the straight line, curvilinear and average path velocities were statistically significantly lower in comparison with the control group. There was no effect of thiamethoxam on straightness.

Sperm morphology:

There was no effect of thiamethoxam on sperm morphology in either generation. For the F0 males given 2500 ppm the mean percentage of abnormal sperm was statistically significantly higher than in the control group. There was a high percentage of abnormal sperm, with abnormal/detached heads predominantly, which was due solely to males number 117 and 129; these males had already been shown to have poor quality sperm samples associated with macroscopic abnormalities in the testis and/or cauda epididymis. Exclusion of these animals from evaluation of the group mean data confirmed that there was no effect of thiamethoxam on the morphology of the sperm of the F0 males. This conclusion was also supported by the sperm morphology data for the F1 males where there was no evidence for any effect of thiamethoxam.

No macroscopic findings were observed in any tissue from the F0 or F1 animals that could be attributed to treatment with thiamehtoxam.

Uterine implantations: There was no effect of thiamethoxam on the number of uterine implantation sites or on post-implantation loss for either the F0 or F1 females.

Microscopic findings: increased tubular hyaline droplet formation was observed in the kidney of the majority of FFF0 males given 2500 ppm thiamethoxam and in a few given 100 ppm. Similar findings were observed in F1 males except that the incidence in F1 males given 1000 ppm (17/26) was much higher than in the F0 generation (3/26). This change was not observed in any control male or in males given 20 or 50 ppm, in either generation or in any female.

Other minor kidney changes observed at an increased incidence in males given 2500 ppm were hyaline eosinophilic casts, tubular dilatation with granular eosinophilic casts at the cortico-medullary junction, tubular basophilia and interstitial mononuclear cell infiltration. Slight increases in the incidences of hyaline eosinophilic casts and tubular basophilia compared with controls were also observed in F1 males given 1000 ppm

Germ cell loss, recorded under 4 morphologies, according to degree and distribution was observed in the testis of some treated and control males. Since the 4 categories clearly represented variable degrees of a single pathological process they were maerged to a single finding i.e. germ cell loss/disorganisation +/- sertoli cell vacuolation for the purposes of tabulation and evaluation.

An increased incidence (14/26) of minimal germ cell loss/disorganisation +/- sertoli cell vacuolation was observed in the testis of F1 males given 2500 ppm compared with the control and lower dose groups (1/26-3/26). The change was extremely minor affecting a few scattered tubules; minimal change being defined as affecting an average of not more than 10 tubules/section for the 4 sections examined from each testis. It was characterised by variable germ cell loss ranging from complete depltion to minimal loss of a few germ cells in one segment of a tubular cross section. In some instances, partial germ cell loss was associated with germ cell disorganisation and Sertoli cell vacuolation indicating a degenerative process. The change was not associated with any discernible reduction in epididymal sperm or increase in epididymal desquamated germcells. The average total number of tubular cross sections affected in all 4 sections per testis examined from affected males receiving 2500 ppm was 10.2 which represents approximately 0.4% of tubular cross sections in 4 average

testis sections. This calculation is based on an approximate count of tubular cross sections in the 4 standard testis sections from a representative control male conservatively estimated at 2500. the change was most frequently observed in regions adjacent to the rete testis.

A small number of F0 males given 20 ppm (2/26) or 2500 ppm (3/26) showed severe germ cell loss, affecting neraly all tubules which amounted to near total tubular atrophy. These were considered to be spontaneous lesions, unrelated to treatment, as the incidence was los, unrelated to dose and not repeated in the F1 generation and the changes were identical to those observed in control animals of this strain.

For the main study males, only one testis was available for histological examination and so it was not possible to determine whether changes were unilateral or bilateral. For this reason, a satellite group of F1 males was used from which both testes were available. Minimal testicular tubular changes were observed as on the main study. However, a treatment-related increase in incidence of germ cell loss/disorganisation +/- sertoli cell vacuolation was confined to minimal bilateral change at 2500 ppm only. The average number of tubular cross sections affected/testis in the 4 sectons/testis examined from satellite males receiving 2500 ppm thiamethoxam which showed bilateral change was 9.1 i.e similar to the corresponding figure from the main study F1 males and again representing approximately 0.4% of tubular cross sections in the 4 sections examined.

Unilateral change (in the satellite groups) was observed at a low incidence, unrelated to dose, in control and treated groups suggesting that, in contrast to the bilateral change, this was a spontaneous lesio. It was not possible to distinguish between unilateral and bilateral changes in the main study animals since only one testis was examined.

Therefore,, the incidence of lesions reported for the main study animals represents a summation of spontaneous and treatment-related lesions.

The testicular histology data from main study F1 and satellite F1males are summarised below and show the overall incidence of germ cell loss/disorganisation +/- sertoli cell vacuolation in both groups. When the incidences of change in both groups are added together regardless of unilateral or bilateral status the data confirms the increase at 2500 ppm thiamethoxam with a clear no effect level at 1000 ppm. Although the incidence was clearly increased at 2500 ppm the nature and severity of the lesion was identical to that observed at a low incidence in control and lower dose group F1 animals and in all goups of F0 males.

Incidence of Tubules with Germ Cell Loss/Disorganisation +/- Sertoli Cell Vacuolation

	Dose level of Thiamethoxam (ppm)				
	0	20	50	1000	2500
Total number of F1 Males (main study + satellites)	40 (26+14)	40 (26+14)	40 (26+14)	40 (26+14)	40 (26+14)
Main study: uni/bilateral status unknown	3/26	1/26	1/26	3/26	15/26
Satellites: unilateral	1/14	4/14	2/14	3/14	0/14
Satellites: bilateral	1/14	0/14	0/14	1/14	5/14
Total incidence	5/40	5/40	3/40	7/40	20/40

No changes were detected in any other tissue from F0 or F1 animals that could be attributed to treatment with thiamethoxam.

Oocyte count: the numbers of small follicles in the ovaries of F1 females receiving 2500 ppm thiamethoxam were comparable to the numbers observed in control females

Investigations post mortem – litters:

No macroscopic changes were observed in F1A or F2A pups, which could be related to treatment with thiamethoxam.

Organ weights: there was no effect of thiamethoxam on theweight of the brain, spleen or thymus of the F1A or F2A litters.

9 APPLICANT'S SUMMARY AND CONCLUSION

5.1. Materials and methods

5.2. Results and discussion

The purpose of this study was to investigate the effect of continuous feeding of diets containing thiamethoxam on the propagation of two generations of theTif: RAIf rat was successfully accomplished.

Analysis of the diets showed that the achieved concentrations, homogenicity and chemical stability were satisfactory throughout the study. There was no effect of thiamethoxam on the clinical condition of the F0 or F1 animals.

There was an effect of thiamethoxam on bodyweight with lower bodyweights being recorded for the F0 males given 2500 ppm in comparison with control group. This effect was accompanied by a reduction in food consumption and food utilisation during weeks 1-4. the lower bodyweights of the F0 males given 1000 ppm which were not statistically significantly different from the control bodyweights (with one exception at week 4), were not associated with any reduction in food consumption and were therefore considered to be of no toxicological significance.

There was no effect of 1000 ppm or 2500 ppm thiamethoxam on the bodyweight or food consumption of the F1 males or on the bodyweight or food consumption of the F0 and F1 females during the pre-mating period, gestation or *post partum*.

There was no effect of 20 or 50 ppm thiamethoxam on the bodyweight or food consumption of the F0 or F1 animals.

For females with litters, food consumption was lower in the 2500 ppm group in comparison with the control group for week 3 post partum for the F0 females but not the F1 females. At this time the pups are also consuming diet and so it cannot be determined whether the mother, the pups or both had a reduced food consumption.

There was no effect thiamethoxam on oestrus cyclicity, pre-coital interval, the duration of gestation or on the success of mating.

There was no effect of thiametoxam on the proportion or percentage of pups live born or on the proportion of litters with all pups born alive for either the F1A or F2A litters.

There was no effect of thiamethoxam on mean litter size or pup bodyweight on days 1, 5 and 8. however, on days 15 and 22, a lower total litter weight for the F1A pups given 2500 ppm was distributed to a slightly smaller litter size and a slightly lower up bodyweight (rather than a slightly higher pup bodyweight typically associated with smaller litters). This was considered due to the direct consumption of diet containing 2500 ppm by the pups and therefore a treatment-related effect. A similar effect was not seen with the F2A litters.

The incidence of whole litter losses was unrelated to treatment with thiamethoxam and there was no effect of thiamethoxam on pup survival.

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

In summary, the fertility and reproductive performance of two generations of parental animals (F0 and F1) were not affected by theadministration of thiamethoxam on the survival or development of the pups born to the parental animals.

Increased kidney weights adjusted for bodyweight were noted for the F0 males given 2500 ppm. Treatment-related histological change was seen in the kidneys of the F0 and F1 males given 1000 or 2500 ppm. The histological change was consistent with α-2μ globulin neophropathy seen previously in rats treated thiamethoxam (Weber, 2000a, b, c, d). This effect is a phenomenon specific to the male rat, and of no relevance to human risk assessment. The primary change was increased hyaline droplet accumulation within the proximal tubular epithelium and in the tubular lumen. The other minor changes described resulted from mild tubular damage associated with excess α-2μ globulin accumulation. The changes were less pronounced in males given 1000 ppm. Evidence of tubular damage in F1 males given 1000 ppm was greater than that in F0 males which, probably reflected the longer period of exposure to thiamethoxam compared with F0 males.

Te following changes in organ weights were not seen consistently in both sexes or were not accompanied by any histological change and were therefore considered unlikely to be of toxicological significance:

- Higher liver weight adjusted for bodyweight in the F1 males and females given 2500 ppm
- Lower pituitary weight, absolute andadjusted for bodyweight, in the F0 females given 1000 or 2500 ppm
- Higher spleen weight adjusted for bodyweight in the F1 males given 2500 ppm
- Higher adrenal weight adjusted for bodyweight in the F0 males given 2500 ppm

there was no effect of thiamethoxam on the weight of the brain, ovaries, prostate gland, seminal vesicles, or uterus with cervix in either generation.

Testis weights were statistically significantly higher in the F1 males given 20, 1000 or 2500 ppm in comparison with the control males, but not in those given 50 ppm. This was not associated with any histological change considered likely to result in increased weight. In addition, all group mean values for testis weight were within the range of values obtained for control animals from two contemporary studies conducted to the same design in the same strain of rat. In the abscenc of any dose response, or an association with any histological change considered likely to result in increased weight, these higher testis weights at 20, 1000 and 2500 ppm were considered to be incidental to treatment.

	Current study July 2002		Control study July 2002		Control study August 2002		Control range	Current study July 2002
Organ weight (g)	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 + F1 0 ppm	F1 20, 50, 1000, 2500 ppm
Testes								
Mean	4.18	3.89	4.11	4.01	4.33	4.01	3.89 – 4.33	4.15, 4.02, 4.13, 4.19
S.D.	0.34	0.29	0.34	0.82	0.41	0.42		
N	26	25	26	26	26	25		

epidydimis weights were statistically significantly higher in the F1 males given 1000 or 2500 ppm in comparison with the control males. This increase was not associated with any histological change considered likely to result in increased weight. In addition, all group mean values for epididymis weight were within the range of values obtained in the same strain of rat. Thus, the slightly higher epididymis

weights, possibly related to treatment with thiamethoxam, were in the absence of any associated histological findings considered unlikely to represent an adverse effect.

	Current study July 2002		Control study July 2002		Control study August 2002		Control range	Current study July 2002
Organ weight (g)	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 + F1 0 ppm	F1 20, 50, 1000, 2500 ppm
Epididymides								
Mean	1.661	1.580	1.549	1.627	1.755	1.668	1.549 - 1.755	1.627, 1.616, 1.656, 1.659
S.D.	0.169	0.124	0.179	0.131	0.141	0.198		
N	26	25	26	25	26	25		

A very minor testicular tubular lesion (minimal germ cell loss /disorganisation +/- Sertoli cell vacuolation) was confined to the F1 males given 2500 ppm thiamethoxam. Findings in the satellite group of F1 males where both testes were available for histological examination indicated that the treatment-related lesion was bilateral. The change itself was of a vry minor nature, identical to that observed at a lower incidence in control animals and localised to a very small number of tubules. On the basis of the small number of tubules. On the basis of the small number of tubular cross sections affected (in the order of 0.4%) it was considered highly unlikely that this finding would have any effect on organ weight or any functional effect on spermatogenesis or fertility. This study demonstrated that were no functional effects on spermatogenesis or fertility.

There was no effect of treatment on sperm motility. For sperm from the F1 males given 2500 ppm, the straight line, curvilinear and averae path velocities were statistically significantly lower in comparison with the concurrent control group. The magnitude of difference from control was very small (approximately 5% reduction). The data from this study, in context with the historical range of values obtained for control animals from two contemporary studies conducted to the same design in the same strain of rat are tabulated below.

	Current study July 2002		Control study July 2002		Control study August 2002		Control range	Current study July 2002
	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 + F1 0 ppm	F1 2500 ppm
Straight line velocity								
Mean	76.7	71.6	75.2	64.9	72.7	73.7	64.9-76.7	68.1
S.D.	5.5	5.6	4.9	7.0	6.7	6.7		5.4
N	25	25	26	25	26	25		25
Curvilinear velocity								
Mean	316.1	305.0	279.7	277.9	301.7	306.2	277.9-316.1	291.9
S.D.	18.6	16.7	14.2	20.1	23.6	17.1		14.7
N	25	25	26	25	26	25		25
Average path velocity								
Mean	130.7	123.9	118.9	112.6	124.6	124.0	112.6-130.7	117.2
S.D.	7.2	7.5	6.6	8.6	7.9	8.0		6.1
N	25	25	26	25	26	25		25

Data do not include exclusions (i.e. poor sperm samples)

The data show that the mean velocities for the 2500 ppm group were within normal variability. It was therefore concluded that thiamethoxam at dose levels up to and including 2500 ppm had no effect on sperm velocity.

