined		50	50	50	50	50	50	49	49	50	50
benign Schwannoma	b	-	-	-	-	-	-	-	-	-	1
Harderian glands - no. examined		50	50	50	50	50	50	49	49	50	50
metastatic carcinoma	m	-	-	-	-	-	-	-	1	-	-
Heart - no. examined		50	50	50	49	50	50	48	49	50	50
benign endo. Schwannoma	b	1	-	-	1	-	1	-	-	-	1
metastatic carcinoma	m	-	-	-	-	-	1	-	-	-	-
Kidneys - no. examined		50	50	49	50	50	50	50	49	50	50
carcinoma	m	-	-	-	-	1	-	-	-	-	-
Large intestine - no. examined		50	49	49	49	50	50	50	48	50	49
adenocarcinoma	m	-	-	-	-	-	1	-	-	-	-
lipoma	b	1	-	-	-	-	-	-	-	-	-
Liver - no. examined		50	50	49	50	50	50	49	49	50	50

hepatocellular adenoma	b	-	1	-	1	1	2	-	1	2	2
hepatocellular adenocarcinoma	m	-	-	1	1	-	-	-	1	-	-
hepatocellular neoplasia		-	1	1	1	1	2	-	2	2	2
metastatic carcinoma	m	-	-	-	-	-	1	-	-	-	-
Lung - no. examined		50	50	50	50	50	50	48	49	50	50
bronchial-alveolar adenoma	b	-	1	-	-	1	-	-	-	-	-
bronchial-alveolar carcinoma	m	-	-	1	-	-	-	-	-	-	-
squamous cell carcinoma	m	-	-	-	-	-	-	-	1	-	-
metastatic tumour	m	-	-	1	-	-	-	-	-	-	-
metastatic carcinoma	m	-	-	1	1	-	3	-	-	-	-
Lymph node - no. examined		2	2	2	5	6	1	1	1	1	1
hemangioma	b	-	-	-	-	-	1	-	-	-	-

Table 5: Continued

		Males					Females				
Dose Level [ppm]	**m/b**	**0**	**10**	**30**	**500**	**1500**	**0**	**10**	**30**	**1000**	**3000**
Mammary gland - no. examined		46	45	42	44	45	50	50	48	50	50
adenocarc. in fibroadenoma	m	-	-	-	-	-	-	1	1	-	-
adenocarcinoma	m	1	-	-	-	1	7	3	1	2	6
1st fibroadenoma	b	-	2	-	1	1	18	20	25	31	17
2nd fibroadenoma	b	-	-	-	-	-	8	8	5	6	4
multiple adenoma	b	-	-	-	-	-	3	3	1	2	3
Mesenteric lymph node - no. examined		50	50	49	50	50	49	50	49	50	50
hemangioma	b	-	-	2	-	1	-	-	1	-	-
hemangiosarcoma	m	-	1	1	-	1	-	-	1	-	-
metastatic carcinoma	m	-	1	-	-	-	1	-	-	-	-
Nasal cavities - no. examined		49	50	50	50	50	50	50	49	50	50
adenoma	b	-	-	-	-	-	-	1	-	1	-
infiltrating osteosarcoma	m	-	-	-	-	1	-	-	-	-	-
fibroadenoma	b	-	-	-	-	-	-	-	-	-	-
Oral cavity - no. examined		49	49	50	50	50	50	50	49	50	50
squamous carcinoma	m	-	-	-	-	-	-	1	-	-	-
Ovaries - no. examined		0	0	0	0	0	50	50	49	50	50
benign granulosa tumour	b	-	-	-	-	-	2	-	-	-	2
malignant granulosa tumour	m	-	-	-	-	-	-	-	-	-	1
benign sex cord tumour	b	-	-	-	-	-	-	1	-	-	1
metastatic carcinoma	m	-	-	-	-	-	1	-	-	-	-
Pancreas - no. examined		49	49	48	50	49	50	49	49	50	49
acinar adenocarcinoma	m	-	-	1	-	1	-	-	-	-	-

1st acinar adenoma	b	7	8	5	8	9	1	1	1	1	-
2nd acinar adenoma	b	1	-	3	1	2	-	-	-	-	-
multiple acinar adenoma	b	-	-	-	1	-	-	-	-	-	-
1st benign islet cell tumour	b	9	5	8	4	6	4	-	3	1	2
2nd benign islet cell tumour	b	-	-	1	-	-	-	-	-	-	-
multiple islet cell tumour	b	-	-	1	-	-	-	-	-	-	-
malignant islet cell tumour	m	1	4	1	3	1	1	1	1	1	-
metastatic carcinoma	m	-	-	-	-	-	1	-	-	-	-
Parathyroid gland - no. examined		47	48	49	47	49	49	47	47	49	43
adenoma	b	-	-	1	-	-	-	-	-	-	-
Pituitary gland - no. examined		50	50	49	49	50	49	50	49	49	50
adenoma pars distalis	b	14	14	17	15	19	16	18	20	16	20
benign pituicytoma	b	-	-	-	-	-	-	-	-	1	-
Preputial gland - no. examined		0	2	0	0	0	0	0	0	0	0
adenoma	b	-	1	-	-	-	-	-	-	-	-
Prostate gland - no. examined		50	49	49	50	50	0	0	0	0	0
adenoma	b	1	-	1	1	1	-	-	-	-	-
Salivary glands - no. examined		49	50	49	49	50	50	49	48	50	49
malignant Schwannoma	m	-	-	-	1	-	-	-	-	-	-
metastatic sarcoma	m	-	-	-	1	-	-	-	-	-	-
Seminal vesicles - no. examined		50	49	49	50	50	0	0	0	0	0
metastatic mesothelioma	m	1	-	-	-	-	-	-	-	-	-
Skeletal muscle - no. examined		50	50	50	50	50	50	50	49	50	50
rhabdomyoma	m	-	-	-	1	-	-	-	-	-	-

Table 5: Continued

		Males					Females				
Dose Level [ppm]	**m/b**	**0**	**10**	**30**	**500**	**1500**	**0**	**10**	**30**	**1000**	**3000**
Skin/subcutis - no. examined		50	50	50	50	50	50	50	49	50	50
basal cell carcinoma	m	-	-	-	-	1	-	-	-	-	1
squamous cell carcinoma	m	-	1	-	-	1	-	-	-	1	-
1st fibroma	b	6	6	7	4	5	1	4	1	4	2
2nd fibroma	b	-	-	-	1	-	-	1	-	1	-
fibrosarcoma	m	2	-	-	1	1	-	-	-	-	-
benign hair follicle tumour	b						-	1	-	-	-
hemangiosarcoma	m	2	-	-	1	-	-	-	-	-	-
benign fibrous histiocytoma	b	-	-	1	-	-	-	-	-	-	-
malignant fibr. histiosarcoma	m	-	2	2	3	2	-	-	-	-	-
keratoacanthoma	b	-	3	-	-	-	-	-	-	-	-
lipoma	b	-	1	-	1	3	-	-	-	-	-
squamous cell papilloma	b	-	2	-	1	-	-	-	-	-	-
sarcoma nos	m	1	-	-	2	1	-	-	-	-	-
benign Schwannoma	b	2	-	-	1	-	-	-	-	-	-
malignant Schwannoma	m	-	-	-	1	1	-	-	-	-	-
unclassified malignant tumour	m	-	-	1	-	-	-	-	-	-	-
Small intestine - no. examined		50	49	49	50	50	50	48	49	50	47
adenocarcinoma	m	-	-	1	-	-	-	-	-	-	-
leiomyosarcoma	m	-	-	-	-	-	-	-	-	-	1
Spleen - no. examined		50	50	49	50	50	50	49	49	50	49
hemangioma	b	-	1	-	1	-	-	-	-	-	-
hemangiosarcoma	m	1	-	-	1	1	-	-	-	-	-
Systemic neoplasias - no. examined		50	50	50	50	50	50	50	49	50	50
malignant fibrous histiocytoma	m	-	1	-	-	-	-	-	-	-	-
myeloid leukaemia	b	1	-	-	-	-	1	-	-	-	-
malignant lymphoma	m	1	2	1	-	-	1	1	1	1	-
hystiocytic sarcoma	m	-	-	-	-	1	-	-	-	-	-
Testes - no. examined		50	50	50	50	50	0	0	0	0	0
benign Leydig cell tumour	b	3	1	2	1	2	-	-	-	-	-
malignant Leydig cell tumour	m	-	-	-	-	1	-	-	-	-	-
Thoracic cavity - no. examined		50	50	50	50	50	50	50	49	50	50
malignant fibrous histiocytoma	m	-	-	-	1	-	-	-	-	-	-
metastatic carcinoma	m	-	-	-	-	-	1	-	1	-	-
Thymus - no. examined		49	48	48	48	49	48	45	45	48	48
benign thymoma	b	1	-	-	-	-	-	1	1	1	-
malignant thymoma	m	-	-	-	1	-	-	-	-	-	1
Thyroid gland - no. examined		49	50	49	48	49	50	49	49	50	49
follicular adenocarcinoma	m	-	-	-	-	-	-	1	-	-	1
follicular adenoma	b	1	-	1	1	-	-	1	-	3	-
1st benign c-cell tumour	b	9	5	5	8	7	6	9	4	1	4
2nd benign c-cell tumour	b	-	1	-	-	-	3	-	1	-	-

malignant c-cell tumour	m	1	2	1	2	-	2	1	-	-	-
metastatic carcinoma	m	-	-	-	-	-	1	-	-	-	-
Urinary bladder - no. examined		48	48	49	49	50	50	48	49	50	49
leiomyoma	b	-	-	-	-	-	-	-	1	-	-
Uterus - no. examined		0	0	0	0	0	50	50	49	50	50
hemangioma	b	-	-	-	-	-	1	1	-	1	1
Zymbal's glands - no. examined		50	48	49	49	50	50	49	47	50	49
squamous cell carcinoma	m	-	-	-	-	-	1	-	-	-	-
Total no. with one neoplasm[a]		17	16	18	19	19	17	12	19	22	17
Total no. with multiple neoplasms[a]		22	28	20	23	28	26	25	22	25	20
Total no. bearing-bearing animals		39	44	38	42	47	43	37	41	47	37

- zero incidence; [a] primary

Conclusion: Based on no excess tumours occurring at any dose level, there was no indication of a carcinogenic potential.