The number of sperm in the right cauda epididymis when expressed as a total number and per gram of the right cauda was statistically significantly higher in the F1 males given 2500 ppm in comparison with the concurrent control group. When considered along-side the historical

data, the values for te 2500 ppm group were seen to be slightly higher than the range of historical control values. However, histological examination of the epididymis did not indicate any treatment-related effects and morphological examination of the sperm did not reveal any treatment-related abnormality. Although possibly treatment-related, the higher number of sperm in the right cauda epididymis was considered unlikely to be of toxicological significance and thus not an adverse finding.

	Current study July 2002		Control study July 2002		Control study August 2002		Control range	Current study July 2002
	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 + F1 0 ppm	F1 2500 ppm
Total no. sperm in cauda								
Mean	148	153	146	137	154	170	137-170	192
S.D.	39	38	38	51	45	61		37
N	25	25	26	25	26	25		25
No. sperm per g cauda								
Mean	472	505	502	458	479	545	458-545	620
S.D.	112	94	159	172	135	170		122
N	25	25	26	25	26	25		25

Data do not include exclusions (i.e. poor sperm samples)

The number of sperm in the right testis when expressed as a total number and per gram of tissue was statistically significantly lower in the F1 males given 50, 1000 or 2500 ppm in comparison with the concurrent control group. There was no evidence for a dose-related effect and when considered along-side the historical data, the values for the 50 and 2500 ppm groups were seen to be within the control range and the values for the 1000 ppm group were only marginally below. From the lack of dose-response, despite the 50 fold difference in dose between 50 and 2500 ppm, and because of the consistency of the values with the historical control data it was concluded that this did not represent an effect of treatment on the number of sperm in the testis. In addition, the minimal histological effect of treatment seen in the testis of the F1 males given 2500 ppm was considered not to have any potential for impact on sperm number.

	Current study July 2002		Control study July 2002		Control study August 2002		Control range	Current study July 2002
	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 0 ppm	F1 0 ppm	F0 + F1 0 ppm	F1 50, 1000, 2500 ppm
Total no. sperm in testis								
Mean	98	87	93	85	103	69	69-103	70, 63, 74
S.D.	20	22	13	16	15	18		19, 16, 18
N	25	25	26	25	26	25		26, 26, 25
No. sperm per g testis								
Mean	55	52	52	48	56	42	42-56	42, 36, 43
S.D.	11	14	5	8	7	10		10, 9, 10
N	25	25	26	25	26	25		26, 26, 25

Data do not include exclusions (i.e. poor sperm samples)

In summary, 1000 and 2500 ppm thiamethoxam in diet was associated with histological change in the kidney of the F0 and the F1 males. Histological change in the testis was seen in F1 males given 2500 ppm. The change was considered to be minimal, affecting only a very small number of tubles, with no effect on reproductive function or spermatogenesis. There was a possible treatmet-related increase in epididymal weight in F1 males given 1000 or 2500 ppm, and increase in epididymal sperm number in F1 males given 2500 ppm, both were considered not to be adverse.

5.3. Conclusion

The fertility and reproductive performance of two generations of parental animals (F0 and F1) was not affected by the administration of thiamethoxam and there was no effect on the survival or development of the pups born to the prental animals. The no observd effect level for reproductive effects was 2500 ppm thiamethoxam.

Minimal histological change in the testis of the F1 males given 2500 ppm was considered not to represent an adverse effect of treatment.

Possible treatment-related increases in epididymal weight in F1 males given 1000 or 2500 ppm, and in epididymal sperm number in F1 males given 2500 ppm, were also considered not to be adverse.

Thiamethoxam at 1000 and 2500 ppm in diet was associated with histological change in the kidney of the F0 and F1 males. The no effect level in this study based on pathological change in the kidney was 50 ppm thiamethoxam.

5.3.1. LO(A)EL

5.3.1.1. Parent males

5.3.1.2. Parent females

5.3.1.3. F1 males

5.3.1.4. F1 females

5.3.1.5. F2 males

5.3.1.6. F2 females

5.3.2. NO(A)EL

5.3.2.1. Parent males

5.3.2.2. Parent females

5.3.2.3. F1 males

5.3.2.4. F1 females

5.3.2.5. F2 males

5.3.2.6. F2 females

5.3.3. Reliability

5.3.4. Deficiencies

Section A6.8.2/05	**Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2	**Rats**

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007

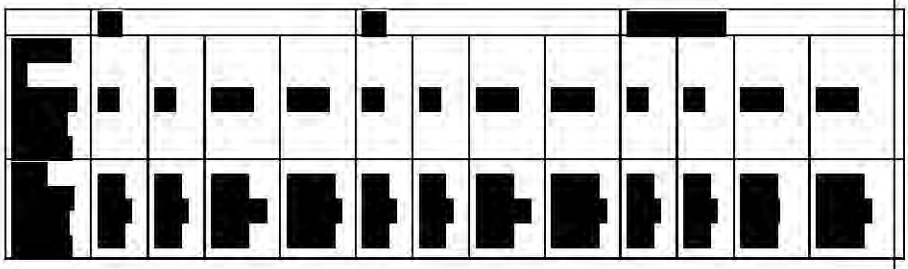
Section A6.8.2/05	**Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2	**Rats**

Materials and Methods

[redacted]

Results and discussion

[redacted]



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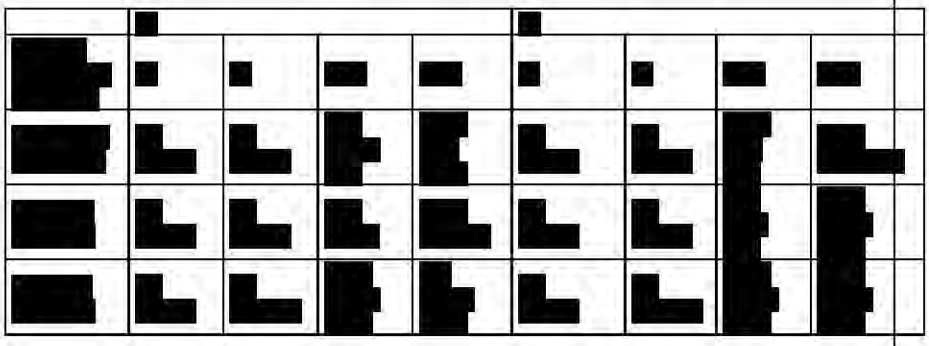

[redacted]

After Thiamethoxam administration, there was no increase of gestation length in

Section A6.8.2/05 **Multigeneration Reproduction Toxicity Study**

Annex Point IIA6.8.2 **Rats**

[redacted]

[redacted]

[redacted]

[redacted]

	[redacted]				
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] [redacted] [redacted]	[redacted] [redacted] [redacted]	[redacted] [redacted] [redacted]	[redacted] [redacted] [redacted]	[redacted] [redacted]	[redacted] [redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted] [redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted] [redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

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Section A6.8.2/05	**Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2	**Rats**

Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

Animal assignment to dosage groups

The following regime was used for both the F0 and F1 parental generations.

Group	Dietary Concentration of Thiamethoxam (ppm)	Colour code	Identities of rats	
			Males	Females
1	0 (control)	blue	1-26	131-156
2	20	green	27-52	157-182
3	50	yellow	53-78	183-208
4	1000	red	79-104	209-234
5	2500	black/white	105-130	235-260

Satellite F1 males: (14 per group) were selected for the study as described in section 4.11.2, to generate histological data on the testis only. These males were given the same identity number as their parent female.

Group	Dietary Concentration of Thiamethoxam (ppm)	Colour code	Identities of rats
1	0 (control)	blue	134, 136, 137, 138, 140, 144, 145, 146, 147, 150, 151, 152, 153, 156
2	20	green	157, 158, 159, 160, 161, 165, 166, 168, 172, 174, 175, 180, 181, 182
3	50	yellow	183, 184, 186, 188, 189, 190, 191, 196, 198, 199, 201, 202, 205, 207
4	1000	red	209, 211, 212, 214, 215, 217, 218, 224, 225, 227, 230, 231, 232, 233
5	2500	black/white	235, 237, 238, 240, 241, 242, 243, 244, 246, 251, 257, 258, 259, 260

98/8 Doc IIIA section No. 6.10 / 01	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: *in vivo* hepatic cell proliferation; 28-days feeding**
91/414 Annex II Point addressed 5.8.2 / 02	Studies on tumour promotion

1. Annex point(s)	**IIA, 5.8.2 Studies on tumour promotion**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2
3. Authors (year) **Title** **Owner, Date**	[redacted] Assessment of replicative DNA synthesis in the course of a 28-day oral (feeding) toxicity study in male rats. Syngenta Crop Protection AG, unpublished report No. CB94/47, February 27, 1995
4. Testing facility	[redacted]
5. Dates of work	November 3, 1994 - December, 1994
6. Test substance	CGA 293343 tech., [redacted] (, 1994, Syngenta File No. CGA293343/0904)
7. Test method	Not applicable – investigative study
8. GLP	Partially fulfilled (laboratory certified by the Eidgenössisches Department des Inneren (Federal Department of Home Affairs), Bern, Switzerland); no procedural quality assurance inspections or report audits were performed.

Material and methods: Thiamethoxam ([redacted]%), was administered orally for 28 days to groups of 5 male and 5 female Tif: RAIf (SPF) rats, by admixture in the diet, at concentrations of 0, 100, 1000, 2500 and 10000 ppm, as reported in detail in unpublished report no. 942088 ([redacted][4], 1995). Liver samples from male rats in this study were used for the assessment of proliferating cell nuclear antigen (PCNA). Four liver tissue slices from the left, median and right lobes, from all male animals were fixed and embedded in 2 paraffin blocks. Three 5µm sections of one paraffin block containing samples of the left and median liver lobes, from all male control and high dose (10000ppm) animals, were used for the immunohistochemical localisation of PCNA, essentially as described by Cattoretti[5] *et. al.* (1992), Foley[6] *et. al.* (1991) and Greenwell[7] *et. al.* (1991). They were incubated with monoclonal anti-PCNA antibody clone PC10 and further processed with an Avidin Biotin Alkaline Phosphatase detection kit. One of the sections was counter-stained with haematoxylin for photo-micrography. One further section from each animal was used as a negative control for PCNA staining and was similarly processed but without incubation with anti-PCNA antibody. A section of control group testicular tissue was immunoincubated in parallel with the liver sections as a positive control for PCNA staining. Labelling indices were not calculated. Cells

[4] [redacted]: see Chapter 5.3.1.1

[5] Cattoretti, G. *et. al.* (1992): Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB1 and MIB3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections, J. Pathol., 168, 357 - 363.

[6] Foley, JF. *et. al.* (1991): Detection and evaluation of proliferating cell nuclear antigen (PCNA) in rat tissue by an improved immunohistochemical procedure, J. Histotech., 14, 237 - 241.

[7] Greenwell, A *et. al.* (1991): An enhancement method for immunohistochemical staining proliferating cell nuclear antigen in archival rodent tissues, Cancer Letters, 59, 251 - 256.

in S-phase were identified by uniform dark red nuclear staining. Hepatocytes, but not sinusoidal cells, were evaluated by light microscopy for PCNA positive nuclei.

Findings: Prominent PCNA staining of spermatogonia occurred in control testis sections and no nuclear staining occurred in the negative control liver slices, demonstrating the suitability for purpose of the technique employed. Evaluation of hepatocyte nuclei from control group animals revealed small numbers of PCNA-stained nuclei in each section. Treatment with thiamethoxam at 10000ppm did not increase the number of PCNA-stained nuclei in hepatocytes.

Conclusion: No-observed-effect-level (NOEL) for hepatocyte proliferation: >10000ppm, equivalent to a dose level of >710.6mg/kg bw/day, based on the finding of no increase in the number of PCNA-stained nuclei in hepatocytes.

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.	**6.10 / 02**	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: *in vitro* cytotoxicty; hepatocytes**
91/414 Annex II Point addressed	**5.8.2**	Studies on tumour promotion

2.	**Reference point:**	Volume 7, Section 3, Annex IIA, 5.8.2 / 02
3.1	**Authors:**	[redacted]
3.2	**Title:**	The effects of CGA 293'343 tech. and CGA 256'084 in primary cultured rat and mouse hepatocytes
3.3	**Owner:**	Syngenta Crop Protection AG
3.4	**Published:**	no
3.5	**Report No:**	CB 97/36
3.6	**Date of report:**	November 20, 1997
4.1	**Testing facility:**	[redacted]
4.2	**Lab. report No:**	CB 97/36
5.1	**Dates of experimental work:**	July 08, 1997 to July 14, 1997
5.2	**Objectives:**	
6.1	**Test substance:**	thiamethoxam, [redacted]
6.2	**Specification:**	not applicable
6.3	**Storage stability:**	fresh stock solutions and dilutions were prepared just prior to the treatment
6.4	**Stability in vehicle:**	was not studied
6.5	**Homogeneity in vehicle:**	was not checked
6.6	**Validity:**	not applicable
6.7	**Physical form:**	solution added to cell culture medium
6.8	**Vehicle/solvent:**	dimethylsulfoxide (DMSO)
7.1	**Test method:**	Primary cultures of hepatocytes were prepared from young adult male Tif:RAIf (SPF) strain rats and Tif:MAGf mice (supplied by [redacted]). Hepatocytes were isolated by *in situ* perfusion of the liver with collagenase solution. Hepatocyte viability was confirmed to be >90% by the trypan blue exclusion method. Isolated hepatocytes were suspended in Williams Medium E with supplements and seeded at confluency on collagen-coated 24-well plates at an initial cell density of 2 x 10^5 cells/well. The experiment was started 24 hours after establishment and attachment of the monolayers. Solutions of thiamethoxam in DMSO solvent (0.1 or 0.5%) at

		concentrations of 10, 30, 60, 100, 300, 600, 1000, 2000 and 5000 µM were added to the culture medium. A positive control material, dibucaine was also tested at concentrations of 50, 100, 200 and 300 µM. The morphology of hepatocyte monolayers was evaluated by light microscopy after 1, 4 and 24 hours exposure. Results of the evaluations, no morphological effect (0), or irregular cell surface, formation of blebs, cell spreading, intracellular granulation or vacuolation, and cell disaggregation were recorded and the severity graded (1 to 3). Treatments resulting in 100% cell death and/or detachment of the monolayer (K) were also described. Lactate dehydrogenase (LDH) activity in the culture medium was assayed, in triplicate, spectrophotometrically after 4 and 24 hours exposure. Total intracellular LDH activity was determined in 3 additional cultures before exposure to thiamethoxam.
7.2	**Justification:**	The method is considered suitable for the purpose of the study.
7.3	**Copy of method:**	Description of the method is included in the report.
7.4	**Choice of method:**	not applicable
7.5	**Deviations:**	not applicable
8.1	**Certified laboratory:**	yes
8.2	**Certifying authority:**	Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland
8.3	**GLP:**	no
8.4	**Justification:**	non-standard exploratory study
9.1	**GEP:**	not applicable
9.2	**Type of Facility (official or officially recognized):**	not applicable
9.3	**Justification:**	not applicable
10	**Test system -**	primary hepatocyte cultures from rats and mice, for details see under 7.1, Test methods
11	**Statistics:**	not applicable
12.1	**References:**	no published data cited in this summary
13	**Unpublished data:**	no unpublished data cited in this summary

Findings: The positive control material, dibucaine, induced morphological changes in both rat and mouse cultures at concentrations between 50 and 300µM. The severity of cytotoxicity was both time and concentration dependent. Morphological effects observed in rat hepatocytes were slight to severe granulation, vacuolation and hypertrophy of cells and 100% cell death with detachment of the monolayer. In the mouse, slight granulation, vacuolation and hypertrophy of cells and 100% cell death with detachment of the monolayer occurred. LDH release in both species in response to dibucaine paralleled the morphological changes. LDH release increased dose- and time-dependently from approximately 15% of total intracellular activity at 50µM to approximately 95% at 300µM. In contrast, thiamethoxam-exposed cultures were unaffected morphologically and did not release intracellular LDH activity into the medium at any concentration up to the maximum of 5000µM.

Conclusion: Thiamethoxam is not cytotoxic to rat and mouse primary hepatocytes in short-term culture at concentrations up to 5000µM.

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. 6.10 / 03	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: *in vivo* liver biochemical parameters; 14-days feeding**
91/414 Annex II Point addressed 5.8.2	**Studies on tumour promotion**

1. Annex point(s)	**IIA, 5.8.2 Studies on tumour promotion**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2
3. Authors (year) **Title** **Owner, Date**	[redacted] CGA 293343 tech. - Effects on biochemical parameters in the liver following administration to male and female mice Syngenta Crop Protection AG, unpublished report No. CB98/11, September 15, 1998
4. Testing facility	[redacted]
5. Dates of work	April 2, 1998 - July 20, 1998
6. Test substance	CGA 293343 tech., [redacted]
7. Test method	Not applicable – investigative study
8. GLP	Yes - laboratory certified by the Eidgenössisches Department des Inneren (Federal Department of Home Affairs), Bern, Switzerland).

Materials and methods: Groups of 6 male and 6 female young adult mice (Tif:MAGf, SPF strain, body weight range 22.6 - 39.7g, supplied by [redacted]) were treated orally for 14 days with thiamethoxam by admixture in the diet at concentrations of 0, 100, 500 and 2500ppm. Clinical signs, food consumption and body weights were recorded daily. After 14 days treatment the animals were exsanguinated under ether anaesthesia, the carcasses weighed and the livers removed for weighing. Liver tissue was immediately chopped, weighed and frozen in liquid nitrogen and stored at -80°C until further processing. Thawed livers were subjected to subcellular fractionation at 100 x g and supernatants, microsomal and cytosolic fractions collected, frozen in liquid nitrogen and stored at -80°C. The biochemical parameters in Table 1 were determined.

Table 1: Biochemical parameters determined and methodology references

Parameter	Fraction	Published method
Protein content	S, M, C	Smith[8] *et.al.* (1985)
Cytochrome P450	M	Omura & Sato[9] (1964)
7-ethoxyresorufin-O- dealkylase 7-pentoxyresorufin-O-dealkylase 7-benzyloxyresorufin-O-dealkylase	M	Burke *et. al*[10]. (1985) Kennedy & Jones [11](1994)
Coumarin 7-hydroxylase	M	Aitio[12] (1978)
Regioselective & stereoselective hydroxylation of testosterone	M	Van den Hoeven[13] (1984) Purdon & Lehman- McKeeman[14] (1997)
Lauric acid 11-hydroxylation Lauric acid 12-hydroxylation	M	Orton & Parker ([15]1992)
UDP-glucuronosyltransferase	M	Mulder & van Doorn[16] (1975)
Glutathione S-transferase	C	Habig *et. al.*[17] (1974)
Epoxide hydrolase	M	Oesch *et. al*[18]. (1971)
Cyanide-insensitive peroxisomal-β-oxidation	S	Lazarow[19] (1981)

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8 Smith, PK., *et.al.* (1985): Measurement of protein using bicinchoninic acid, Anal. Biochem., 150, 76 - 85.

9 Omura, T & Sato, R (1964): The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature, J. Biol. Chem., 239, 2370 - 2378.

10 Burke, MD., *et. al.* (1985): Ethoxy- pentoxy- and benzyloxy-phenoxazones and homologues; a series of substrates to distinguish between different induced cytochromes P450, Biochem. Pharmacol., 34, 3337 - 3345.

11 Kennedy, SW & Jones, SP (1994): Simultaneous measurement of cytochrome P4501A catalytic activity and total protein concentration with a fluorescence plate reader, Anal. Biochem., 222, 217 - 223.

12 Aitio, A (1978): A simple and sensitive assay of 7-ethoxycoumarin deethylation, Anal. Biochem., 85, 488 - 491.

13 Van den Hoeven, T (1984): Assay of hepatic microsomal testosterone hydroxylases by high performance liquid chromatography, Anal. Biochem., 138, 57 - 65.

14 Purdon, MP & Lehman-McKeeman, LD (1997): Improved high performance liquid chromatographic procedure for the separation and quantification of hydroxytestosterone metabolites, J. Pharmacol. Toxicol. Methods, 37, 67 - 73.

15 Orton, TC & Parker, GL (1992): The effect of hypolipidaemic agents on the hepatic microsomal drug-metabolising enzyme system of the rat, Drug Metab. Dispos., 10, 110 - 115.

16 Mulder, GJ & van Doorn, AB (1975): A rapid NAD+-linked assay for microsomal uridine diphosphate glucuronyl-transferase of rat liver and some observations on substrate specificity of the enzyme, Biochem. J., 151, 131 - 140.

17 Habig, WH., *et. al.* (1974): Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation, J. Biol. Chem., 249, 7130 - 7139.

18 Oesch, F., *et. al.* (1971): A radiometric assay for hepatic epoxide hydrase activity with [7-3H]-styrene oxide, Biochim. Biophys. Acta., 227, 685 - 691.

19 Lazarow, PB (1981): Assay of peroxisomal β-oxidation of fatty acids, Meth. Enzymol., 72, 315 - 319.

S = supernatant, M = microsomal, C = cytosolic

Findings: Analysis of diets demonstrated that achieved concentrations were all within the range 94 - 108% nominal, and homogeneity of multiple samples of each diet was within ±15% of the mean concentration. Achieved mean dose levels, based on analytically determined dietary concentrations, were 0, 17, 74 and 376mg/kg bw/day (males) and 0, 20, 92 and 486mg/kg bw/day (females), in order of increasing concentration.

There were no clinical signs of an adverse reaction to treatment, and body weight development and food consumption were unaffected by treatment at all dose levels. There were no treatment-related gross necropsy findings and exsanguinated carcass weights were similar in all groups. There was a treatment-related, slight increase in absolute and relative liver weights of both sexes at 2500ppm. These values were 7 - 12% higher than control values (Table 2).

Table 2: Absolute and relative liver weights

Sex	Dose level (ppm)	Carcass weight (g)	Absolute liver weight (g)	Relative liver weight (% carcass weight)
Males:	0	33.77	1.79	5.31
	100	34.65	1.80	5.20
	500	34.82	1.89	5.41
	2500	34.97	1.99	5.69
Females:	0	26.20	1.38	5.24
	100	26.50	1.35	5.10
	500	25.80	1.35	5.23
	2500	25.57	1.51	5.88

Results of the protein and enzyme assays, expressed as percentage increase over control values, are summarised in Table 3.

Table 3: Summary of enzyme activities

Assay	Result
Protein content	no effect in either sex at any dose in supernatant and cytosolic fractions 24% inc. in females at 2500ppm in microsomal fraction
Cytochrome P_{450}	59% inc. in males at 2500ppm 52% inc. in females at 2500ppm
7-ethoxyresorufin-O-deethylase	no effect in males at all dose levels 45% inc. in females at 500ppm 157% inc. in females at 2500ppm
7-pentoxyresorufin-O-depentylase	42% inc. in males at 500ppm 725% inc. in males at 2500ppm 51% inc. in females at 100ppm 103% inc. in females at 500ppm 711% inc. in females at 2500ppm
7-benzyloxyresorufin-O-debenzylase	72% inc. in males at 500ppm 1021% inc. in males at 2500ppm 53% inc. in females at 100ppm 109% inc. in females at 500ppm 782% inc. in females at 2500ppm
Coumarin 7-hydroxylase	55% inc. in males at 2500ppm no effect in females at all dose levels
Regioselective & stereoselective hydroxylation of testosterone	total oxidation rates inc. in males by 41% due to increased hydroxylation at positions 2β, 6α, 6β, 15α and oxidation to androstenedione. total oxidation rates inc. in females by 37%, due to increased hydroxylation at positions 6α, 16α, 16β, and oxidation to androstenedione.
Lauric acid 11-hydroxylation	47% inc. in males at 2500ppm 104% inc. in females at 2500ppm
Lauric acid 12-hydroxylation	no effect in males at all dose levels no effect in females at all dose levels
UDP-glucuronosyltransferase	33% inc. in males at 2500ppm 31% inc. in females at 2500ppm
Glutathione S-transferase	61% inc. in males at 2500ppm 198% inc. in females at 2500ppm
Epoxide hydrolase	50% inc. in males at 2500ppm 133% inc. in females at 2500ppm
Cyanide-insensitive peroxisomal-β-oxidation	no effect in males at all dose levels no effect in females at all dose levels

Conclusion: Thiamethoxam is a moderate inducer of liver phase I and phase II xenobiotic metabolising enzymes. The induction phenotype has similarities with that produced by the model inducer and rodent liver tumour promoter phenobarbital.

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.	**6.10 / 04**	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: *in vivo* hepatic cell proliferation; Up to 59 days feeding**
91/414 Annex Point addressed	**II 5.8.2**	Studies on tumour promotion

1. **Annex point(s)** **IIA, 5.8.2 Supplementary studies on the active substance**

2. **Reference point (location) in dossier** Volume 7, Section 3, Annex IIA, point 5.8.2/01

3. **Authors (year)** [redacted]
   Title CGA 293343 tech. - Assessment of hepatic cell proliferation in mice
   Owner, Date Syngenta Crop Protection AG, Basel, Switzerland, unpublished Study Report No. CB 98/12, Syngenta File N° CGA 293343/0718, September 24, 1998

4. **Testing facility** [redacted]

5. **Dates of work** April 2, 1998 to August 18, 1998

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

7. **Test method** Not applicable

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

Materials and methods: Groups of 5 male and 5 female young adult mice (Tif:MAGf, SPF strain, approximately 8 weeks old, supplied by [redacted]) were treated orally for 3, 7, 13, 27 and 59 days with thiamethoxam (batch no. P.506006, purity 98.6%) by admixture in the diet at each dietary concentration of 0, 100, 500 and 2500ppm. Mortality was checked twice daily and clinical signs, body weights and food consumption were recorded daily. Two hours before scheduled sacrifice, each animal was dosed intraperitoneally with 100mg/kg bw bromodeoxyuridine (BrdU) in 0.9% saline. The animals were exsanguinated under ether anaesthesia and a limited necropsy with gross examination performed. The exsanguinated carcass weight was recorded and the liver removed and weighed. Three liver slices and one sample of small intestine were preserved and prepared as one paraffin block. Serial sections were prepared and stained for detailed histology, and stained/incubated for BrdU-immunohistochemistry/Feulgen reaction and BrdU-immunohistochemistry (without anti-BrdU antibody) as a negative control. Intestinal sections served as a positive control. Morphometric evaluation of hepatocyte nuclei was performed by image analysis, on between 17607 and 75358 nuclei/animal. The total number and number of BrdU-stained nuclei were counted and a labelling index (LI) calculated. BrdU-labelling indices were used as a measure of proliferative activity. A non-parametric statistical test, the Mann-Whitney rank test, was performed on morphometric parameters with p values <0.05 considered significant. Liver samples from selected animals were also stained with PAS, Ziehl-Neelsen and Schmorl to demonstrate the presence of glycogen and lipogenic pigments.

Findings: Analysis of diet samples demonstrated adequate stability, homogeneity (-9% to +11%) and achieved concentrations (93.6 to 107.7% nominal). Mean achieved dose levels were 0, 15.84, 71.57 and 385.98mg/kg bw/day (males) and 0, 19.85, 86.57 and 463.26mg/kg bw/day (females).