No-observed-effect-levels (toxicity): 30ppm (males) and 1000ppm (females), equivalent to mean dose levels of 1.3mg/kg bw/day (males) and 50.3mg/kg bw/day (females), based on increased incidence of renal chronic tubular lesions and basophilic proliferation in males at ≥500ppm, and foci of cellular alteration in the liver, and increased severity of splenic hemosiderosis, in females at 3000ppm. Since the renal alterations observed in males at 500 and 1500ppm are considered to be mediated by alpha-2μ-globulin, a mechanism specific to male rats, the observed renal toxicity has no relevance to human risk assessment. Therefore, it is concluded that a no-observed-adverse-effect-level (NOAEL) of >1500ppm, equivalent to a dose level of >63.0mg/kg bw/day, is applicable for males. Therefore, for both sexes, the overall NOAEL has to be set at 50.3mg/kg.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2005
Materials and Methods	[redacted]

Results and discussion

[redacted]

[redacted]

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

98/8 Doc IIIA section No.	**6.8.1 / 01**	**Teratogenicity test**
Annex	**II**	Developmental toxicity studies
Point addressed	**5.6.2 / 01**	

1. Annex point(s)	**IIA, 5.6.2 Reproductive toxicity - Developmental toxicity studies**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.6.2 / 01
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - Rat oral teratogenicity study. Syngenta Crop Protection AG, unpublished report No. 942118, August 07, 1996
4. Testing facility	[redacted]
5. Dates of work	July 04, 1995 - October 24, 1995
6. Test substance	ISO common name: Thiamethoxam, [redacted]
7. Test method	OECD 414 ≅ EEC B.31 ≅ FIFRA 83-3 ≅ 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 24 mated female Sprague-Dawley rats (Tif: RAIf, SPF strain, at least 8 weeks old, supplied by [redacted]) were treated orally, by gavage, with thiamethoxam (batch no. [redacted]) in 0.5% aqueous carboxy-methylcellulose at dose levels of 0, 5, 30, 200 and 750 mg/kg bw from day 6 through 15 of gestation (day 0 = plug or sperm observed in vaginal smear). Mortality was recorded twice daily, clinical signs and body weight daily. Food consumption was measured throughout gestation. Dams were sacrificed on day 21 and the foetuses delivered by caesarean section. The uterine tract and contents were removed and weighed. Main organs of the thoracic and abdominal cavities, and ovaries (including corpora lutea count), uteri (implantation site count) and placentas were examined macroscopically. Foetuses were sexed, examined for external malformations and variations, and weighed. Approximately one half of the foetuses from each litter were examined for soft-tissue malformations and variations by fixation in Bouin's solution and subsequent micro-dissection of the head, thoracic and abdominal viscera. The remaining foetuses were subjected to skeletal evaluation using Dawson's technique and examined for skeletal malformations, anomalies and variations. A malformation was defined as a very rare, permanent structural change that may adversely affect foetal survival, development or function. A distinction was made between skeletal anomalies and skeletal variations. A skeletal anomaly is a rare, slight to moderate, permanent or reversible structural change that is not considered to impair foetal survival, development or function. A variation is a relatively frequent, transient structural deviation from normal development that is considered not to have any detrimental effect on foetal survival, development or function. Variations occur regularly in control foetuses. Continuous data were analysed statistically using ANOVA and Dunnett's t test, quantal data by the Chi-square test followed by Fisher's exact test, and non-parametric data by Kruskal-Wallace non-parametric ANOVA followed by the Mann-Whitney U test.

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

No treatment-related deaths occurred, but one dam at 750mg/kg was sacrificed on day 9 for animal welfare reasons. Transient hypoactivity, piloerection and in 2 females, regurgitation of substance were the only clinical findings

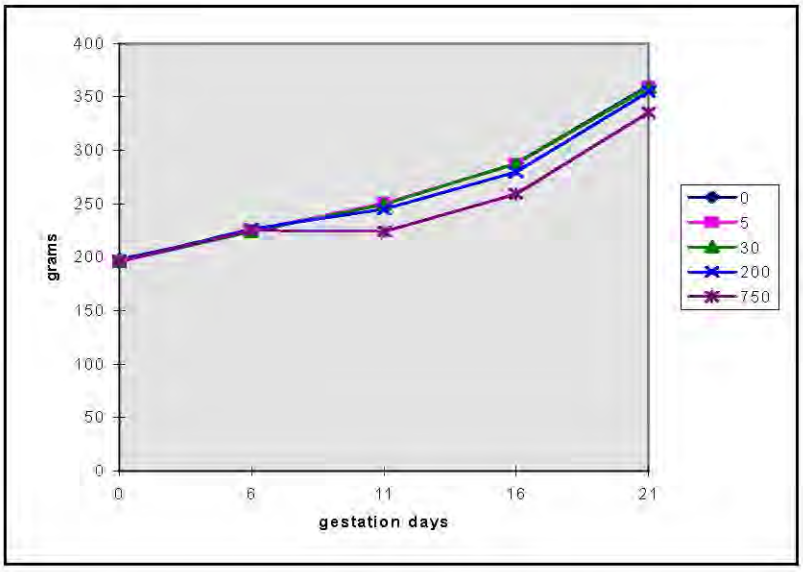
related to treatment. Maternal body weight gain was moderately depressed during the treatment period at 750mg/kg and minimally decreased at 200mg/kg early in the treatment period. (Table 1 and Fig. 1). No effects on body weight were observed at dose levels ≤30mg/kg. Food consumption was depressed during the treatment period at 750mg/kg.

Table 1: Mean body weight and body weight change

Dose Level	0mg/kg bw	5mg/kg bw	30mg/kg bw	200mg/kg bw	750mg/kg bw
Body weight (g):					
day 0	197.8	196.4	196.0	196.5	196.0
day 6	224.3	225.1	223.9	226.3	225.1
day 16	287.7	287.1	287.7	279.7	259.0**
day 21	360.1	358.9	358.5	354.8	335.4*
Body weight change (g):					
day 0 - 5 (pre-treatment)	26.5	28.7	27.9	29.8	29.1
day 6 - 15 (treatment)	63.4	62.0	63.8	53.4**[a]	33.9**[a]
day 16 - 20 (post-treatment)	72.4	71.8	70.8	75.1	76.4
overall change (gestation period)	162.3	162.5	162.5	158.3	139.4

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett's t test); [a] change day 6 - 11

Figure 1: Body weight development of pregnant rats administered thiamethoxam



Pregnancy incidence, mean number of corpora lutea, pre-implantation loss, number of implantation sites, early and late post-implantation losses, mean numbers of live foetuses and late resorptions and the sex ratio were unaffected by treatment at all dose levels (Table 2). A significant reduction in the mean body weight of live foetuses occurred at 750mg/kg. This was attributed to the maternal toxicity observed at this dose level.

Table 2: Intrauterine data

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

a Affected implants include dead foetuses, resorptions and malformed live foetuses.

b Mean value determined for each female.

c 100 minus the percentage of corpora lutea that are reflected in the implantation sites.

d 100 minus the percentage of implants that are viable at the time of intrauterine inspection.

e (Number of malformed live foetuses/total live foetuses) x 100.

f [(Dead foetuses + resorptions + malformed live foetuses)/total implants] x 100.

g Body weight (gestation day 21) - Weight of reproductive tract plus contents.

h Body weight (gestation day 21 - gestation day 0) - Weight of reproductive tract plus contents.

i Body weight (gestation day 21 - gestation day 7) - Weight of reproductive tract plus contents.

k *p ≤ 0.05, two -tailed; ** p ≤ 0.01, two -tailed

The incidence and type of external, visceral and skeletal malformations were not affected by treatment at any dose level (Table 3). Increased incidences of skeletal anomalies and variants occurred at 750mg/kg. Treatment-related anomalies were asymmetric sternebrae and irregular, poor or absent ossification of the occipital bone. Other commonly occurring skeletal anomalies were present at similar incidences in all treated and control groups. Skeletal variants were recorded in all foetuses from all dose groups. Treatment-related increases in the incidence of skeletal variants occurring at 750mg/kg were poor ossification of sternebra 5, shortened 13th rib, absent ossification of metatarsal 1, and poor or absent ossification of one or more phalanges (commonly absence of proximal phalanges, incidences not shown in Table 3). These findings are considered to represent a treatment-related delay of ossification, secondary to reduced pup weight, in turn a reflection of maternal toxicity. The incidences of skeletal variants were unaffected by treatment at dose levels lower than 750mg/kg.

Table 3: Incidence of malformations, and treatment-related anomalies and variants

Dose level [mg/kg bw]	0	5	30	200	750
Litters evaluated	**22**	**23**	**23**	**22**	**22**
Foetuses evaluated	**309**	**302**	**322**	**321**	**305**
External malformations:					
runt foetus	1	0	2	0	0
mouth: agnathia	0	0	1	0	0
skin: generalized oedema	1	0	0	0	0
Visceral malformations:					
diaphragmatic hernia	0	0	0	0	1
Skeletal malformations:					
unossified mandibula and os pubis	0	0	1	0	0
Total no. foetuses with any malformation:	1	0	2	0	1
Skeletal anomalies:					
Total no. (%) of foetuses with any anomaly:	23 (14.9)	14 (9.3)	16 (10.1)	12 (7.2)	40 (26.7)
No. (%) foetuses with:					
asymmetric sternebra 6	4 (2.6)	3 (2.0)	2 (1.3)	3 (1.8)	11 (7.3)
irregular /absent ossification of occipital bone	3 (1.9)	0	2 (1.3)	2 (1.2)	12 (8.0)*
Skeletal variants:					
Total no. (%) of foetuses with any variant:	154 (100)	150 (100)	158 (100)	166 (100)	150 (100)
No. (%) foetuses with:					
poor ossification of sternebra 5	0	0	1 (0.6)	0	10 (6.7)**
shortened 13th rib	13 (8.4)	5 (3.3)	4 (2.5)*	21 (12.7)	27 (18.0)*
absent ossification of metatarsal 1	15 (9.7)	31 (20.7)*	24 (15.2)	16 (9.6)	51 (34.0)**

* = $p<0.05$, ** = $p \leq 0.01$

Conclusion: A dose level of 750mg/kg is toxic to dams, resulting in mild foetotoxicity. There was no evidence of teratogenicity at dose levels up to 750mg/kg bw, the highest dose level employed.

No-observed-effect-levels (NOEL): 30mg/kg bw/day (maternal), based on minimally reduced weight gain at 200mg/kg bw, and 200 mg/kg bw (offspring), based on reduced live birth weight and an excess incidence of pups with delayed skeletal ossification at 750mg/kg bw

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date April 2005

Materials and Methods [redacted]

Results and discussion [redacted]

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

98/8 Doc IIIA section No.	**6.8.1 / 02**	**Teratogenicity test**
Annex	**II**	Developmental toxicity studies
Point addressed	**5.6.2 / 02**	

1. Annex point(s)	**IIA, 5.6.2 Reproductive toxicity - Developmental toxicity studies**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.6.2 / 02
3. Authors (year) Title Owner, Date	[redacted] CGA 293'343 tech. - Rabbit oral teratogenicity. Syngenta Crop Protection AG, unpublished report No. 942119, August 13, 1996
4. Testing facility	[redacted]
5. Dates of work	August 07, 1995 - November 14, 1995
6. Test substance	ISO common name: Thiamethoxam, [redacted]
7. Test method	OECD 414 ≅ EEC B.31 ≅ FIFRA 83-3 ≅ EPA TSCA 798.4900 ≅ 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 19 mated female rabbits (Russian Chbb:HM strain, at least 3 months old, supplied by [redacted]) were treated orally, by gavage, with thiamethoxam ([redacted]) in 0.5% aqueous carboxymethylcellulose from day 7 through 19 of gestation (day 0 = plug or sperm in smear) at daily dose levels of 0, 5, 15, 50 and 150mg/kg. Females were observed twice daily for mortality and once daily for clinical signs. Body weights were recorded daily. Food consumption was recorded on days 4, 7, 12, 16, 20, 24 and 29. Dams were sacrificed on day 29 and the reproductive tract plus contents removed by hysterectomy and weighed. Main organs, in particular ovaries including the number of corpora lutea, uteri and placentas were examined. In dams that died or were sacrificed before scheduled necropsy, the number and location of implantation and/or abortion sites in the uterus were examined. In dams sacrificed at scheduled necropsy the number and location of live and dead foetuses or early and late (embryonic or foetal) losses were recorded. Foetuses were numbered, tagged individually, weighed, sexed, and examined for external malformations and variations. All foetuses were examined for thoracic / abdominal soft-tissue or skeletal malformations, anomalies and variations. A malformation was defined as a very rare, permanent structural change that may adversely affect foetal survival, development or function. A distinction was made between skeletal anomalies and skeletal variations. A skeletal anomaly is a rare, slight to moderate, permanent or reversible structural change that is not considered to impair foetal survival, development or function. A variation is a relatively frequent, transient structural deviation from normal development that is considered not to have any detrimental effect on foetal survival, development or function. Variations occur regularly in control foetuses. Approximately one-half of the foetuses were examined for cranial soft tissue malformations. All foetal trunks and approximately half of the foetal heads per litter were assigned to skeletal assessment according to the staining technique of Dawson. Continuous data were analysed statistically using ANOVA and Dunnett's t test, quantal data by the Chi-square test followed by Fisher's exact test, and non-parametric data by Kruskal-Wallace non-parametric ANOVA followed by the Mann-Whitney U test.

Findings: Analysis of formulations revealed the test article to be stable in the vehicle and homogeneously dispersed (-2% to +3% mean concentration). Overall achieved mean concentrations were within the range 97.2% to 101.4%.

Three animals treated at 150mg/kg died or were killed for humane reasons at the end of the treatment period or shortly thereafter. Treatment-related clinical signs at 150mg/kg comprised a bloody discharge in the perineal area of 13 animals, including all animals that died or were killed. Deaths and clinical signs of adverse effects of treatment were not apparent at lower dose levels. Overall mean body weight was markedly reduced by treatment at 150mg/kg, due primarily to weight loss during the early part of the treatment period (Table 1 and Fig. 1). A minimal reduction in weight gain during the treatment period also occurred at 50mg/kg but overall weight gain was not adversely affected. Food consumption was markedly reduced during the treatment period at 150mg/kg and to a minimal extent at 50mg/kg (Table 1). Food consumption and body weight gain were unaffected by treatment at lower dose levels.

At necropsy, the uterine contents of the three animals that died or were killed prematurely at 150mg/kg were haemorrhagic and one animal also showed a haemorrhagic vagina. There were no other treatment-related necropsy findings.

Table 1: Mean body weight gain and food consumption (± SD)

Dose level [mg/kg bw]	0	5	15	50	150
Mean body weight (g):					
day 0	2733	2833	2840	2833	2718
day 7	2726	2854	2834	2819	2740
day 20	2778	2899	2873	2838	2667
day 29	2840	2998	3001	2952	2777
Mean body weight gain (g):					
day 0 - 6 (pre-treatment)	-7	21	-6	-14	22
day 7 - 19 (treatment)	52	45	39	19	-73**
day 20 - 29 (post-treatment)	62	99	128	114	110
overall gain (gestation period)	107	165	161	119	59
Food consumption (g/day):					
- Pre-treatment (days 0-4)	93.5 ±14.4	94.1 ±19.1	91.6 ±17.4	95.4 ±10.0	98.3 ±11.4
- Pre-treatment (days 4-7)	88.6 ±11.2	97.9 ±20.0	95.3 ±12.6	92.0 ±13.2	100.4±17.3
- Treatment (days 7-12)	88.7 ±14.1	92.4 ±15.1	88.5 ±15.8	69.2**±9.9	21.4** ± 9.4
- Treatment (days 12-16)	86.7 ±13.6	91.2 ±17.4	79.1 ±20.7	64.2**±19.1	38.5** ±21.0
- Treatment (days 16-20)	96.3 ±21.7	95.9 ±20.2	88.5 ±22.0	80.6 ±20.6	56.8** ±27.7
- Post treatment (days 20-24)	96.3 ±28.7	99.2 ±21.1	93.1 ±17.2	100.9±21.2	125.1*±16.6
- Post treatment (days 24-29)	93.4 ±26.1	96.8 ±20.4	100.3±14.5	103.3±23.9	121.6*±17.2

* $p \leq 0.05$;

** $p \leq 0.01$

Figure 1: Body weight development of pregnant rabbits

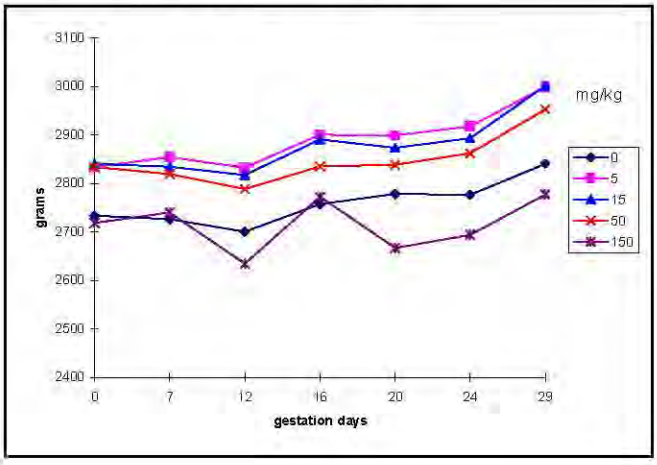


Table 2: Intrauterine data

Dose level (mg/kg bw)	0	5	15	50	150
Does with implants	15	19	19	19	18
Does with live foetuses	15	19	19	18	12
Does with resorptions (%)	9 (47)	4 (21)	9 (47)	8 (42)	13 (68)
Does with affected implants[a] (%)	10 (53)	8 (42)	10 (53)	10 (5.3)	16 (84)
	Mean[b] ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Corpora lutea/doe	6.7 ± 1.2	7.2 ±1.2	7.2 ± 1.4	6.9 ± 1.3	7.1 ± 1.6
Implants/doe	4.7 ± 2.1	5.4 ± 1.2	5.9 ± 1.7	5.4 ± 2.0	5.4 ± 2.1
Preimplantation loss[c] [%]	32.6 ± 25.2	23.1 ± 21.7	17.5 ± 12.2	23.7 ± 18.4	25.1 ± 19.0
Postimplantation loss[d] [%]	21.0 ± 24.3	6.3 ± 16.1	9.9 ± 12.8	16.3 ± 25.9	45.6 ± 35.4
Live foetuses/doe	3.7 ± 2.2	5.1 ± 1.5	5.4 ± 1.7	4.6 ± 2.2	3.0 ± 2.4
Dead foetuses/doe	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Resorptions/doe - Early	1.0 ± 1.1	0.3 ± 0.6	0.5 ± 0.7	0.6 ± 1.0	2.4 ± 2.7
Resorptions/doe - Late	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.2	0.2 ± 0.5	0.0 ± 0.0
Malformed live foetuse[e] [%]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Affected implants[f] [%]	1.0 ± 1.1	0.3 ± 0.6	0.6 ± 0.7	0.8 ± 1.1	2.4 ± 2.7
Sex ratio (% females)	43.6	50.5	54.9	46.5	51.1
Mean pup body weight [g] - all	44.0 ± 4.3	41.5 ± 3.2	41.7 ± 2.9	42.1 ± 3.9	37.5**± 4.5
- males	44.4 ± 4.9	41.7 ± 3.6	43.0 ± 3.3	42.2 ± 4.1	38.8**± 4.9
- females	41.8 ± 2.0	40.9 ± 3.2	40.8 ± 3.2	41.1 ± 4.3	36.6**± 5.4
Reproductive tract weight [g] - all	226 ± 115	303 ± 82	316 ± 80	294 ± 99	210 ± 100
Corrected body weight[g]	2614	2695	2685	2658	2567
Corr. bw change: gestation[h]	-119	-138	-155	-175	-151
Corr. bw change: treatment[i]	-112	-159	-149	-161	-173

a Affected implants include dead foetuses, resorptions and malformed live foetuses.

b Mean value determined for each female.

c 100 minus the percentage of corpora lutea that are reflected in the implantation sites.

d 100 minus the percentage of implants that are viable at the time of intrauterine inspection.

e (Number of malformed live foetuses/total live foetuses) x 100.

f [(Dead foetuses + resorptions + malformed live foetuses)/total implants] x 100.

g Body weight (gestational day 29) - Weight of reproductive tract plus contents.

h Body weight (gestational day 29 - gestational day 0) - Weight of reproductive tract plus contents.

i Body weight (gestational day 29 - gestational day 7) - Weight of reproductive tract plus contents.