No deaths occurred and there were no effects on clinical condition, food consumption and body weight development, for any treatment duration, at any dose level. Absolute and relative liver weights in males and females at 2500ppm were increased by 9 - 20% by treatment for 7 days or more (Table 1). Occasional, slightly higher mean liver weights at 100 and 500ppm are considered not to be treatment-induced since carcass weight were slightly higher at these dose levels and relative liver weights were similar to control values.

Table 1: Absolute and relative liver weights

Sex/days of treatment	Liver weight expressed as % of control value					
	100ppm		500ppm		2500ppm	
	Absolute	Relative	Absolute	Relative	Absolute	Relative
Males						
3	102	99	99	101	97	99
7	108	105	99	102	109	111
13	98	101	94	103	110	112*
27	104	97	103	102	109	110
59	106	100	97	98	115	112*
Females						
3	94	95	104	100	103	102
7	113*	106	110	101	112*	108
13	95	96	105	101	110	111*
27	109	108	101	98	120*	119*
59	101	95	98	94	116	115*

* $p < 0.05$ (Dunnett's pairwise comparison)

Macroscopically, speckled liver occurred in all but 3 animals at 2500ppm and also in 2 males at 500ppm. This observation correlated microscopically with hepatocellular glycogenosis/fatty change and was characterised by enlarged centrilobular/midzonal hepatocytes containing increased amounts of glycogen, fat, and to a lesser extent, smooth endoplasmic reticulum. In males, there was a dose-related increase in incidence at all dose levels and the severity was increased on day 59. In females, there was a clear increase in incidence and severity at 2500ppm and increased severity with time on days 27 and 59. In both sexes at 2500ppm, the alteration was apparent in some animals after 3 days treatment. A treatment-related increased incidence and/or severity of hepatocellular necrosis (necrotic and apoptotic) occurred in both sexes at 2500ppm only, predominantly after 27 or 59 days treatment. Cytoplasmic, granular, lipogenic pigmentation of centrilobular hepatocytes occurred almost exclusively in both sexes at 2500ppm, and after 5 days, with occasional involvement of Kupffer cells. There were no other hepatic alterations considered to be treatment-related.

The BrdU staining of proliferating enterocyte nuclei confirmed the suitability of the method. The BrdU labelling index (LI) was increased in males at 2500ppm by 110, 151 and 97% after 3, 7 and 59 days treatment, and in females by 349 and 328% after 7 and 59 days treatment, respectively (Table 2). Treatment of males at 500ppm also increased the LI by 68% after 13 days treatment. The LIs of females at 500ppm and of both sexes at 100ppm were unaffected by treatment. The pattern of elevated BrdU-labelling indices indicate a persistent effect which is not compatible with the contention of a mitogenic effect of thiamethoxam. Therefore, the data suggest that increased proliferative activity has a regenerative aetiology in response to thiamethoxam-induced hepatocellular necroses and/or apoptosis.

Table 2: Mean labelling indices (LI) expressed as % of control

Days of treatment	Mean labelling index (% BrdU-positive hepatocyte nuclei) relative to controls					
	100ppm		500ppm		2500ppm	
	Males	Females	Males	Females	Males	Females
3	96	87	115	94	210**	151
7	69	197	110	154	251*	449**
13	79	77	168*	193	161	317
27	101	127	176	85	97	88
59	74	91	138	126	197**	428**

* $p < 0.05$; ** $p < 0.01$ (Mann-Whitney rank test)

Conclusion: Thiamethoxam at 2500ppm produced a well-defined sequence of effects in the liver. A perturbation of liver homeostasis manifested as glycogenosis/fatty change after 3 days of treatment was followed by increased liver weight after 7 days treatment and frank necrosis and/or apoptosis after 27 days or more treatment. Lipogenic pigmentation occurred as a late event in the sequence. From day 3 in males and from day 7 in females, thiamethoxam induced a concomitant increase in persistent proliferative activity of hepatocytes as a regenerative response to enhanced hepatocellular necrosis and/or apoptosis.

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 6.10 / 05 section No.	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: *in vivo* acute hepatic cell damage**
91/414 Annex II Point addressed 5.8.2	Studies on tumour promotion

1. **Annex point(s)** **IIA, 5.8.2 Supplementary studies on the active substance**

2. **Reference point (location) in dossier** Volume 7, Section 3, Annex IIA, point 5.8.2/01

3. **Authors (year)** [redacted]
   Title Histopathologic evaluation of the liver in male mice upon treatment with a single high dose of CGA 293343 tech. (thiamethoxam)
   Owner, Date Syngenta Crop Protection AG, Basel, Switzerland
   Unpublished Study Report No. CB 99/60, Syngenta File N° CGA 293343/1168, 17.12.1999

4. **Testing facility** [redacted]

5. **Dates of work** 17.11.1999 – 13.12.1999

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

7. **Test method** Not applicable

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

Material and methods: Male mice (Tif:Ibm MAG (SPF)) were treated with thiamethoxam at a single high dose of 500 mg/kg body weight by gavage. Three animals each were sacrificed at 6 and 24 hours after treatment and portions of the liver were taken for paraffin embedding or stored deep frozen. Paraffin-embedded liver sections were examined histopathologically. Three control animals received the vehicle only and were sacrificed at 24 hours.

Findings: Except for animal number 1 (control), which died accidentally after application of the gavage vehicle, no mortality occurred in this study. There were no clinical signs and no treatment-related effects on carcass or liver weight (Table 1).

Upon histopathological examination (Table 2), a moderate cytoplasmic condensation of mostly periportal hepatocytes was seen in animals killed at 6 hours after treatment. The severity of this change dropped back to minimal at 24 hours after treatment. It therefore represents an acute and transient or reversible change. From its morphologic appearance, the cytoplasmic condensation seems to be due to a depletion of stored glycogen in periportal hepatocytes. The second treatment-related change in this study was a clear suppression of the naturally high hepatocellular mitotic activity (Table 3) in these young animals at 24 hours after single dose administration. No such effect was seen at 6 hours after treatment. Beside these changes, no other treatment-related findings were observed, particularly there were no signs of induced necroses in spite of the very high dose applied.

Table 1: Carcass and liver weights

	Control			**Thiamethoxam: 500 mg/kg**					
				6 Hours			**24 Hours**		
Carcass Weight	25.55	±1.34	n = 2	26.10	±0.87	n = 3	25.53	±1.99	n = 3
Absolute Liver Weight	1.686	±0.298	n = 2	1.801	±0.109	n = 3	1.652	±0.263	n = 3
Relative Liver Weight	6.575	±0.813	n = 2	6.910	±0.644	n = 3	6.463	±0.552	n = 3

Values are means ± standard deviations of carcass or absolute liver weights in gram or relative liver weights in percent of carcass weight; n = number of animals.

Table 2: Liver histopathology

	Control	Thiamethoxam: 500 mg/kg	
		6 Hours	24 Hours
Animals Examined	2	3	3
Extramedullary Hematopoiesis	1 (1.0)		
Increased Mitotic Activity	2 (3.0)	3 (3.3)	1 (2.0)
Necrosis	1 (2.0)		
Periportal Cytoplasmic Condensation		3 (3.0)	3 (1.0)

Values are incidences (i.e. number of animals affected) and mean severities (among animals affected) in parenthesis.

Table 3: Hepatocellular mitotic activity

Control	CGA 293343 tech. (500 mg/kg), 6 Hours	CGA 293343 tech. (500 mg/kg), 24 Hours
0.44 ± 0.12, n = 2	0.64 ± 0.43, n = 3	0.10 ± 0.15, n = 3

Values are means ± standard deviations of number of mitotic figures per mm^2; n = number of animals.

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 6.10 / 06 section No.	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: *in vivo* hepatic cell apoptosis**
91/414 Annex II Point addressed 5.8.2	Supplementary studies on the active substance

1. **Annex point(s)** **IIA, 5.8.2 Supplementary studies on the active substance**

2. **Reference point (location) in dossier** Volume 7, Section 3, Annex IIA, point 5.8.2/02

3. **Authors (year)** [redacted]
   Title Histochemical assessment of hepatic apoptosis upon treatment of male mice with CGA 293'343 tech. (thiamethoxam) for up to 9 months
   Owner, Date Syngenta Crop Protection AG, Basel, Switzerland
   Study Report No. CB 99/57, Syngenta File N° CGA 293343/1168, 06.12.1999

4. **Testing facility** [redacted]

5. **Dates of work** 12.11.1999 – 03.12.1999

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

7. **Test method** Not applicable

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

Material and methods: In this retrospective investigation, hepatic apoptosis in mice treated with thiamethoxam at dietary concentrations of 0, 100, 500, and 2500 ppm for 3, 7, 13, 27, and 59 days and for 9 months (0 and 2500 ppm only) was histochemically identified and quantitatively assessed. Paraffin liver sections from the subchronic cell proliferation study[20] and from the interim sacrifice of the 18-month long-term study[21] were submitted to a TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end labelling) histochemical procedure, followed by morphometry to quantify the extent of apoptotic activity.

Hepatocellular apoptosis assessed by TUNEL: During apoptosis, the DNA is cut into multiples of 180 base-pair DNA fragments. The TUNEL assay detects DNA strand breaks by enzymatically coupling the free 3'-OH termini with label-conjugated (e.g. digoxigenin, fluorescein) nucleotides, followed by an anti-label antibody based immunohistochemistry. TUNEL staining is typically localized in morphologically identifiable apoptotic nuclei and apoptotic bodies. In contrast, non-apoptotic cells, which have relatively insignificant numbers of DNA 3'-OH ends, usually do not stain with this method.

The staining procedure, which is described in detail in a respective Standard Operating Procedure, comprised basically the following steps. A trypsin pre-incubation was used as antigen unmasking procedure, followed by quenching of endogenous peroxidase by hydrogen peroxide. Labelling buffer containing label-conjugated nucleotides was applied. The label-nucleotides were linked to the DNA by terminal deoxynucleotidyl transferase (TdT), which catalyses a template independent addition of deoxyribonucleotide triphosphate to the 3'-OH ends of double- or single-stranded DNA. For negative control slides, the assay was performed without the TdT step. The

20 Study Number CB 98/12: CGA 293343 tech.: Assessment of hepatic cell proliferation in mice [redacted] 1998). Doses: 0, 100, 500, and 2500 ppm. Treatment periods: 3, 7, 13, 27, and 59 days, 5 animals per dose group and time point

21 Study Number 942109: 18-month oncogenicity study in mice ([redacted] 1998). Doses: 0, and 2500 ppm. Treatment period: 9 months (interim sacrifice, only control and top dose animals), 10 animals per dose group

incorporated nucleotides form a random heteropolymer of label-conjugated dUTP and dATP, in a ratio that has been optimised for anti-label antibody binding. The antibody caries a conjugated enzyme (peroxidase) to the reaction site. Diaminobenzidine (DAB, the chromogen) was oxidized by peroxidase, resulting in a reddish brown precipitate in the sections.

As a counterstain of TUNEL histochemistry, an eosin staining was used as a basis for measurement of the hepatic tissue area, to which the number of apoptotic figures is related to.

Morphometrical assessment of apoptosis was performed by counting hepatocellular apoptotic figures (apoptotic hepatocyte nuclei and clusters of apoptotic fragments, for which a hepatocellular origin could be established) and by image analysis (analySIS Pro, Soft Imaging System GmbH, Münster, Germany) based measurement of the reference (i.e. total hepatic tissue) area. As a measure of apoptotic activity, the number of apoptotic figures per unit area (mm^2) was evaluated.

Findings: In small intestine sections, serving as positive controls, TUNEL staining was found to label dying enterocytes at the villus tips, indicating the method's ability to detect apoptotic cells. The negative control sections (without the DNA label-linking enzyme "terminal deoxynucleotidyl transferase") revealed no TUNEL-specific staining at all.

Significantly increased numbers of apoptotic figures per unit area were seen at 500 and 2500 ppm after 59 days and at 2500 ppm after 9 months of treatment. No significant increase was seen at 100 ppm or before day 59. These results are in line with the reported late appearance of apoptosis and fit into the overall concept of persistent and exacerbating cell loss in the liver of mice after prolonged treatment with high doses of thiamethoxam. The data are presented in the Table 1 below.

Table 1: Number of apoptoses

Study	Time point	Control	Thiamethoxam 100 ppm	Thiamethoxam 500 ppm	Thiamethoxam 2500 ppm
CB 98/12	3 days	22.46 ± 31.41	23.48 ± 1.56	34.48 ± 22.22	40.35 ± 46.50
	7 days	21.45 ± 16.47	28.94 ± 23.03	48.04 ± 42.85	37.91 ± 17.38
	13 days	57.13 ± 54.73	41.91 ± 19.49	40.13 ± 46.24	85.75 ± 57.56
	27 days	43.51 ± 35.27	61.20 ± 54.67	63.16 ± 31.97	34.75 ± 19.45
	59 days	49.45 ± 37.46	89.47 ± 36.63	184.40 ± 115.17*	568.16 ± 196.46***
942109	9 months	123.87 ± 79.68	nd	Nd	1'223.63 ± 367.76****

Values are means of apoptotic numerical density ± standard deviation (number of apoptotic figures per mm^2 $x10^3$). Five animals per group and time point except for study 942109 with 10 animals per group. nd = no data (100 and 500 ppm groups were not included in 9-month interim sacrifice of study 942109). * $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$ (Mann-Whitney Rank Sum Test).

Conclusion: Treatment of male mice with thiamethoxam at dietary concentrations of 500 and 2500 ppm caused an increase in apoptotic activity after 59 days and at 2500 ppm after 9 months. No effects were seen at 100 ppm or at any feeding level before day 59.