Total resorption occurred in 3 animals at 150mg/kg and in one animal at 50mg/kg. Therefore, there was a treatment-related reduction in the number of animals with live foetuses and a concomitant increase in post-implantation loss at 150mg/kg. The mean pup weight of both sexes was reduced as a result of treatment at 150mg/kg. Gravid uterus weight, number of corpora lutea, pre-implantation loss, number of implantation sites and the number of dead foetuses were unaffected by treatment at all dose levels (Table 2).

No external or skeletal foetal malformations occurred in the study, and no treatment-related visceral malformations were evident. There were 0, 1, 2, 0 and 1 foetuses, in order of ascending dosage, with flexure of the forepaw. This, however, is not considered a malformation, but due to restriction of movement within the uterus. The distribution of visceral malformations did not indicate an effect of treatment at any dose level. Small gall bladder occurred in some groups treated with thiamethoxam but not in the control group. Based on the scattered incidence and absence of a dose-relationship, this anomaly is considered incidental and not related to treatment. The foetal incidence of the skeletal anomaly fused sternebrae, was increased at 150mg/kg, but not at lower dose levels. This is considered to be treatment-related as a consequence of reduced birth weight, in turn a reflection of maternal toxicity. The distribution of other skeletal anomalies did not indicate an effect of treatment. Skeletal variants occurred in a majority of foetuses from most litters in all treatment groups. The overall foetal incidences were 70.9, 78.4, 79.4, 79.5 and 75.6% in order of increasing dose level. One skeletal variation, absent ossification of the medial phalanx of anterior digit-5, occurred at a foetal incidence of 8.9% at 150mg/kg (control incidence 0%). Although the observed incidence was within the historical control range of 0 - 9.2%, the finding may be an indication of treatment-related delayed ossification (Table 3).

Table 3: Foetal incidence and nature of malformations and treatment-related skeletal anomalies and variations

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

Conclusion: A dose level of 150mg/kg is toxic to does, and results in mild foetotoxicity. There was no evidence of teratogenicity at dose levels up to 150mg/kg bw, the highest dose level employed.

No-observed-effect-levels (NOEL): 15mg/kg bw (maternal), based on the findings of minimally reduced weight gain and food consumption at 50mg/kg, and 50mg/kg (offspring), based on reduced birth weight and increased incidence of pups with delayed ossification at 150mg/kg.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date April 2005

Materials and Methods [redacted]

Results and discussion [redacted]

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

98/8 Doc IIIA section No.	**6.8.2 / 01**	**Two generations reproduction study**
Annex	**II**	Multigeneration studies
Point addressed	**5.6.1 / 02**	

1. Annex point(s)	**IIA, 5.6.1 Reproductive toxicity - Multi-generation studies**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.6.1/02
3. Authors (year) Title **Owner, Date**	[redacted] CGA 293'343 tech. - Rat dietary two-generation reproduction study; includes report: Effects on sperm cell parameters. Syngenta Crop Protection AG, unpublished report Nos. 942121 and 982015, July 20, 1998. Amended October 22, 1998 (Amendment 1), amended November 12, 1998 (Amendment 2).
4. Testing facility	[redacted]
5. Dates of work	November 06, 1995 - September 23, 1996, and March 16, 1998 - May 27, 1998.
6. Test substance	ISO common name: Thiamethoxam, [redacted]
7. Test method	OECD 416 ≅ FIFRA 83-4 ≅ OPPTS 870.3800 ≅ 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 (batch no. [redacted] was administered orally to 2 successive generations (F_0 and F_1) of male and female Sprague-Dawley-derived rats (Tif:RAIf, SPF strain, age range 6 - 7 weeks, supplied by [redacted]) by admixture in the diet at nominal concentrations of 0, 10, 30, 1000 and 2500ppm. Treatment of F_0 animals (30/sex/group) was initiated 10 weeks pre-mating and continued throughout the production of 2 litters/generation until necropsy. Litters were culled to 4 pups/sex, where possible, on day 4 *post partum*. The F_1 generation was selected from the first litters of the F_0 generation. Clinical signs, body weights, food consumption, mating, gestation and parturition parameters, pup survival and developmental/behavioural landmarks were recorded. A gross necropsy examination was performed on all pups not selected for mating. All parental animals were necropsied after weaning of the second litters and subjected to macroscopic examination and histopathology of the sex and target organs. Sperm analysis (motility, morphology and spermatid counts) was performed on 15 males/group of the F_0 and F_1 parental generations. Male and female mating and fertility indices, female gestation and parturition indices, and litter live birth, viability and lactation indices were calculated. Continuous data were analysed statistically using ANOVA and Dunnett's t test, quantal data by the Chi-square test followed by Fisher's exact test, and non-parametric data by Kruskal-Wallace non-parametric ANOVA followed by the Dunn test.

In the ancillary sperm cell parameter study, groups of 30 virgin male Sprague-Dawley-derived rats (Tif:RAIf, SPF strain, age 6 - 7 weeks, supplied by [redacted]) were fed thiamethoxam in the diet for 10 weeks at nominal concentrations of 0, 10, 30, 1000 or 2500 ppm. Body weight, food consumption and clinical signs were recorded. After 10 weeks, the animals were killed and necropsied, the testes, epididymides, prostate, and seminal vesicles (with coagulating glands) were weighed, and epididymis sperm cells and/or testis spermatids were evaluated for number, motility, and morphology. Changes in procedure from the first study were

implemented to reduce and standardise the time for sperm collection, to refine the technique for opening the cauda epididymis, and to randomise the order in which sperm evaluations were performed to minimise inter-day bias.

Findings (multigeneration study): Analysis of representative diet samples demonstrated that thiamethoxam was stable in diet for at least 5 weeks at room temperature. Homogeneity varied in the range -4% - +5% nominal. Analysis of representative diet samples from 7/12 mixes showed the overall mean concentrations to be 9.6, 29.1, 1022 and 2590ppm in order of increasing concentration. Achieved dose level ranges, based on nominal concentrations, were 0, 0.4-1.5, 1.4-4.3, 45.6-144.0 and 117.6-362.9mg/kg bw/day (males) and 0, 0.6-2.1, 1.8-6.4, 59.3-219.6 and 147.8-541.3mg/kg bw/day (females) in order of ascending concentration.

F_0 Parental Animals: There were no treatment-related deaths or clinical signs of an adverse reaction to treatment. Body weight gain was slightly reduced throughout the study in males at 2500ppm. (Table 1). There was no effect on weight gain in the other male groups, or in any female group, either before mating or during gestation and lactation. Males at 2500ppm showed transiently reduced food consumption (-7.5%) during 2 weeks of the first pre-mating treatment period only. No other groups of either sex were affected.

Table 1: Body weight development - F_0 generation

Day	Timepoint	Parameter Dose Level	0ppm	10ppm	30ppm	1000ppm	2500ppm
				Males			
1	Treatment start	Mean body weight (g)	199.5	198.8	198.2	199.2	196.8
68	End 1st premating	Mean body weight (g)	460.7	454.6	453.6	450.1	430.8
		Cumulative body weight gain[a]	261.2	255.8	255.4	250.9	234.0
		Body weight gain % of control		-2.1	-2.2	-3.9	-10.4
190	End 2nd postmating	Mean body weight (g)	578.1	566.9	562.3	557.2	546.5
		Cumulative body weight gain	378.6	368.1	364.1	358.0	349.7
		Body weight gain % of control		-2.8	-3.8	-5.4	-7.6
				Females			
1	Treatment start	Mean body weight (g)	154.5	153.7	153.5	152.7	154.2
68	End 1st premating	Mean body weight (g)	265.4	271.1	272.5	267.4	266.4
		Cumulative body weight gain	110.9	117.4	119.0	114.7	112.2
		Body weight gain % of control		+5.9	+7.3	+3.4	+1.2
211	End 2nd lactation	Mean body weight (g)	348.4	357.3	349.8	346.3	348.5
		Cumulative body weight gain	193.9	203.6	196.3	193.6	194.3
		Body weight gain % of control		+5.0	+1.2	-0.2	+0.2

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

At necropsy, there was no effect on the gross appearance of organs in male and female F_0 parental animals at any dose level. There were no effects on the microscopic appearance of the reproductive organs of F_0 males and females at 2500ppm, or in non-pregnant females and in males that failed to mate. An increased incidence of minimal to marked hyaline change in renal tubules was associated with treatment at 1000 and 2500ppm in males (Table 2). A slightly increased incidence of renal tubular casts occurred in males at 2500ppm. Both findings are attributed to the treatment.

Table 2: Treatment-related microscopic findings - F_0 animals

Dose Level [ppm]	Males					Females				
	0	10	30	1000	2500	0	10	30	1000	2500
Kidney No. exam	30	30	30	30	30	30	0	0	0	30
tubular hyaline change	1	2	3	16	25	0	-	-	-	0
grade 1	1	2	3	12	1	-	-	-	-	-
grade 2	-	-	-	4	17	-	-	-	-	-
grade 3	-	-	-	-	7	-	-	-	-	-
average grade	1.0	1.0	1.0	1.3	2.2	-	-	-	-	-
tubular cast	22	20	19	23	28	14	-	-	-	8
grade 1	8	12	10	15	10	10	-	-	-	6
grade 2	14	6	8	7	17	3	-	-	-	2
grade 3	-	2	1	1	-	1	-	-	-	-
grade 4	-	-	-	-	1	-	-	-	-	-
average grade	1.6	1.5	1.5	1.4	1.7	1.4	-	-	-	1.3

Sperm analyses revealed no treatment-related changes in the concentration of spermatids in testes at any dose level (Table 3). Sperm morphology was unaffected by treatment.