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 6.10 / 07 section No.	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: immunohistochemical assessment of α_{2u}-Globulin in the rat kidney**
91/414 Annex II Point addressed 5.8.2	**Supplementary studies on the active substance**

1. **Annex point(s)** IIA, 5.8.2 Supplementary studies on the active substance

2. **Reference point (location) in dossier** Volume 7, Section 3, Annex IIA, point 5.8.2/02

3. **Authors (year)** [redacted]
   Title Immunohistochemical Assessment of α_{2u}-Globulin in the Rat Kidney upon Administration of CGA 293343 for 28 Days
   Owner, Date Syngenta Crop Protection AG, Basel, Switzerland
   Study Report No. CB 00/16, Syngenta File N° CGA 293343/1231, 03.07.2000

4. **Testing facility** [redacted]

5. **Dates of work** 27.01.2000 to 11.02.2000

6. **Test substance** Thiamethoxam tech.
   [redacted] ([redacted], 1995, Syngenta File No. CGA293343/0006)

7. **Test method** Not applicable

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

Material and methods: In this retrospective investigation, groups of 5 male and 5 female rats, treated with thiamethoxam at 0, 2500, and 10000 ppm for 28 days were used for immunohistochemical identification of the nature of hyaline accumulation in kidneys of male animals. Material from a 28-day rat study[22] was submitted to α_{2u}-globulin immunohistochemistry followed by morphometric assessment of the extent of α_{2u}-globulin accumulation.

Findings: In male control rats, immunohistochemistry showed a considerable staining in the renal cortex, where P_1 and mainly P_2 segments of proximal convoluted tubules were identified as positively stained. A faint staining was also found in the outer stripe of the outer medulla, where the chromogen was located in segment P_3 of proximal tubules. No staining was seen in the inner medulla, including papilla. The considerable intensity of staining of the renal cortex in α_{2u}-globulin immunohistochemistry in male control animals reflects the known natural large amount of α_{2u}-globulin present in the male rat kidney.

An increased staining intensity in the renal cortex (proximal tubular segments P_1 and P_2) was found in male rats, treated at 2500 ppm. The increased staining was mainly due to the occurrence of patches of strong staining intensity (clearly above surrounding tissue). These patches could be shown to correspond to hyaline droplets in H&E stained sections. Upon morphometric examination of immunohistochemically stained sections, the number per mm^2 and the percent-area of these strong staining patches (i.e. hyaline droplets) were clearly increased and their mean area was slightly increased (Table 1). These morphometric results reflect the occurrence of renal tubular hyaline change (original diagnosis) in male rats at 2500 ppm. In the outer stripe of outer medulla, a slightly increased staining

22 [redacted] CGA 293343 tech.: 28-days range finding study in rats (administration in food); [redacted]; report no. 942088; May 5, 1995; dates of experimental work: October 05, 1994 - November 03, 1994. Doses: 0, 100, 1000, 2500, and 10000 ppm. 5 animals per dose group and sex.

intensity was seen in segment P_3 of proximal tubule in male rats, treated at 2500 ppm. This increase was due to a more prominent cytoplasmic staining without formation of strong staining patches, as observed in cortical segments.

At 10000 ppm, neither an increased staining intensity in α_{2u}-globulin immunohistochemistry, nor highly positive patches were seen in male rats. It is speculated that the liver might have been functionally overloaded at 10000 ppm and therefore produced lower amounts of α_{2u}-globulin, which might have balanced the modified protein's slower lysosomal degradation in the kidney. In consequence, no renal accumulation of α_{2u}-globulin was observed in male rats at 10000 ppm.

In contrast to male rats, neither control nor treated female rats showed any specific staining in α_{2u}-globulin immunohistochemistry.

Table 1 Morphometry of α_{2u}-globulin-positive droplets, 28 days, males

	Control 0 ppm	Thiamethoxam 2500 ppm	Thiamethoxam 10000 ppm
Number of droplets per mm^2	4.5 ± 2.1	132.4 ± 24.9 *	Ndm
Droplet size [μm^2]	189 ± 22	238 ± 23 *	Ndm
Droplet %area	0.085 ± 0.042	3.177 ± 0.769 *	Ndm

Values are means ± standard deviation. "Droplets" are specifically and strongly stained particles (α_{2u}-globulin immunohistochemistry-positive aggregates with a staining intensity above surrounding tissue). Groups 2-3 (100 and 1000 ppm) not determined according to protocol. Data from males only, females did not show any specific staining. ndm = not determined morphometrically due to absence of strongly stained particles.

* $p < 0.01$ (Mann-Whitney Rank Sum or t-test).

Conclusion: Treatment of male rats with thiamethoxam at 2500 ppm for 28 days resulted in an increased accumulation of α_{2u}-globulin in the kidney, as characterized by immunohistochemistry. The immunohistochemical staining pattern, with respect to distribution and extent, as well as the sex-specificity correspond to effects of known α_{2u}-globulin nephropathy-inducing compounds. Therefore, the results of the present investigation confirm the hypothesis that the hyaline change seen in renal tubules of males of the 28-day study represent a mild α_{2u}-globulin nephropathy.

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 6.10 / 08 section No.	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: immunohistochemical assessment of α_{2u}-Globulin in the rat kidney**
91/414 Annex II Point addressed 5.8.2	**Supplementary studies on the active substance**

1. **Annex point(s)** **IIA, 5.8.2 Supplementary studies on the active substance**

2. **Reference point (location) in dossier** Volume 7, Section 3, Annex IIA, point 5.8.2

3. **Authors (year)** [redacted])
Title Immunohistochemical Assessment of α_{2u}-Globulin in the Rat Kidney upon Administration of CGA 293343 for 3 Months
Owner, Date Syngenta Crop Protection AG, Basel, Switzerland
Study Report No. CB 99/55, Syngenta File N° CGA 293343/1232, 03.07.2000

4. **Testing facility** [redacted]

5. **Dates of work** 27.01.2000 to 11.02.2000

6. **Test substance** Thiamethoxam tech.
Batch number: [redacted] ([redacted] 1996, Syngenta File No. CGA293343/0033)

7. **Test method** Not applicable

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

Material and methods: In this retrospective investigation, groups of 10 male and 10 female rats, treated with thiamethoxam at 0 or 5000 ppm for 3 months were used for immunohistochemical identification of the nature of hyaline accumulation in kidneys of male animals. Material from a 3-month rat study[23] was submitted to α_{2u}-globulin immunohistochemistry followed by morphometric assessment of the extent of α_{2u}-globulin accumulation.

Findings: In male control rats, immunohistochemistry showed a considerable staining in the renal cortex, where P_1 and mainly P_2 segments of proximal convoluted tubules were identified as positively stained. A very faint staining was also found in the outer stripe of the outer medulla, where the chromogen was located in segment P_3 of proximal tubules. No staining was seen in the inner medulla, including papilla. The considerable intensity of staining of the renal cortex in α_{2u}-globulin immunohistochemistry in male control animals reflects the known natural large amount of α_{2u}-globulin present in the male rat kidney.

An increased staining intensity in the renal cortex (proximal tubular segments P_1 and P_2) was found in male rats, treated at 5000 ppm. The increased staining was mainly due to the occurrence of patches of strong staining intensity (clearly above surrounding tissue). These could be shown to correspond to hyaline droplets (or hyaline change, the original diagnostic term) in H&E stained sections. Upon morphometric examination of immunohistochemically stained sections, the number per mm^2 and the percent-area of these strong staining patches (i.e. hyaline droplets) were clearly increased (Table 1). In the outer stripe of outer medulla, a slightly increased staining intensity was seen in segment P_3 of proximal tubules in male rats, treated at 5000 ppm. This increase was due to a more prominent

23 [redacted] (1996) CGA 293343 tech.: 3-month oral toxicity study in rats (administration in food), [redacted] report no. 942089, January 23, 1996; dates of experimental work: December 27, 1994 to March 30, 1995. Doses: 0, 25, 250, 1250, 2500, and 5000 ppm. 10 animals per dose group and sex.

cytoplasmic staining without formation of strong staining patches, as observed in cortical segments. In addition, near the junction of the straight portion of the proximal tubule with the descending loop of Henle, granular casts were seen in male rats treated at 5000 ppm.

In contrast to male rats, neither control nor treated female rats showed any specific staining in α_{2u}-globulin immunohistochemistry.

Table 1: Morphometry of α_{2u}-globulin-positive droplets, 3 months, males

	Control 0 ppm	Thiamethoxam 5000 ppm
Number of droplets per mm^2	15.3 ± 15.1	79.8 ± 15.2*
Droplet size [μm^2]	229.0 ± 38.2	253.2 ± 17.7
Droplet %area	0.39 ± 0.43	2.03 ± 0.49*

Values are means ± standard deviation. "Droplets" are specifically and strongly stained particles (α_{2u}-globulin immunohistochemistry-positive aggregates with a staining intensity above surrounding tissue). Groups 2-5 (25, 250, 1250, 2500 ppm) not determined according to protocol. Data from males only, females did not show any specific staining. * $p < 0.001$ (t-test).

Conclusion: Treatment of male rats with thiamethoxam at 5000 ppm for 3 months resulted in an increased accumulation of α_{2u}-globulin in the kidney, as characterized by immunohistochemistry. The immunohistochemical staining pattern, with respect to distribution and extent, as well as the sex-specificity correspond to effects of known α_{2u}-globulin nephropathy-inducing compounds. Therefore, the results of the present investigation confirm the hypothesis that the hyaline change seen in renal tubules of males of the 3-month study represent a mild α_{2u}-globulin nephropathy.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date April 2005

Materials and Methods [redacted]

Results and discussion [redacted]

Conclusion [redacted]

	[redacted]
Reliability	[redacted]
Acceptability	[redacted]

98/8 Doc IIIA 6.10 / 09 section No.	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: immunohistochemical assessment of α_{2u}-Globulin in the rat kidney**
91/414 Annex II Point addressed 5.8.2	**Supplementary studies on the active substance**

1.	**Annex point(s)**	**IIA, 5.8.2 Supplementary studies on the active substance**
2.	**Reference point (location) in dossier**	Volume 7, Section 3, Annex IIA, point 5.8.2
3.	**Authors (year)**	[redacted]
	Title	Immunohistochemical Assessment of α_{2u}-Globulin in the Rat Kidney upon Administration of CGA 293343 for 12 Months
	Owner, Date	Syngenta Crop Protection AG, Basel, Switzerland Study Report No. CB 00/14, Syngenta File N° CGA 293343/1233, 03.07.2000
4.	**Testing facility**	[redacted]
5.	**Dates of work**	27.01.2000 to 11.02.2000
6.	**Test substance**	Thiamethoxam tech. Batch number: [redacted], 1998, Syngenta File No. CGA293343/0652)
7.	**Test method**	Not applicable
8.	**GLP**	No laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs, Bern, Switzerland)

Material and methods: In this retrospective investigation, groups of 6-9 male and 5 female rats, treated with thiamethoxam at 0 and 1500 ppm (males) or 3000 ppm (females) for 12 months were used for immunohistochemical assessment of long term renal α_{2u}-globulin accumulation. Material from the 12-month interim sacrifice of a 24-month carcinogenicity and chronic toxicity study[24] was submitted for α_{2u}-globulin immunohistochemistry followed by morphometric assessment of the extent of α_{2u}-globulin accumulation.

Findings: In male control rats, α_{2u}-globulin immunohistochemistry showed a moderate staining of the renal cortex, where P_1 and mainly P_2 segments of proximal convoluted tubules were identified as positively stained. A faint staining was also found in the outer stripe of the outer medulla, where the chromogen was located in segment P_3 (pars recta) of proximal tubules. No specific staining was seen in all other nephron parts, like glomerulus, distal tubule, Henle's loop or collecting duct.

A slightly increased staining intensity in the renal cortex (proximal tubular segments P_1 and P_2) was found in male rats, treated at 1500 ppm. The increased staining was mainly due to the occurrence of patches of strong staining intensity (clearly above surrounding tissue). Upon morphometric examination of immunohistochemically stained sections, the number per mm^2, the size and the percent-area of these strong staining patches were increased (Table 1). Occasionally, a slightly increased staining intensity was also seen in segment P_3 of proximal tubules. This increase was due to a more prominent cytoplasmic staining without formation of strong staining patches, as typical for cortical segments.

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24 [redacted] CGA 293343 tech. - 24-month carcinogenicity and chronic toxicity study in rats: [redacted] report no. 942110, July 27, 1998; dates of experimental work: August 7, 1995- August 21, 1997. Doses: 0, 10, 30, 500 (males)/1000 (females), or 1500 (males)/3000 (females) ppm. 10 animals per dose group and sex for interim sacrifice.

In contrast to male rats, neither control nor treated female rats showed any specific staining in α_{2u}-globulin immunohistochemistry.

Table 1: Morphometry of α_{2u}-globulin-positive droplets, 12 months, males

	Control 0 ppm	Thiamethoxam 1500 ppm
Number of droplets per mm^2	0.507 ± 0.216	6.955 ± 9.757*
Droplet size in μm^2	134.5 ± 15.1	190.6 ± 28.4**
Droplet percent-area	0.007 ± 0.003	0.151 ± 0.226*

Values are means ± standard deviation. "Droplets" are specifically and strongly stained particles (α_{2u}-globulin immunohistochemistry-positive patches with a staining intensity above surrounding tissue). Groups 2-4 (10, 30, 500 (males)/1000 (females)) not determined according to protocol. Data from males only, females did not show any specific staining. * $p < 0.05$, ** $p < 0.001$ (Mann-Whitney Rank Sum Test or t-test).