Sperm motility in all thiamethoxam-treated groups was reduced significantly by approximately 20%. However, variability, reflected in large standard deviations, was high in all groups, indicating probable technical flaws. In view of the absence of effects on spermatid concentration, histological morphology, sperm morphology, fertility during both pairings and the absence of a dose-relationship for reduced motility, the toxicological relevance of these results is considered equivocal. The results of a further study of sperm parameters, reported below, supports the contention that technical flaws were the cause of observed differences. One technical flaw considered to have contributed to the variability between samples was poor standardization of the interval between sacrifice and sperm evaluation. A flaw possibly accounting for the significant differences among groups was the conduct of sperm evaluations in group order rather than in randomised order. In the second sperm analysis study, procedural changes were implemented to reduce and standardize the time for sperm collection, to refine the technique for opening the cauda epididymis, and to randomise the order in which sperm evaluations were performed to minimize inter-day bias.

Table 3: Sperm parameters - F_0 parental generation

Dose Level	0ppm		10ppm		30ppm		1000ppm		2500ppm	
Number used for evaluation	15		15		15		15		15	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total spermatids ($x10^6$ /g testis)	67.6	14.1	70.4	11.6	64.6	11.8	77.2	15.7	73.8	11.1
Percent abnormal sperm	22.0	18.3	16.6	8.8	14.1	3.8	19.0	6.3	16.0	4.6
Percent motile sperm cells	73.0	21	54.5*	15	51.5**	20	56.0*	16	54.5*	14

* = $p \leq 0.05$, ** $p \leq 0.01$ (ANOVA + Dunnett)

There were no treatment-related differences in reproductive parameters among the groups (Table 4). The number of animals that mated, the pregnancy incidence, the mean time to mating and the number of males and females that failed to mate or mated unsuccessfully were comparable in all treated and control groups for both matings, with the exception of the group treated at 1000ppm that showed slightly lower insemination and pregnancy incidences at both matings. There was no effect of treatment at any dose level, at either mating, on the duration of gestation or on gestation and parturition indices.

Table 4: Reproductive parameters - F_0 parental animals

Dose Level	0ppm	10ppm	30ppm	1000ppm	2500ppm
First Mating					
Females placed with males (n)	30	30	30	30	30
Males placed with females (n)	30	30	30	30	30
Days until evidence of mating	5.3	4.9	4.0	3.7	4.4
Total inseminated (n)	28	29	29	26	29
Total pregnant (n)	25	28	28	23	27
Pregnant females died or sacrificed moribund (n)	0	0	1	0	0
Duration of gestation (days)	22.1	21.8	22.2	22.0	22.0
Total delivering with liveborn pups (n)	25	28	27	23	27
Second Mating					
Females placed with males (n)	30	30	29	30	30
Males placed with females (n)	30	30	29	30	30
Days until evidence of mating	3.3	3.0	4.3	4.1	3.2
Total inseminated (n)	28	29	29	25	29
Total pregnant (n)	24	26	27	22	27
Duration of gestation (days)	22.2	22.0	22.1	21.9	22.2
Pregnant females died or sacrificed moribund (n)	0	0	0	0	0
Total delivering with liveborn pups (n)	24	26	27	22	27

F_{1a} generation: Litter size at birth was slightly, but not significantly lower than the control group at 2500ppm (Table 5.6.1-5). Mean birth weight was unaffected by treatment at all dose levels, but slightly reduced pup weight gain occurred at 2500ppm during the last 2 weeks of lactation. Sex ratios, viability and lactation indices, clinical signs, achievement of physical/behavioural developmental landmarks and macroscopic findings at necropsy were unaffected by treatment at all dose levels.

F_{1b} generation: Treatment-related effects occurring in F_{1b} offspring were confined to slightly reduced pup weight gain at 2500ppm during the last 2 weeks of lactation (Table 5). Due to a lower number of dams in the 1000ppm group, the total number of pups born was lower. Loss of one litter (9 stillborn pups) was responsible for an increased number of stillborn pups in the 1000ppm group. All other litter parameters in the F_{1b} generation were comparable to the controls.

Table 5: Litter data - F_{1a} and F_{1b} litters

Parameter	Dose Level	0ppm	10ppm	30ppm	1000ppm	2500ppm
Number of litters - **F_{1a}**		25	28	27	23	27
Total pups born		341	368	357	300	314
Mean litter size		13.6	13.0	13.1	13.0	11.4
Live birth index		99.4	98.6	99.2	99.7	98.4
Viability Index		96.2	96.1	98.3	99.0	98.4
Sex ratio (% females day 0)		51.6	50.4	53.7	49.8	47.6
Mean pup weight						
- on day 0		6.2	5.9	6.1	6.1	6.1
- on day 4	precull	9.0	8.9	9.3	9.1	9.2
- on day 4	postcull	9.2	9.1	9.5	9.2	9.4
- on day 7		14.7	14.6	15.2	14.8	14.6
- on day 14		30.5	29.8	30.7	29.8	28.9
- on day 21		53.1	50.9	52.5	51.4	48.6**
Number of litters - **F_{1b}**		24	26	27	22	27
Total pups born		333	360	360	305	330
Mean litter size		13.8	13.7	13.2	13.3	12.1
Live birth index		99.7	98.6	98.9	96.1	99.1
Viability Index		94.3	97.7	97.8	98.3	95.7
Sex ratio (% females day 0)		50.0	47.9	51.4	54.9	46.5
Mean pup weight						
- on day 0		6.1	6.0	6.2	5.9	6.1
- on day 4	precull	9.0	8.9	9.0	9.0	8.7
- on day 4	postcull	9.3	9.1	9.2	9.3	8.8
- on day 7		14.7	15.1	14.8	15.2	13.7
- on day 14		30.5	30.5	30.3	30.9	28.4*
- on day 21		52.6	51.8	51.8	52.9	47.3**

* $p \leq 0.05$, ** $p \leq 0.01$, ANOVA + Dunnett

F_1 Parental Animals: There were no treatment-related deaths or clinical signs in the F_1 parental animals, although 2 animals at 2500ppm and one at 10ppm were killed in a moribund condition. The overall weight gain of selected F_1 parental animals (both sexes) was unaffected by treatment at all dose levels, although the initial weight of 2500ppm animals was slightly reduced, reflecting reduced weight gain during lactation (Table 6). Food consumption was also unaffected by treatment. Minimally higher apparent food consumption at 2500ppm, occasionally observed throughout the treatment period, is considered likely to reflect increased wastage.

Table 6: Body weight development - F_1 parental animals

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

a Body weights and body weight gains in grams; * $p \leq 0.05$, ANOVA + Dunnett

None of the differences between control and treated group organ weights of F_1 parental animals was considered to be treatment-related (Table 7). Combined weights of the testes were significantly lower in 2500ppm animals compared to controls, but histopathological examination showed the incidences of tubular atrophy not to be distributed in a dose related manner among groups. These findings in F_1 males were considered not of toxicological significance. Lower thymus weights occurred in females at 30, 1000 and 2500ppm, but there was no histological correlate, suggesting the differences were incidental to treatment. The relative spleen and liver weights of males at 2500ppm were significantly higher than the controls, but the absolute weights were not significantly elevated.

Table 7: Organ weights - F_1 parental animals

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

a All absolute weights in grams; [b]Relative organ weights are organ weight % of body weight x 100;

* $p \leq 0.05$, ** $p \leq 0.01$, ANOVA + Dunnett

The gross appearance of tissues and organs at necropsy in F_1 parental animals was unaffected by treatment at all dose levels. No treatment-related histological lesions occurred in the reproductive organs of parental animals at

2500ppm or in non-pregnant females and males that failed to mate. Tubular atrophy of the testes was increased in the treated groups, but the incidences were not dose-related and were considered not of toxicological significance. Treatment of males at 1000 or 2500ppm produced increased incidences of casts and minimal to marked hyaline changes in the renal tubule (Table 8). This finding can be attributed to male rat-specific α2μ-globulin nephropathy, not considered relevant for humans.

Table 8: Treatment-related microscopic findings - F_1 animals

	Males					Females				
Dose Level [ppm]	**0**	**10**	**30**	**1000**	**2500**	**0**	**10**	**30**	**1000**	**2500**
Kidney No. exam.	30	30	30	30	30	30	10	0	0	30
Tubular hyaline change	3	5	3	24	28	0	-	-	-	1
grade 1	3	4	3	4	1	-	-	-	-	1
grade 2	-	1	-	16	12	-	-	-	-	-
grade 3	-	-	-	4	12	-	-	-	-	-
grade 4	-	-	-	-	3	-	-	-	-	-
average grade	1.0	1.2	1.0	2.0	2.6	-	-	-	-	1.0
Tubular cast	21	20	21	27	29	20	0	-	-	20
grade 1	7	5	11	11	6	7	-	-	-	6
grade 2	9	13	7	15	13	11	-	-	-	9
grade 3	5	2	3	1	9	1	-	-	-	3
grade 4	-	-	-	-	1	1	-	-	-	2
average grade	1.9	1.9	1.6	1.6	2.2	1.8	-	-	-	2.1

Sperm analyses revealed no treatment-related changes at any dose level in morphology and the concentration of spermatids in testes (Table 9). Sperm motility at 10 and 2500ppm was reduced significantly by approximately 18%. However, variability as reflected by large standard deviations was high in all groups, indicating probable technical flaws. In view of the absence of effects on spermatid concentration, histological morphology, sperm gross morphology and fertility during both pairings, the toxicological relevance of these results is considered equivocal.

Table 9: Sperm parameters - F_1 parental generation

Dose Level	**0ppm**		**10ppm**		**30ppm**		**1000ppm**		**2500ppm**	
Number used for evaluation	**14/15[a]**		**15**		**15**		**14/15[a]**		**15**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total spermatids (x1,000,000 /g testis)	63.9	8.1	60.3	9.7	68.8	12.0	60.1	14.7	77.2	37.2
Percent abnormal sperm	17.4	21.8	15.0	24.3	10.2	4.2	18.3	23.9	16.6	22.8
Percent motile sperm cells	65.5	14	53.0*	12	56.0	14	60.0	11	53.5*	11

[a] Morphology evaluated for 14 animals, motility for 15; * = $p \leq 0.05$, ANOVA + Dunnett

There were no treatment-related effects on reproductive parameters at any dose level (Table 10). The number of animals that mated, the pregnancy incidence, the mean time to mating and the number of males and females that failed to mate or mated unsuccessfully were comparable in all treated and control groups for both matings, with the exception of the group treated at 30ppm that showed slightly lower insemination and pregnancy incidences at both matings. There was no effect of treatment at any dose level, at either mating, on the duration of gestation or on gestation and parturition indices.