Conclusion: Treatment of male rats with thiamethoxam at 1500 ppm for 12 months resulted in a slightly increased accumulation of α_{2u}-globulin in the kidney, as characterized by immunohistochemistry. The immunohistochemical staining pattern, with respect to distribution and extent, as well as the sex-specificity correspond to effects of known α_{2u}-globulin nephropathy-inducing compounds. Therefore, the results of the present investigation confirm the hypothesis that the regenerative changes seen in renal tubules of male rats treated for 12 months with thiamethoxam represent the sequelae of a mild α_{2u}-globulin nephropathy.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date April 2005

Materials and Methods [redacted]

Results and discussion [redacted]

Conclusion [redacted]

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

98/8 Doc IIIA 6.10 / 10 section No.	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies: immunohistochemical assessment of α_{2u}-Globulin in the rat kidney**
91/414 Annex II Point addressed 5.8.2	**Supplementary studies on the active substance**

1. **Annex point(s)** **IIA, 5.8.2 Supplementary studies on the active substance**

2. **Reference point (location) in dossier** Volume 7, Section 3, Annex IIA, point 5.8.2

3. **Authors (year)** [redacted]
   Title Immunohistochemical Assessment of α_{2u}-Globulin in the Rat Kidney upon Administration of CGA 293343 for 24 Months
   Owner, Date Syngenta Crop Protection AG, Basel, Switzerland
   Study Report No. CB 00/15, Syngenta File N° CGA 293343/1234, 03.07.2000

4. **Testing facility** [redacted]

5. **Dates of work** 27.01.2000 to 11.02.2000

6. **Test substance** Thiamethoxam tech.
   Batch number: [redacted], 1998, Syngenta File No. CGA293343/0652)

7. **Test method** Not applicable

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

Material and methods: In this retrospective investigation, groups of 10 male and 5-10 female rats, treated with thiamethoxam at 0 and 1500 ppm (males) or 3000 ppm (females) for 24 months were used for immunohistochemical assessment of long term renal α_{2u}-globulin accumulation. Material from the terminal sacrifice of a 24-month carcinogenicity and chronic toxicity study[25] was submitted to α_{2u}-globulin immunohistochemistry.

Findings: In male control rats, immunohistochemistry showed no or only minimal to slight staining intensity in the renal cortex and no specific staining in the medulla. An increased staining intensity in renal proximal tubules (segments P_1 and mainly P_2, occasionally also including segment P_3) was found in male rats, treated at 1500 ppm (Table 1).

In contrast to male rats, neither control nor treated female rats showed any specific staining in α_{2u}-globulin immunohistochemistry.

Table 1: α_{2u}-Globulin immunohistochemical staining intensity, 24 months

Males	Control, 0 ppm	Thiamethoxam, 1500 ppm
Grading (mean ± standard deviation)	1.0 ± 0.9	2.1 ± 1.1*
Females	**Control, 0 ppm**	**Thiamethoxam, 3000 ppm**

[25] [redacted] CGA 293343 tech. - 24-month carcinogenicity and chronic toxicity study in rats; [redacted] report no. 942110, July 27, 1998; dates of experimental work: August 7, 1995- August 21, 1997. Doses: 0, 10, 30, 500 (males)/1000 (females), or 1500 (males)/3000 (females) ppm. 50 animals per dose group and sex for terminal sacrifice.

Grading (mean ± standard deviation)	0.0 ± 0.0	0.0 ± 0.0

Values are mean ± standard deviation of grading of intensity of α_{2u}-globulin immunohistochemistry (0 = no or subminimal, 1 = minimal, 2 = slight, 3 = moderate staining intensity, semi-quantitative evaluation). Groups 2-4 (10, 30, 500 (males)/1000 (females)) not determined according to protocol. 10 animals per group except female controls with 5 animals. * $p < 0.05$ (Mann-Whitney Rank Sum Test).

Conclusion: Treatment of male rats with thiamethoxam at 1500 ppm for 24 months resulted in a slightly increased accumulation of α_{2u}-globulin in the kidney, as characterized by immunohistochemistry. The immunohistochemical staining pattern and the sex-specificity correspond to effects of known α_{2u}-globulin nephropathy-inducing compounds. Therefore, the results of the present investigation confirm the hypothesis that the chronic changes seen in renal tubules of males treated for 24 months with thiamethoxam represent the sequelae of a mild α_{2u}-globulin nephropathy.

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. 6.10 / 11	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 03	Studies on tumour promotion

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/03
3. Authors (year) **Title** **Source** **Owner**	B.E. Butterworth, R.B. Conolly, K.T. Morgan (1995) A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. Cancer Letters, 93, 129-146 (1995) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 12	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 05	Studies on tumour promotion

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/05
3. Authors (year) **Title** **Source** **Owner**	P. Grasso, R.H. Hinton (1990) Evidence for and possible mechanisms of non-genotoxic carcinogenesis in rodent liver. Mutation Research, 248, 271-290 (1991) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 13	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 04	Studies on tumour promotion

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/04
3. Authors (year) **Title** **Source** **Owner**	N.G. Carmichael, H. Enzmann, I. Pate, F. Waechter (1997) The Significance of Mouse Liver Tumor Formation for Carcinogenic Risk Assessment: Results and Conclusions from a Survey of Ten Years of Testing by the Agrochemical Industry. Environ. Health Perspec., 105 (11), 1196-1203 (1997) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 14	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 01	Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/16
3. Authors (year) Title Source Owner	I.F.H. Purchase (1994) Current Knowledge of Mechanisms of Carcinogenicity: Genotoxins versus Non-genotoxins. Human & Experimental Toxicology, 13, 17-28 (1994) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	**6.10 / 15** **Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed	**5.8.2 / 01** Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/21
3. Authors (year) **Title** **Source** **Owner**	J. Whysner, P.M. Ross, G.M. Williams (1996) Phenobarbital Mechanistic Data and Risk Assessment: Enzyme Induction, Enhanced Cell Proliferation, and Tumor Promotion. Pharmacol. Ther., Vol. 71, Nos.1/2, 153-191 (1996) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 16	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 01	Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies – Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/09
3. Authors (year) **Title** **Source** **Owner**	E.E. McConnell (1990) Mouse Liver Tumors: The Problem. In: Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons. Stevenson, D.E., McClain, R.M., Popp, J.A., Slaga, T.J., Ward, J.M., Pitot, H.C. (eds.), New York: Alan R. Liss, Inc., 1-3 (1990) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 17	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 01	Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/14
3. Authors (year) **Title** **Source** **Owner**	S.S. Olin, ed. (1991) Meeting Summary. Third Workshop on Mouse Liver Tumors; Arlington, VA; October 29-30, 1991. ILSI Health and Environmental Sciences Institute, Washington, DC; ILSI Risk Science Institute (1991) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 section No.	**Doc IIIA**	**6.10 / 18**	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Point addressed	**Annex II**	**5.8.2 / 01**	Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/19
3. Authors (year) **Title** **Source** **Owner**	S.F. Velazquez, R. Schoeny, G.E. Rice, V.J. Cogliano (1996) Cancer Risk Assessment: Historical Perspectives, Current Issues, and Future Directions. Drug and Chemical Toxicology, 19 (3), 161-185 (1996) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 19	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 01	Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/01
3. Authors (year) **Title** **Source** **Owner**	R.P. Beasley (1988) Hepatitis B Virus. The Major Etiology of Hepatocellular Carcinoma. Cancer, 61, 1942-1956 (1988) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 20	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 07	Studies on tumour promotion

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/22
3. Authors (year) **Title** **Source** **Owner**	T.L. Wright, A.P. Venook, G.H. Millward-Sadler (1992) Hepatic Tumours. Wright's Liver and Biliary Disease, Chapter 39; London: W.B. Saunders Company; 1079-1121 (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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]e
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 21	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 07	Studies on tumour promotion

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/07
3. Authors (year) Title Source Owner	R. Mazzanti, L. Monsacchi, P. Gentilini (1994) Epidemiology and natural history of hepatocellular carcinoma. Trends in Exp. Clin. Med., 4, 161-171 (1994) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted] on
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 22	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 01	Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/13
3. Authors (year) Title Source Owner	K. Okuda, T. Nakashima, M. Kojiro, Y. Kondo, K. Wada (1989) Hepatocellular Carcinoma Without Cirrhosis in Japanese Patients. Gastroenterology, 97, 140-146 (1989) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 23	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 01	Other toxicological studies - Supplementary studies on the active substance

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/17
3. Authors (year) **Title** **Source** **Owner**	R.G. Simonetti, C. Cammà, F. Fiorello, F. Politi, G. D'Amico, L. Pagliaro (1991) Hepatocellular Carcinoma - A Worldwide Problem and the Major Risk Factors. Digestive Diseases and Sciences, Vol. 36, No. 7, 962-972 (1991) 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)

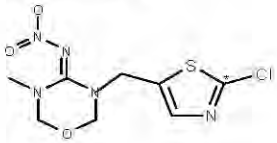
Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted] on
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No. 6.10 / 24	**Mechanistic study - any studies necessary to clarify effects reported in toxicity studies**
91/414 Annex II Point addressed 5.8.2 / 08	Studies on tumour promotion

1. Annex point(s)	**IIA, 5.8.2 Other toxicological studies - Supplementary studies on the active substance**
2. Reference point (location) in dossier	Volume 7, Section 3, Annex IIA, point 5.8.2/08
3. Authors (year) **Title** **Source** **Owner**	R.M. McClain (1990) Mouse Liver Tumors and Microsomal Enzyme-Inducing Drugs: Experimental and Clinical Perspectives With Phenobarbital. In: Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons. Stevenson, D.E., McClain, R.M., Popp, J.A., Slaga, T.J., Ward, J.M., Pitot, H.C. (eds.), New York: Alan R. Liss, Inc., 345-365 (1990) 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	May 2005
Materials and Methods	
Results and discussion	
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	7.1.1.1.1 / 01	Hydrolysis as a fuction of pH and identification of breakdown products
91/414 Annex Point addressed	II 7.2.1.1 / 01	Rate of hydrolysis of relevant metabolites

1.	Annex point(s)	IIA, 7.2.1.1	**Rate of hydrolysis of relevant metabolites**
2.	Location in Dossier	Section 5,	
3.	Authors (year) Title	Adora Clark (1998c) Final Report: Hydrolysis of 2-^{14}C-thiazolyl CGA 293343 under Laboratory Conditions	
	Report No., Date Syngenta File N° Owner	ABR-96106, 17 September 1998 293343/753 Syngenta Crop Protection AG	
4.	Testing facility	Novartis Crop Protection Inc Environmental Safety Department Greensboro, NC 27419, USA	
5.	Dates of work	12 June 1995 - 17 September 1995	
6.	Test substance	ISO common name: Batch: ^{14}C-labelled test substance Specific activity of [^{14}C] Radiochemical purity of the test substance: Structural formula: (position of label)	thiamethoxam company code: CGA 93343 [redacted] Yes , 2-^{14}C-thiazolyl label 1.09 MBq/mg [redacted] 
7.	Test method	The study was conducted in compliance with: "Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 161-1: Hydrolysis Studies", U.S. Environmental Protection Agency, October 18, 1982. and under consideration of: "OECD Guideline for Testing Chemicals, 'Hydrolysis as a Function of pH', 111", Adopted: 12 May 1981, Paris/ France "Prüfung des Verhaltens von Pflanzenbehandlungsmitteln im Wasser, Merkblatt Nr. 55, Teil I und II", Biologische Bundesanstalt für Land- und Forstwirtschaft Bundesrepublik Deutschland, Oktober 1980	
8.	Deviations	No deviations have to be reported	
9.	GLP	Yes , EPA Good Laboratory Practice Standards (40 CFR Part 160)	

Test system: The test was performed to determine the rate of hydrolysis of thiamethoxam in sterile aqueous solution at various pH values at 25°C, 40°C and 60°C and to obtain information on the identity and pattern of hydrolysis products. The hydrolysis of thiazolyl-^{14}C-labelled thiamethoxam was investigated at a concentration of approximately 10 mg/l in buffer solutions of pH 1, 5, 7 and 9. Test duration was variable depending on pH and temperature. For pH 7 and 9 at 25°C it was 30 days. Aliquots of the test solutions were radioassayed by liquid scintillation counting. Determination of thiamethoxam and its hydrolysis products were carried out by thin layer chromatography and high performance liquid chromatography of the test solutions. Mass spectrometry was used to confirm identity of thiamethoxam and major hydrolysis products in

incubated solutions. Half-life values of thiamethoxam were calculated using the results from the TLC assays and using pseudo first order reaction kinetics.

Findings: The test substance thiamethoxam is base labile as demonstrated by the rapid degradation in the pH 9 buffer. It is stable in pH 1 and pH 5 buffers at 60°C. The rate of degradation is both pH and temperature dependent in the pH 7 and pH 9 buffers. The analytical details of the experiment at pH 9 and 40 °C are presented in table 2. For pH 7 and pH 9 half-lives at 20°C were calculated using the Arrhenius parameters based on the experimentally obtained rate constants (Table 1). There were three major degradates identified by mass spectral analysis as CGA 355190, CGA 404617 and CGA 309335 formed by hydrolysis from NOA 404617 at a maximum of 54.3%, 35.2% and 9.1% at 25°C and 30 days, respectively. CGA 322704 was a minor component of <1% in all samples.

Table 1 Hydrolytic half-lives of thiazolyl-^{14}C-labelled thiamethoxam

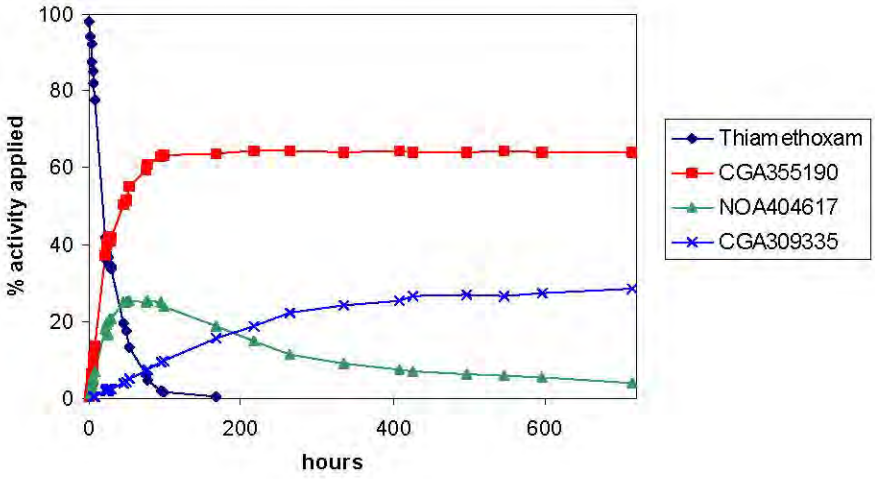
	20°C (calculated)	25°C (experimental)	40°C (experimental)	60°C (experimental)
pH 1	stable	-	-	stable
pH 5	stable	stable	-	stable
pH 7	1114 days	572 days	46.4 days	9.7 days
pH 9	7.3 days	4.2 days	0.75 days	0.12 days

Table 2 TLC distribution of components for hydrolysis at pH 9, 40°C of thiazolyl - ^{14}C-labelled thiamethoxam as percent of applied dose

Incubation time [hours]	Thiame-thoxam [%]	CGA 355190 [%]	NOA 404617 [%]	CGA 309335 [%]
0	98.11	0.36	nd	nd
2	94.00	2.41	1.15	nd
3	92.16	4.32	2.24	nd
4	87.62	6.28	3.42	nd
5	85.23	8.58	4.66	0.36
6	82.14	11.07	5.63	0.34
7	77.90	13.73	6.94	0.51
21	41.69	37.25	18.13	1.86
23	37.78	39.69	19.22	2.11
24	36.13	40.31	19.56	2.24
25	36.78	42.29	16.50	2.51
26	35.33	40.77	19.75	2.10
27	34.82	40.99	20.63	2.30
28	34.28	41.37	20.59	2.45
29	34.36	41.47	20.77	2.23
30	33.59	41.86	20.63	2.33
46	19.38	50.49	24.94	3.84
50	17.58	51.57	24.92	4.18
53	13.09	55.09	25.42	5.00
74	6.27	59.57	25.10	6.86
77	4.51	60.95	25.56	7.47
95	1.90	62.72	24.81	9.24
99	1.62	63.28	23.73	9.75
168	0.21	63.68	18.64	15.69
216	nd	64.40	14.75	18.63
264	nd	64.26	11.27	22.44
336	nd	64.01	8.99	24.25
408	nd	64.59	7.51	25.58
426	nd	64.23	7.11	26.47
498	nd	64.21	6.34	27.01
546	nd	64.55	5.74	26.57
595	nd	63.92	5.46	27.20
715	nd	63.97	4.06	28.69

nd: not determined

Figure 1 Hydrolysis at pH 9, 40°C of thiazolyl -^{14}C-labelled thiamethoxam



Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 9/9/2004

Materials and Methods [redacted]

Results and discussion [redacted]

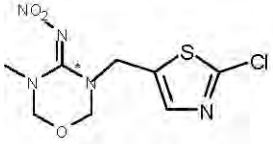
Conclusion [redacted]

Reliability [redacted]

Acceptability [redacted]

Remarks [redacted]

98/8 Doc IIIA section No.	7.1.1.1.1 / 02 Hydrolysis as a fuction of pH and identification of breakdown products
91/414 Annex Point addressed	II 7.2.1.1 / 02 Rate of hydrolysis of relevant metabolites

1.	**Annex point(s)**	**IIA, 7.2.1.1 Rate of hydrolysis of relevant metabolites**
2.	**Location in Dossier**	Section 5,
3.	**Authors (year)** **Title** **Report No., Date** **Syngenta File N°** **Owner**	Edward Lowery (1997) Hydrolysis of ^{14}C-Guanidine CGA 293343 under Laboratory Conditions ABR-97013, 03 November 1997 293343/373 Syngenta Crop Protection AG
4.	**Testing facility**	Novartis Crop Protection Inc Environmental Safety Department Greensboro, NC 27419, USA
5.	**Dates of work**	27 August 1996 - 03 November 1997
6.	**Test substance**	ISO common name: thiamethoxam company code: CGA 293343 Batch: 1) [redacted] ^{14}C-labelled test substance: Yes , ^{14}C-guanidine label Specific activity of [^{14}C]: 1)1.21 Mbq/mg, 2) 1.96 Mbq/mg 3) 2.20 Mbq/mg Radiochemical purity of the test substance: [redacted] Structural formula: (position of label) 
7.	**Test method**	The study was conducted in compliance with: "Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 161-1: Hydrolysis Studies", U.S. Environmental Protection Agency, October 18, 1982. and under consideration of: "OECD Guideline for Testing Chemicals, 'Hydrolysis as a Function of pH', 111", Adopted: 12 May 1981, Paris/ France "Prüfung des Verhaltens von Pflanzenbehandlungsmitteln im Wasser, Merkblatt Nr. 55, Teil I und II", Biologische Bundesanstalt für Land- und Forstwirtschaft Bundesrepublik Deutschland, Oktober 1980
8.	**Deviations**	No deviations have to be reported
9.	**GLP**	Yes , EPA Good Laboratory Practice Standards (40 CFR Part 160)

Test system: In this second study the hydrolysis of thiamethoxam was investigated under the identical conditions as above (Clark 1998c) but using the guanidine-^{14}C-labelled test substance.

Findings: The test substance thiamethoxam is base labile as demonstrated by the rapid degradation in the pH 9 buffer. Preliminary studies at pH 1 and at pH 5 at 60°C showed little or no breakdown of the parent compound (less than 10% in five days). No further studies were performed at pH 1 and pH 5. For pH 7 and pH 9 the hydrolysis was described by pseudo first order reaction kinetics. Rate constants were determined at three temperatures of 25°C, 40°C and 60°C. Half-lives at 20°C were calculated using the Arrhenius parameters based on the experimentally obtained rate constants. The following half-lives of hydrolysis of thiamethoxam at environmental relevant temperatures were obtained:

Table 1: Hydrolytic half-lives of guanidine-^{14}C-labelled thiamethoxam (Lowery 1997)

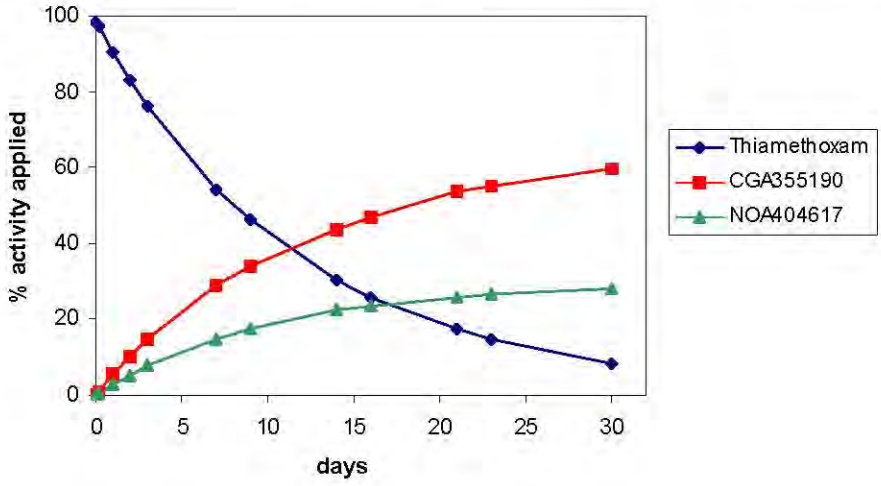
	20°C (calculated)	25°C (experimental)
pH 7	1253 days	643 days
pH 9	15.6 days	8.4 days

The main degradation products which were generated at pH >5 were identified as CGA 355190 and NOA 404617. They represented an average maximum of 59.5% and 27.9% of the applied dose after 30 days at pH 9 and 25°C. The analytical details of the experiment at pH 9 and 25 °C are presented in table 2 and figure 1.

Table 2: TLC distribution of components for hydrolysis at pH 9, 25°C of guanidine -^{14}C-labelled thiamethoxam as percent of applied dose (Lowery 1997)

Incubation time [days]	Thiamethoxam [%]	CGA355190 [%]	NOA404617 [%]
0	97.99	0.14	0.12
0.17	97.35	0.98	0.29
1	90.48	5.44	2.79
2	83.24	10.07	5.17
3	75.95	14.57	7.64
7	53.91	28.75	14.68
9	46.40	33.77	17.51
14	30.29	43.63	22.49
16	25.81	46.97	23.54
21	17.25	53.45	25.88
23	14.60	55.23	26.80
30	8.45	59.65	27.93

Figure 1: Hydrolysis at pH 9, 25°C of guanidine -^{14}C-labelled thiamethoxam



Evaluation by Competent Authorities	
	7.1.1.1.1/02
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	9/9/2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

98/8 Doc IIIA section No.	7.1.1.1.1 / 03 Hydrolysis as a fuction of pH and identification of breakdown products
91/414 Annex Point addressed	II 7.2.1.1 Rate of hydrolysis of relevant metabolites

1.	Annex point(s)	**IIA, 7.2.1.1**	**Rate of hydrolysis of relevant metabolites**
2.	Location in Dossier	Section 5,	
3.	Authors (year) Title Report No., Date Syngenta File N° Owner	Ulbrich, R. (1999) Hydrolysis of ^{14}C-Labelled-CGA 322704 under Laboratory Conditions, 98UL03, February 19, 1999. 322704/0020 Syngenta Crop Protection AG	
4.	Testing facility	Novartis Crop Protection AG, Environmental Safety Department 4002 Basel, Switzerland	
5.	Dates of work	June 23, 1998 – January 22, 1999	
6.	Test substance	ISO common name: Batch: ^{14}C-labelled test substance Specific activity of [^{14}C] Radiochemical purity of the test substance:	Not available (metabolite of thiamethoxam) company code: CGA 322704 [redacted] Thiazol-2-^{14}C label 2.04 Mbq/mg [redacted]
7.	Test method	European Community Commission Directive 94/37/EC of 22 July 1994 and 95/36/EC of 14 July 1955, both amending Council Directive 91/414/EEC: Annex I: 2.9.1 Hydrolysis rate, and Annex II: 7.2.1.1 Hydrolytic degradation. "OECD Guideline for Testing Chemicals, 'Hydrolysis as a Function of pH', 111", Adopted: 12 May 1981, Paris/ France.	
8.	Deviations	No deviations have to be reported	
9.	GLP	yes (Novartis Crop Protection AG, 4002 Basel, Switzerland)	

Test system: The hydrolytic behavior of thiazole-2-^{14}C-labelled CGA 322704 was investigated in aqueous buffer solutions at pH 4, pH 5, pH 7 and pH 9 at a concentration of about 5 mg/l in the dark at 20°C under sterile conditions. Samples were incubated over a testing period of 31 days.

Findings: The total recoveries for the different experimental parts were between 97.3% and 101.3% of the applied radioactivity. No degradation was observed over the testing period of 31 days at the various pH values. It can be concluded that CGA 322704 is hydrolytically stable at the environmentally relevant temperature of 20°C over a range of pH 4 to pH 9.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 10/09/2004

Materials and Methods

[redacted]

Results and discussion

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

Acceptability

[redacted]

Remarks

98/8 Doc IIIA section No.	7.1.1.1.2 / 01	Phototransformation in water including identitiy of products of transformation
91/414 Annex Point addressed	II 7.2.1.2 / 04	Quantum yield

1.	Annex point(s)	**II, 7.2.1.2 and II, 2.9.3**	**Quantum yield**
2.	Location in Dossier	Section 5	
3.	Authors (year) Title Report No., Date Syngenta File N°(Desire) Owner	Prof. Dr. C. Zetzsch (1997) Quantum Yield of the Photochemical Degradation of CGA 293343 in Aqueous Solution 11G97014 (September 9, 1997) 293343/469 Syngenta Crop Protection AG, Product Safety/Ecochemistry, 4002 Basel, Switzerland	
4.	Testing facility	Frauenhofer Institute of Toxicology and Aerosol Research, 30625 Hannover, Germany	
5.	Dates of work	Study Initiation: Study Termination:	July 24, 1997 September 12, 1997
6.	Test substance	CGA 293343, Lot No. [redacted]	
7.	Test method	UBA Test Guideline "Phototransformation of Chemicals in Water, Part A, Direct Phototransformation", Berlin, FRG January 1990. ECETOC-technical report No. 12, Brussels 1984: "The Phototransformation of Chemicals in Water: Results of a Ring Test.	
8.	Deviations	none	
9.	GLP	yes, German Chemiclas Law (Chemikaliengesetz) of July 25, 1994 (Anhang 1 zu Par. 19a, Abs.1 ChemG, Bundesgesetzblatt Teil 1, S. 1724-1732).	

Test system: The direct photolytic degradation of thiamethoxam was studied using polychromatic light (xenon lamp) above 290 nm. Starting concentrations varied between 0.27 and 1.92×10^{-5} mol/l in water. Within the error of the experiments no influence of concentration, irradiation time and filtration on the rate of the photoreaction was found.

Quantum yields were calculated from the absolute intensities of the xenon light source (obtained by actinometry) in the wavelength region 290 to 400 nm, from the molar absorption coefficients (obtained by UV-spectroscopy) and from the concentrations of thiamethoxam (analysed by HPLC) for different irradiation times from 3 experimental runs.