Table 10: Reproductive parameters - F_1 parental animals

Dose Level	0ppm	10ppm	30ppm	1000ppm	2500ppm
First Mating					
Females placed with males (n)	30	30	30	30	30
Males placed with females (n)	30	30	30	30	30
Days until evidence of mating	4.2	3.9	4.7	5.4	5.1
Total inseminated (n)	30	28	28	30	30
Total pregnant (n)	28	27	25	26	27
Pregnant females died or sacrificed moribund (n)	0	0	0	0	0
Duration of gestation (days)	22.1	22.2	22.0	22.0	22.0
Total delivering with liveborn pups (n)	28	27	25	26	27
Second Mating					
Females placed with males (n)	30	30	30	30	30
Males placed with females (n)	30	30	30	30	30
Days until evidence of mating	4.0	4.2	3.4	2.8	3.2
Total inseminated (n)	29	28	23	29	28
Total pregnant (n)	28	25	21	28	26
Duration of gestation (days)	22.2	22.1	22.1	22.1	22.1
Pregnant females died or sacrificed moribund (n)	0	1	0	0	1
Total delivering with liveborn pups (n)	28	25	21	28	25

F_{2a} generation: The weight gain of the pups was slightly reduced throughout lactation at 2500ppm, and minimally reduced during the latter part of lactation at 1000ppm (Table 5.6.1-11). Litter size at birth, mean birth weight, sex ratios, viability and lactation indices, clinical signs, achievement of physical/behavioural developmental landmarks and macroscopic findings at necropsy were unaffected by treatment at all dose levels.

F_{2b} generation: The weight gain of these pups was slightly reduced during the latter part of lactation at 1000 & 2500ppm (Table 11). The mean time to establishment of the surface righting reflex was slightly, but significantly, longer in pups at 2500ppm (2.3 days) compared with other groups including the controls (2.1 - 2.2 days), possibly reflecting lower body weights in this group. There were no treatment-related effects on other litter parameters or on clinical signs, achievement of physical/behavioural developmental landmarks and macroscopic findings at necropsy.

Table 11: Litter data - F_{2a} and F_{2b} litters

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

* = $p \leq 0.05$, ** = $p \leq 0.01$, ANOVA + Dunnett

Second sperm cell study findings: The homogeneity of representative diet samples were shown by analysis to be acceptable, and in the range -7% to +6% nominal. Analysis of representative diet samples showed the overall mean concentrations to be 0, 9.7, 31.8, 1044 and 2540ppm. Overall achieved dose levels were 0, 0.5 - 0.7, 1.6 - 2.3, 53.4 - 74.8 and 141.5 - 174.7mg/kg bw/day in order of ascending concentration.

There were no treatment-related deaths or clinical signs of an adverse effect of treatment. Significantly lower body weight gains occurred during weeks 1 through 3 at 2500ppm resulting in a 14% reduction in weight at the end of treatment (Table 12). Overall weight gain was unaffected by treatment at lower dose levels although at 1000ppm, transiently reduced weight gain was observed in week 2 only. Food consumption was slightly reduced at 2500ppm during the first 6 weeks of treatment.

Table 12: Body weight development - males

Day	Timepoint	Parameter	Dose Level	0ppm	10ppm	30ppm	1000ppm	2500ppm
1	Treatment start	Mean body weight[a]		284.1	286.8	278.0	279.2	280.9
71	Treatment end	Mean body weight		484.9	487.2	483.1	473.0	453.7
		Cumulative body weight gain		200.8	200.4	205.1	193.8	172.8**
		Body weight gain % of control			-0.1	+2.1	-3.5	-13.9

[a] Body weights and body weight gains in grams; ** $p \leq 0.01$, ANOVA + Dunnett

There were no treatment-related effects on sex organ weights (Table 5.6.1-13). Higher mean relative testis and right cauda epididymis weights at 2500ppm are attributed to the reduced mean exsanguinated body weights of this group.

Table 13: Organ weights

Organ[a]	Dose Level	0ppm	10ppm	30ppm	1000ppm	2500ppm
Carcass		465	465	464	452	434
Testis, left	absolute	2.01	2.01	1.97	1.91*	2.07
	relative[b]	43.50	43.83	42.95	42.46	48.00**
Testis, right	absolute	2.00	2.01	1.96	1.89*	2.01
	relative	43.20	43.75	42.74	42.01	46.69*
Epididymis, right	absolute	0.80	0.79	0.80	0.76	0.80
	relative	17.24	17.21	17.34	17.01	18.54
Cauda epididymis, right	absolute	0.33	0.31	0.32	0.31	0.35
	relative	7.07	6.77	7.06	6.98	7.99*
Prostate	absolute	0.91	0.99	0.89	0.93	0.95
	relative	19.62	21.71	19.36	20.67	22.11
Seminal vesicles	absolute	1.68	1.71	1.72	1.77	1.77
	relative	36.29	37.18	37.57	39.30	40.96

[a] All absolute weights in grams; [b]Relative organ weights are organ weight % of body weight x 100;

* $p \leq 0.05$, ** $p \leq 0.01$, ANOVA + Dunnett

There was no effect on the gross appearance of organs at necropsy. There were no effects on the proportion of motile sperm, sperm morphology, concentration of spermatids in the testes or on the number of sperm cells/mg cauda epididymis fluid (Table 14). The standard deviations calculated for the number of motile epididymal sperm (4 - 12) were substantially lower than in the original study (14 - 21), suggesting that technical flaws were the cause of apparently reduced numbers of motile sperm in the multigeneration study. Sperm motility values were consistent with other published data from the rat (Chapin et al., 1992).

Table 14: Sperm parameters

Dose Level	0ppm		10ppm		30ppm		1000ppm		2500ppm	
Number used for evaluation	30		30		30		30		30	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total spermatids ($x10^6$/g testis)	59.6	9.6	58.2	10.7	55.0	10.8	57.5	10.0	55.8	12.9
Percent abnormal sperm	12.2	6.3	11.7	5.8	11.6	4.9	12.2	5.1	11.0	5.3
Total sperm/mg cauda liquid x 10^6	2.37	0.67	2.67	0.83	2.31	0.88	2.43	0.83	2.39	0.73
Percent motile sperm cells	73.1	12	75.5	4	73.0	7	73.5	7	74.5	8

Conclusion: No-observable-effect-levels (NOEL): 30ppm (females), equivalent to dose levels of 1.8 - 6.4mg/kg bw/day, based on reduced pre-weaning weight gain in pups from F_0 parental females at 2500ppm and in pups from F_1 parental females at 1000 and 2500ppm. The occurrence of an increased incidence of hyaline change and casts in renal tubules in males at ≥1000mg/kg is attributed to male rat-specific α2μ-globulin nephropathy, not considered relevant for humans.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date April 2005

Materials and Methods [redacted]

Results and discussion

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

98/8 Doc IIIA section No.	**6.8.2 / 02**	**Two generations reproduction study**
Annex	**II**	Multigeneration studies
Point addressed	**5.6.1 / 02**	

1. **Annex point(s)** — **IIA, 5.6.1 Reproductive toxicity - Multi-generation studies**

2. **Reference point (location) in dossier** — Volume 7, Section 3, Annex IIA, point 5.6.1/01

3. **Authors (year)** — [redacted]
   Title — Amendments 4 and 5, CGA 293'343 tech. - Rat dietary two-generation reproduction study;
   Owner, Date — includes report: Effects on sperm cell parameters.
   Syngenta Crop Protection AG, unpublished report Nos. 942121 and 982015, Syngenta File No. CGA 293343/626, 20.07.1998.
   Amended 22.10.1998 (Amendment 1), amended 12.11.1998 (Amendment 2), amended 07.01.1999 (Amendment 3), amended 26.07.1999 (Amendment 4, Syngenta file No. CGA 293343/1096), amended 25.08.1999 (Amendment 5, Syngenta file No. CGA 293343/1110)

4. **Testing facility** — [redacted]

5. **Dates of work** — 06.11.1995 - 23.09.1996, and
   16.03.1998 - 27.05.1998.

6. **Test substance** — Thiamethoxam
   [redacted]

7. **Test method** — OECD 416 ≅ FIFRA 83-4 ≅ OPPTS 870.3800 ≅ J-MAFF
   Deviations - none

8. **GLP** — Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: **Additional histopathological investigations of the testes of F_0 and F_1 generation were performed in order to discriminate between the minute *focal tubular changes* and *diffuse tubular atrophy*.**

Findings: In F_0 animals incidence and severity of both findings were similar in control and treated groups. In F_1 animals there was an increase in incidence and severity of *diffuse tubular atrophy* in the group fed 1'000 ppm but not at higher or lower feeding levels. The histological findings and the report were peer reviewed (Prentice, 1999) and the approach taken as well as the interpretation were confirmed by the expert.

Conclusion: It is concluded that the increased incidence in *diffuse tubular atrophy* of the testes at 1'000 ppm is an isolated finding and relation to treatment is unlikely.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2005
Materials and Methods	[redacted]

[redacted]

Results and discussion

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

Acceptability

[redacted]

Remarks

98/8 Doc IIIA section No.	**6.8.2 / 03**	**Two generations reproduction study**
Annex	**II**	Multigeneration studies
Point addressed	**5.6.1 / 02**	

1. **Annex point(s)** **IIA, 5.6.1 Reproductive toxicity - Multi-generation studies**

2. **Reference point (location) in dossier** Volume 7, Section 3, Annex IIA, point 5.6.1/02

3. **Authors (year)** [redacted]
   Title Morphometric Assessment of Thymic Atrophy in F1 Females of a Two-Generation Reproduction Study in the Rat with CGA 293343 tech.
   Owner Syngenta Crop Protection AG, Basel, Switzerland
   Study Report No. CB 00/18, Syngenta File N° CGA 293343/1187
   Date 25.02.2000

4. **Testing facility** [redacted]

5. **Dates of work** 08.02.2000 to 11.02.2000

6. **Test substance** [redacted]

7. **Test method** None –guideline study

8. **GLP** No (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland)

Material and methods: In each haematoxylin & eosin stained slide, a representative area of the thymic section was evaluated and the percentage of the cortical area was calculated. For statistics, t-tests (SigmaStat Version 2.03 for Windows) were applied for comparison of each treated group with the control group. The morphometric evaluation of the thymic compartments was performed on all animals except for animals 92 (10 ppm) and 182 (2500 ppm), which had died intercurrently.

Findings: The analysis revealed mean percentages of thymic cortical area of approximately 65% in all dose groups. There was no statistically significant difference to control and, in addition, there was no indication of an increasing or decreasing dose-related trend.

Conclusion: The results of the present investigation showed no effect of treatment with thiamethoxam at any dose level on the compartmental organization of the thymus in the F_1 female rats of the two-generation reproduction study. In the absence of compartmental changes, the observed treatment-related reduction of thymic weights and increased incidences of the microscopic diagnosis "thymus atrophy" might have been due to a reduced size of the whole organ, which is likely to be related to the lower body weights of all treated female groups compared to the control group.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2005
Materials and Methods	[redacted]

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

98/8 Doc IIIA section No.	**6.8.2 / 04**	**Two generations reproduction study**
Annex	**II**	Multigeneration studies
Point addressed	**5.6.1 / 01**	

1. Annex point(s)	**IIA, 5.6.1 Reproductive toxicity - Multi-generation studies**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.6.1/01
3. Authors (year) **Title** **Source** **Owner**	R.E. Chapin, R.S. Filler, D. Gulati, et al. (1992) Methods for assessing rat sperm motility. Reproductive Toxicology, Vol 6, 267-273 (1992) not applicable (publication)
4. Testing facility	Not applicable (publication)
5. Dates of work	Not applicable (publication)
6. Test substance	Not applicable (publication)
7. Test method	Not applicable (publication)
8. GLP	Not applicable (publication)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Section A6.8.2/05	Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2	Rats	
	• REFERENCE	Official use only
1.1 Reference	[redacted] Rat dietary two-generation reproduction stud in rats, tes N° 942121, [redacted] was conducted at [redacted]	
1.2 Data protection	Yes	
1.2.1. Data owner	Syngenta Ltd,	
1.2.2. Companies with letter of access		
1.2.3. Criteria for data protection		
	6 GUIDELINES AND QUALITY ASSURANCE	
2.1. Guideline study	The study was performed and conducted according to the United States Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.3800 (1988), equivalent to OECD 416 (2001)	
2.2. GLP	Yes	
2.3. Deviations	No	
	7 MATERIALS AND METHODS	
3.1. Test material	Thiamethoxam	
3.1.1. Lot/Batch number	[redacted]	
3.1.2. Specification		X
3.1.2.1. Description	Beige/yellow solid	
3.1.2.2. Purity	[redacted]	
3.1.2.3. Stability	At least 5 years	
3.2. Test Animals		
3.2.1. Species	Rat	
3.2.2. Strain	Tif:RAI	
3.2.3. Source	[redacted]	
3.2.4. Sex	Males and females	
3.2.5. Age/weight at study initiation	5 week of age	
3.2.6. Number of animals per group	140 males and 140 females supplied as 28 litters of 5 males and 5 females	
3.2.7. Mating	One female was housed with one male from the same group for a maximum period of 14 days. This was done by replacing one male with one female from the adjacent cage belonging to the same group. Thus animals with odd individual numbers were housed together and animals with even individual numbers were housed together. Brother/sister matings were avoided. Daily vaginal smears were taken from all females and examined to determine when mating had occurred as indicated by the presence of	

	sperm in the smear. The day of detection of a sperm positive vaginal smear was designated gestation day 1. a female with a sperm positive vaginal smear was separated from the male and no further smears were taken. Satellite males were not housed for mating.
3.2.8. Duration of mating	One female was housed with one male from the same group for a maximum period of 14 days
3.2.9. Deviations from standard protocol	
3.2.10. Control animals	Yes
3.3. Administration/ Exposure	Oral
3.3.1. Animal assignment to dosage groups	See table below
3.3.2. Duration of exposure before mating	The duration of the pre-mating period was ten weeks from the start of the study for the F0 parents and at least ten weeks from selection for the second (F1) generation parents
3.3.3. Duration of exposure in general P, F1, F2 males, females	F0 received test substance ten weeks before mating F1 parents received test substance at least ten weeks
	Oral
3.3.4. Type	Test substance was administered in the diet
3.3.5. Concentration	0 (control), 20, 50, 1000 or 2500 ppm thiamethoxam Feed was available ad libitum
3.3.6. Vehicle	Diet
3.3.7. Concentration in vehicle	0 (control), 20, 50, 1000 or 2500 ppm
3.3.8. Total volume applied	
3.3.9. Controls	Yes
3.4. Examinations	
3.4.1. Clinical signs	All F0 and F1 rats (including satellite F1 males) were checked daily throughtout the study and significant changes in clinical condition were recorded. Detailed observations were recorded at the same time that each rat was weighed
3.4.2. Mortality	
3.4.3. Body weight	Bodyweights were recorded for each F0 and F1 male rat (including satellites) at weekly intervals throughtout the study and at termination and for F0 and F1 female rat during the pre-mating period. Females with positive indication of mating were weighed on days 1, 8, 15 and 22 of gestation and females with litters were weighed on days 1, 5, 8, 15 and 22 *post partum*. All females were weighed prior to termination. A bodyweight was recorded for F1 rats on the day of preputial separation or vaginal opening.
3.4.4. Food/water consumption	Food consumption was recorded for each cage of F0 and F1 rats on a weekly basis (g/food/rat/day) during the pre-mating period. Following completion of the mating period, food consumption was resumed for

	each cage of F0 and F1 male rats on a weekly basis. During gestation, weekly food consumption was recorded for the females with positive indication of mating and post partum, weekly food consumption was recorded for the females with litters. Food consumption for the F1 generation (including satellite males) commenced on the day the first rat selected was placed in each cage.
3.4.5. Oestrus cycle	For 25 days prior to the mating period, daily vaginal smears were taken and examined from all F0 and F1 females and cyclicity assessed. A vaginal smear was taken and examined from each F0 and F1 female on the day of scheduled termination and the stage of oestrus was recorded.
3.4.6. Sperm parameters	*Sperm count, motility and velocity*: the right cauda epididymis was removed and weighed and a sample of sperm prepared for all males at scheduled termination excluding the satellite F1 males. The samples were analysed using a Computer-Assisted Sperm Analyser (CASA, Hamilton Thorne), for total and static count and straight line, curvilinear and average path velocity. Percentage motility and motile count were calculated from the total and static count. A sample of minced cauda epididymis was stained with IDENT, a DNA specific stain and the number of sperm in the sample were counted using the CASA fluorescent mode. *Sperm morphology*: samples of minced cauda epididymis were stained using a multicoloured single step stain (0.8% trypan blue, 0.4% naphtthol yellow, 0.2% eosin Y in 1% acetic acid) and the morphology of the sperm evaluated for all males at scheduled termination excluding the satellite F1 males *Homogenisation resistant testicular sperm*: the right testis was taken from all males at scheduled termination for analysis of homogenisation resistant testicular spermatid head count. Samples from the F0 and F1 males (excluding satellite F1 males) in each of the control and high dose groups were calculated.
3.4.7. Offspring	Examination of pups for anormalies The day of littering was designated day 1 *post partum*. The sex, bodyweight and clinical condition of each pup was recorded as soon as possible after completion of parturition and always within 24 hours and again on days 5, 8, 15 and 22 *post partum*. F1A and F2A pups were not individually identified and data were presented by litter and sex. Each litter was examined daily for dead, unhealthy or abnormal pups. Pups which required euthanasia were killed humanely. These and any pups found dead were given a macroscopic examination. The pups were given a macroscopic examination which involved an examination of the cranial, thoracic and abdominal organs and structures.
3.4.8. Organ weights P and F1	*The weights of the following organs were recorde from all F0 and F1 rats (excluding satellite F1 males) at scheduled termination:* adrenal glands, brain, left epididymis (including cauda), right epididymis (including cauda), right cauda epididymis, kidneys, liver, ovaries, pituitary gland, prostate gland, seminal vesicles (with prostate, coagulating gland and fluids), spleen, left testis, right testis and uterus (with oviducts and cervix) Paired organs were weighed together unless stated otherwise. A total

weight was calculated for paired organs weighed separately.

The weight of the seminal vesicles (with coagulating gland and fluid) was calculated by deducting the weight of the prostate from the combined weight of prostate and seminal vesicles (with coagulating gland and fluid)). The purpose of doing this was to prevent loss fluid through trimming.

The weights of te following organs were recorded from one male and one female pup per litter (selected from the 3 per sex per litter for macroscopic examination) at scheduled termination: brin, spleen and thymus

3.4.9. Histopathology P and F1

The following tissues were taken from all F0 and F1 rats (excluding satellite F1 males): adnormal tissue, adrenal gland, cervix, kidney (target organ), left epididymis, mammary gland (females only), ovary, pituitary gland, prostate gland, seminal vesicle including coagulating gland, left testis, efferent ductules, uterus with oviducts, vagina

For each testis, four transverse sections were examined ant the number of tubular cross sections showing germ cell loss was recorded for each section separately. For each affected tubular cross-section, the degree of germ cell loss was described as either complete or partial/complete. In addition, the distribution of affected tubules within the testis cross-section was described as either focal or multifocal/diffuse.

3.4.10. Histopathology F1 not selected for mating, F2

See 3.4.9.

3.5. Further remarks

8 RESULTS AND DISCUSSION

4.1. Effects

The following numbers of animals died prior to scheduled termination:

	Dose level of Thiamethoxam (ppm)				
	0	20	50	1000	2500
F0 Males	0	0	0	0	0
F0 Females:	2	2	0	4	1
Found dead	0	1	0	0	0
Killed following whole litter loss*	1 (4)	1 (5)	0 (1)	3 (4)	0 (1)
Killed/died difficult parturition	1	0	0	1	1
F1 Males (including satellites):	1	0	0	0	0
Killed due to clinical signs	1	0	0	0	0
F1 Females:	3	7	6	5	4
Killed failed to litter	3	3	2	2	0
Killed following whole litter loss	0	4	4	3	4

* not all F0 females with whole litter loss were killed prior to scheduled termination (see section 4.12.1). The actual number with whole litter loss is given in parentheses.

The F0 female found dead in the 20 ppm group, died on day 9 post partum. No cause of death was established: there were no changes in clinical condition or loss of bodyweight and no abnormalities were detected at examination post mortem.

One F1 control male was killed in week 18 because it had reduced hind limb function and was seen to be dragging its hind legs. Also, it had lost 34g bodyweight in a week. No significant findings were detected at examination post mortem.

None of the intercurrent deaths was considered to be treatment-related

Clinical observations: there was no effect of thiamethoxam on the

clinical condition of the F0 and F1 parent animals or the F1 satellite males

4.1.1. Parent males

Bodyweights:

Bodyweight adjusted for initial weight were statistically significantly lower in the 2500 ppm group, in comparison with the control group, from week 2 through to week 28. At the end of the pre-mating period the bodyweights were approximately 6% lower than in the control group and approximately 7% lower at week 28.

Bodyweights adjusted for initial weight were marginally lower in the 100 ppm group. The differences from control did not exceed 3% and a statistically significantly difference was obtained for week 4 only.

There was no effect of 50 or 20 ppm thiamethoxam on the bodyweight of the F0 males.

Food consumption and food utilisation:

Food consumption was lower in the 2500 ppm group in comparison with the contrl group throughtout the treatment period and statistically significant differences were obtained for the first 7 weeks of treatment and for weeks 14, 16 and 17. No food consumption was measured for weeks 11 and 12, whilst the animals were housed for mating.

Food utilisation during the pre-mating period was statistically significantly lower for the F0 males given 2500 ppm than in control for weeks 1-4 and consequently for the overall period, weeks 1-10.

There was no effect of 20, 50 or 1000 ppm thiamethoxam on food consumption or utilisation

4.1.2. Parent females

Bodyweights:

There was no effect of thiamethoxam on the bodyweight of the F0 females during the pre-mating period. There was no effect of thiamethoxam on the bodyweight of the F0 females during gestation and *post partum*

Food consumption and food utilisation:

There was no effect of thiamethoxam on the food consumption of the F0 females during the pre-mating period or on food utilisation.

There was no effect of thiamethoxam on the food consumption for F0 females durng gestation

For the F0 females with litters, food consumption was statistically significantly lowerin the 2500 ppm group in comparison with the control group for week 3 *post partum*. There was no effect of 100, 50 or 20 ppm thiamethoxam on food consumption of the F0 females *post partum*.

Pre-mating vaginal smears:

There was no effect of thiamethoxam on mean cycle length or on thenumber of cycles in the 25 day period for F0 females

Reproductive performance:

There was no effect of thiamethoxam on pre-coital interval and on the length of the gestation for F0 females

For F0 females, the proportion and percentage of successful matings were lower in the 20 and 2500 ppm groups in comparison with the control group but were not statistically significantly different. In the absence of a relationship with dose, the variation in the proportion of successful matings was considered to be incidental to treatment with

thiamethoxam. None of the reasons for an unsuccessful mating was considered to be treatment related. The outcome of mating of each generation is shown below:

	Dose level of Thiamethoxam (ppm)				
	0	20	50	1000	2500
F0 Matings	26	26	26	26	26
Number of successful matings	21	18	24	20	18
Number of females which littered but no live pups were found ^	1	3	0	4	1
Number of non pregnant females	2	5	1	1	5
Number of females which failed to litter with uterine implantation sites	1	0	1	0	1
Died due to difficult parturition	1	0	0	1	1

	Dose level of Thiamethoxam (ppm)				
	0	20	50	1000	2500
F1 Matings	26	26	26	26	26
Number of successful matings	23	23	24	21	26
Number of females which littered but no live pups were found^	0	0	0	3	0
Number of non pregnant females	1	3	1	1	0
Number of females which failed to litter with uterine implantation sites	2	0	1	1	0
Died due to difficult parturition	0	0	0	0	0

4.1.3. F1 males

Bodyweights:

There was no effect of thiamethoxam on the bodyweight of the F1 males (main study and satellite males) during the study

Food consumption and food utilisation:

There was no effect of thiamethoxam on the food consumption of the F1 males (main study and satellite males) during the study. Statistically significantly higher food consumption values were obtained for the main study males in the 2500 ppm group in comparison with the control for weeks 24, 25 and 26. Statistically significantly higher food consumption values were obtained for the satellite males in the 100 ppm and 2500 ppm groups in comparison with the controls for weeks 26 and 27. these higher values of a transient nature, were considered to be incidental to treatment with thiamethoxam. Other statistically significant differences in the food consumption of the satellite males did not form part of a dose-response and were also considered to be incidental to treatment with thiamethoxam

There was no effect of thiamethoxam on food utilisation during the pre-mating period.

4.1.4. F1 females

Bodyweights:

There was no effect of thiamethoxam on the bodyweight of the F1 females during the pre-mating period. There was no effect of thiamethoxam on the bodyweight of the F1 females during gestation and *post partum*

Food consumption and food utilisation:

There was no effect of thiamethoxam on the food consumption of the F1 females dring the pre-mating period or on food utilisation.

There was no effect of thiamethoxam on the food consumption for F0 females durng gestation

No statistically significant differences from control were observed for

F1 females and litters *post partum*.

Pre-mating vaginal smears:

There was no effect of thiamethoxam on mean cycle length or on thenumber of cycles in the 25 day period for F1 females

Reproductive performance:

There was no effect of thiamethoxam on pre-coital interval and on the length of the gestation for F1 females

There was no effect of 2500, 1000, 50 or 20 ppm thiamethoxam on the proportion of successful matings of the F1 females

4.1.5. F2 males

4.1.6. F2 females

4.2. Other

Investigations post mortem-parents:

Organ weights:

There was no effect of thiamethoxam on the weight of the brain, right cauda epididymis, ovaries, prostate gland, seminal vesicles, or uterus with cervix.

For the F0 males, adrenal and kidney weights adjusted for bodyweight were statistically significantly greater in the 2500 ppm group in comparison with the control group. Similar differences from control were not seen in the F0 males given 20, 50 or 1000 ppm thiamethoxam. There were no statistically significantly significant differences in adrenal or kidney weight, absolute or adjusted for bodyweight, for F1 males, F0 or F1 females in any of the thiamethoxam treated groups.

There were no statistically significantly differences in liver weight, absolute or adjusted for bodyweight, for the F0 animals in the thiamethoxam treated groups other than the F0 males given 1000 ppm. The weight of the liver adjusted for bodyweight for the F1 males and females given 2500 ppm, was statistically significantly greater than in the control group. A similar difference from control was not seen in the F1 males and females given 20, 50 or 1000 ppm thiamethoxam.

For the F0 females given 1000 and 2500 ppm, pituitary weights absolute and adjusted for bodyweight were statistically significantly lower than in the contrl group. Similar differences from control were not seen in th F0 females given 20 or 50 ppm thiamethoxam. There were no statistically significantly differences in pituitary weight, absolute or adjusted for bodyweight, for F0 males or F1 males and females, in the thiamethoxam treated groups.

There were no statistically significant differences in spleen weight, absolute or adjusted for bodyweight, for the F0 animals in the thiamethoxam treated groups. However, the mean spleen weight for the F0 males given 2500 ppm was notably higher than in the control group. This was due to one male (number 120) with an enlarged spleen (weight 4.569 g).

For the F1 males given 2500 ppm the weight of the spleen adjusted for bodyweight was statistically significantly greater than in the control group. A similar result was not recorded for the F1 males given 20, 50 or 1000 ppm or for any of the F1 females given thiamethoxam.

There were no statistically significant differences in the absolute weight of the epididymides (separately or combined), or in the weights adjusted for bodyweight, for the F0 males in the thiamethoxam treated groups. The weight of the combined epididymides for the F1 males given 2500 ppm, was statistically significantly greater than in the control group and

the weight of the combined epididymided adjusted for bodyweight was statistically significantly greater than in thecontol group for the F1 males given 1000 or 2500 ppm. Similar differences from control were not seen for the F1 males given 20 or 50 ppm thiamethoxam.

There were no statistically significant differences in the testis weight, absolute or adjusted for bodyweight, for the F0 males in te thiamthoxam treated groups. For the F1 males given 20, 1000 or 2500 ppm, the absolute weight of the testes (combined and separate) and the weights adjusted for bodyweight were statistically significantly greater than in the control group. There were no statistically significant differences in testis weight, absolute or adjusted for bodyweight, for the F1 males given 50 ppm. There was no evidence for a dose-response on testis weight.

Sperm parameters:

Data were analysed with and without the following males, which were found to have macroscopically abnormal testis and/or cauda epididymis and poor sperm samples. The data excluding the abnormal samples are the most appropriate for evaluation although the data including them are presented for completeness.

Dietary concentration	F0 male number	Comments
0 ppm	8	Right cauda reduced, no sperm visible. Testis reduced few sperm visible.
20 ppm	34	Right cauda reduced, no sperm visible. Testis reduced few sperm visible.
	48	Right cauda reduced, no sperm visible. Testis reduced few sperm visible.

Dietary concentration	F0 male number	Comments
1000 ppm	80	Right cauda reduced, no sperm visible. Testis reduced few sperm visible.
	101	Right cauda reduced, no sperm visible. Testis reduced few sperm visible.
2500 ppm	109	Right cauda reduced, no sperm visible. Testis reduced few sperm visible.
	117	Right cauda reduced, few sperm visible. Testis reduced few sperm visible. Sperm abnormal.
	129	Mass on right cauda. Sperm abnormal.

Dietary concentration	F1 male number	Comments
2500 ppm	122	Right cauda reduced. Sperm abnormal.

The occurence of these males with poor sperm samples, thought to be associated with the abnormalities in thes testis and/or cauda epididymis, was considered to be incidental to treatment with thiamethoxam.

Sperm count, motility and velocity:

There was no effect of thiamethoxam on the number of sperm in the right cauda epididymis of the F0 males. For the F1 males given 2500 ppm the total number of sperm and the number of sperm per gram of right cauda epididymis were statistically significantly higher than in the control group.

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

For the F0 males, there was no effect of thiamethoxam on straight line, curvilinear or averagepath velocities. For the F1 males given 2500 ppm,