Findings: The mean rate constant for direct irradiation of thiamethoxam in water , corrected for dark loss, was $k \pm \sigma = 5.3 \times 10^{-} \pm 0.5 \times 10^{-4}$ min^{-1}. This rate constant leads under the conditions used to a half-life of $t_{1/2} \pm \sigma = 22 \pm 2$ h. The mean quantum yield of thiamethoxam in water obtained from the corrected photolysis data in the wavelength region of 290 to 400 nm is $\Phi \pm \sigma = 0.013 \pm 0.002$.

The half-life for thiamethoxam in natural sunlight was calculated for latitudes 40^0N and 50^0N which corresponds to locations such as Madrid or Denver and Frankfurt or Winnipeg, respectively near the surface of a water body using the program GC-SOLAR. Half-lives found for different seasons vary between 0.8 days in summer and 8 days in winter with an annual half-life of 1.2 days at 40^0N and of 1.6 days at 50^0N, respectively.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 13/08/04

Materials and Methods

[redacted]

Results and discussion

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

Acceptability

[redacted]

Remarks

98/8 Doc IIIA section No.	7.1.1.1.2 / 02	Phototransformation in water including identitiy of products of transformation
91/414 Annex Point addressed	II 7.2.1.2 / 01	Quantum yield

1.	Annex point(s)	II, 7.2.1.2	**Quantum yield of metabolites**
2.	Location in Dossier	Section 5	
3.	Authors (year) Title Report No., Date Syngenta File N°(Desire) Owner	H. Rüdel (1998) Quantum Yield of the Photochemical Degradation of CGA 322704 in Aqueous Solution NOV-001/7-21, 10.11.1998) 322704-18 Syngenta Crop Protection AG, Product Safety/Ecochemistry, 4002 Basel, Switzerland	
4.	Testing facility	ITA Frauenhofer-Inst., Hannover, Germany	
5.	Dates of work	Study Initiation: Study Termination:	July 24, 1997 September 12, 1997
6.	Test substance	CGA 293343, Lot No. [redacted]	
7.	Test method	UBA Test Guideline "Phototransformation of Chemicals in Water, Part A, Direct Phototransformation", Berlin, FRG January 1990. ECETOC-technical report No. 12, Brussels 1984: "The Phototransformation of Chemicals in Water: Results of a Ring Test.	
8.	Deviations	none	
9.	GLP	yes, German Chemicals Law (Chemikaliengesetz) of July 25, 1994 (Anhang 1 zu Par. 19a, Abs.1 ChemG, Bundesgesetzblatt Teil 1, S. 1724-1732).	

Test system: The degree of photolytic degradation and the quantum yield of CGA 322704 were determined by irradiation with xenon light at 296 ± 6 nm at 20°C in accordance with the Draft OECD Test Guideline. For irradiation CGA 322704 was dissolved in water containing 2% acetonitrile. Initial test substance concentrations for quantum yield determination ranged from 29 mg/l to 114 mg/l. Concentrations of CGA 322704 were analyzed by HPLC for different irradiation times (0.5h to 6 h) in the Quantacount apparatus. The quantum yield was determined from the slope of a plot of the absorbed quanta versus the degree of degradation. Environmental half-life calculation was estimated using a computer program utilizing the spectral distribution of the solar intensity determined from global radiation dates and a simple atmospheric model (R.Franck and W.Klöpffer, UBA Berlin).

Findings: The mean quantum yield of CGA 322704 in water was determined to be $\Phi \pm \sigma = 0.0215 \pm 0.0001$. Degradation products determined by HPLC were CGA 353968 and NOA 404617. The half-life for CGA 322704 referring to direct photolysis in natural sunlight for Northern latitude 52^0N were found to vary between 7.2 hours in summer and 8.5 days in winter.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

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98/8 Doc IIIA section No.	7.1.1.1.2 / 02	Phototransformation in water including identitiy of products of transformation
91/414 Annex Point addressed	II 7.2.1.2 / 01	Quantum yield

1.	Annex point(s)	II, 7.2.1.2	**Quantum yield of metabolites**
2.	Location in Dossier	Section 5	
3.	Authors (year) Title Report No., Date Syngenta File N°(Desire) Owner	H. Rüdel (1998) Quantum Yield of the Photochemical Degradation of CGA 322704 in Aqueous Solution NOV-001/7-21, 10.11.1998) 322704-18 Syngenta Crop Protection AG, Product Safety/Ecochemistry, 4002 Basel, Switzerland	
4.	Testing facility	ITA Frauenhofer-Inst., Hannover, Germany	
5.	Dates of work	Study Initiation: Study Termination:	July 24, 1997 September 12, 1997
6.	Test substance	CGA 293343, Lot No. [redacted]	
7.	Test method	UBA Test Guideline "Phototransformation of Chemicals in Water, Part A, Direct Phototransformation", Berlin, FRG January 1990. ECETOC-technical report No. 12, Brussels 1984: "The Phototransformation of Chemicals in Water: Results of a Ring Test.	
8.	Deviations	none	
9.	GLP	yes, German Chemicals Law (Chemikaliengesetz) of July 25, 1994 (Anhang 1 zu Par. 19a, Abs.1 ChemG, Bundesgesetzblatt Teil 1, S. 1724-1732).	

Test system: The degree of photolytic degradation and the quantum yield of CGA 322704 were determined by irradiation with xenon light at 296 ± 6 nm at 20°C in accordance with the Draft OECD Test Guideline. For irradiation CGA 322704 was dissolved in water containing 2% acetonitrile. Initial test substance concentrations for quantum yield determination ranged from 29 mg/l to 114 mg/l. Concentrations of CGA 322704 were analyzed by HPLC for different irradiation times (0.5h to 6 h) in the Quantacount apparatus. The quantum yield was determined from the slope of a plot of the absorbed quanta versus the degree of degradation. Environmental half-life calculation was estimated using a computer program utilizing the spectral distribution of the solar intensity determined from global radiation dates and a simple atmospheric model (R.Franck and W.Klöpffer, UBA Berlin).

Findings: The mean quantum yield of CGA 322704 in water was determined to be $\Phi \pm \sigma = 0.0215 \pm 0.0001$. Degradation products determined by HPLC were CGA 353968 and NOA 404617. The half-life for CGA 322704 referring to direct photolysis in natural sunlight for Northern latitude 52^0N were found to vary between 7.2 hours in summer and 8.5 days in winter.

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 17/08/04

Materials and Methods

[redacted]

Results and discussion

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

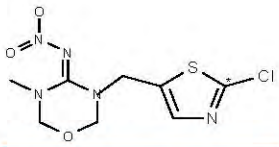
Acceptability

[redacted]

Remarks

[redacted]

98/8 Doc IIIA section No.	7.1.1.1.2 / 03	Phototransformation in water including identitiy of products of transformation
91/414 Annex Point addressed	II 7.2.1.2 / 02	Direct phototransformation

1.	Annex point(s)	IIA, 7.2.1.2 Direct phototransformation
2.	Location in Dossier	Section 5,
3.	Authors (year)	Barbara Schwartz (1998b)
	Title	Final Report: Photodegradation of ^{14}C-Thiazolyly CGA 293343 in pH 5 Buffered Solution Under Artificial Light
	Report No., Date	ABR-98091, 27 October, 1998
	Syngenta File N°(Desire)	293343/798
	Owner	Syngenta Crop Protection AG
4.	Testing facility	Novartis Crop Protection Inc Environmental Safety Department Greensboro, NC 27419, USA
5.	Dates of work	13 February 1996 - 27 October, 1998.
6.	Test substance	ISO common name: thiamethoxam Company code: CGA 293343 Batch: [redacted] ^{14}C-labelled test substance Yes , ^{14}C-thiazolylye label Specific activity of [^{14}C] 1.61 MBq/mg Purity of the test substance: [redacted] Structural formula: (position of label)  Formulation used for study: Yes [ ] No [×]
7.	Test method	The study was conducted in compliance with the Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water, U.S. Environmental Protection Agency, October 18, 1982
8.	Deviations	No deviations have to be reported
9.	GLP	This study was performed in compliance with Good Laboratory Practice (GLP) : EPA Good Laboratory Practice Standards (40 CFR Part 160)

Test system: This study was already presented in Section 2.9.2. Aqueous solutions of thiamethoxam (10.4 mg/l) in 0.01 M phosphate buffer pH 5 were irradiated at 25 °C ± 1 °C with xenon arc light under sterile conditions. The emission spectrum of the artificial light was shown to be comparable to that of natural sunlight, both revealing a cut-off below 290 nm. Samples were exposed to light for 12 hours at an average intensity of 410 W/m^2 per day followed by 12 hours dark intervals with a total incubation time for 30 days. Volatiles were collected and samples assayed for radiochemical balance and degradates pattern. Dark controls were run for the same time intervals under same conditions but protected from light.

Findings: Degradation of thiamethoxam under photolytic conditions in the pH 5 buffered aqueous solution followed first order kinetics with a half-life of 3.1 days and a rate constant of 0.22479. Under non-irradiated control or hydrolytic conditions at pH 5 thiamethoxam did not significantly degrade. The radiochemical balance during the 30 day photodegradation study ranged from 84.5% to 99.6%. Photolytic degradation led to formation of at least 25 components. Volatile radioactivity accounted for up to an average of 56.8% of the total dose. The volatile radioactivity was identified by derivatization with cyclohexylamine as carbonyl sulfide and as isocyanic acid. Carbonyl sulfide was observed to be the predominant component of the volatile fraction. In the aqueous phase one component that was identified as CGA 355190 accumulated between 2% and 10%. Other minor components occurring at levels below 2% were identified as CGA 322704, NOA 407475 and CGA 353968.

Table 1: Degradation pattern of ^{14}C-thiazolyl-thiamethoxam in pH 5 buffered Solution with and without irradiation (Schwartz 1998b)

	Irradiated (in % of total dose)				Non-Irradiated (in % of total dose)		
Time (d)	Volatiles (CO_2)	CGA-293343	CGA-355190	Balance	Volatiles (CO_2)	CGA-293343	Balance
0	0.00	93.18	0.35	96.22	0.00	93.89	97.08
0.25	5.23	88.72	0.52	98.63	0.06	95.11	98.62
0.5	10.22	79.64	1.04	97.03	0.07	94.04	96.97
1	13.06	74.92	0.96	95.51	0.05	92.9	95.31
2	22.40	61.95	3.20	95.59	0.05	93.85	96.79
3	29.60	46.63	2.75	91.97	0.05	93.93	96.67
5	42.39	32.04	4.88	95.62	0.05	94.87	97.43
7	46.76	17.72	6.45	91.68	0.06	94.39	97.04
14	56.76	1.79	7.47	91.69	0.06	95.15	98.23
21	54.87	0.55	9.24	93.27	0.06	95.26	98.18
30	54.30	0.32	8.45	89.80	0.06	93.1	96.11

Values are means of two replicates

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

19/08/04

Materials and Methods

The study was performed to estimate the photodegradation of ^{14}C-[Thiazolyl]-

[redacted]

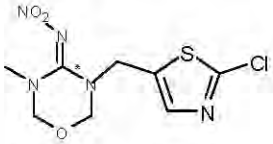
Results and discussion

[redacted]

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

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

98/8 Doc IIIA section No.	7.1.1.1.2 / 04	Phototransformation in water including identitiy of products of transformation
91/414 Annex Point addressed	II 7.2.1.2 / 03	Direct phototransformation

1.	**Annex point(s)**	**IIA, 7.2.1.2**	**Direct phototransformation**
2.	**Location in Dossier**	Section 5,	
3.	**Authors (year)**	Kay Sparrow. (1997c)	
	Title	Final Report: Photodegradation of ^{14}C-Guanidine CGA 293343 in pH 5 Buffered Solution Under Artificial Light	
	Report No., Date	ABR-97023, 27 October, 1997	
	Syngenta File N°(Desire)	293343/375	
	Owner	Syngenta Crop Protection AG	
4.	**Testing facility**	Novartis Crop Protection Inc Environmental Safety Department Greensboro, NC 27419, USA	
5.	**Dates of work**	10 october 1995 - 27 October, 1997.	
6.	**Test substance**	ISO common name:	thiamethoxam
		Company code:	CGA 293343
		Batch:	[redacted]
		^{14}C-labelled test substance	Yes , ^{14}C-guanidine label
		Specific activity of [^{14}C]	1.57 MBq/mg
		Purity of the test substance:	[redacted]
		Structural formula: (position of label)	
		Formulation used for study:	Yes [ ] No [×]
7.	**Test method**	The study was conducted in compliance with the Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, EPA-540/9-82-021, Section 161-2: Photodegradation Studies in Water, U.S. Environmental Protection Agency, October 18, 1982	
8.	**Deviations**	No deviations have to be reported	
9.	**GLP**	This study was performed in compliance with Good Laboratory Practice (GLP) : EPA Good Laboratory Practice Standards (40 CFR Part 160) .	

Test system: This study was already presented in Section 2.9.2. Aqueous solutions of thiamethoxam (10.4 mg/l) in 0.01 M phosphate buffer pH 5 were irradiated at 25 °C ± 1 °C with xenon arc light under sterile conditions. The emission spectrum of the artificial light was shown to be comparable to that of natural sunlight, both revealing a cut-off below 300 nm. Samples were exposed to light for 12 hours at an average intensity of 410 W/m^2 per day followed by 12 hours dark intervals with a total incubation time for 30 days. Volatiles were collected and samples assayed for radiochemical balance and degradates pattern. Dark controls were run for the same time intervals under same conditions but protected from light.

Findings: Degradation of thiamethoxam under photolytic conditions in the pH 5 buffered aqueous solution followed first order kinetics with a half-life of 2.3 days and a rate constant of 0.3025. Under non-irradiated control or hydrolytic conditions at pH 5 thiamethoxam did not significantly degrade. The radiochemical balance during the 30 day photodegradation study ranged from 98.4% to 110.1%. Photolytic degradation led to formation of at least 22 components. Volatile radioactivity was negligible with <1.7% of the total dose after 30 days and was not further characterized. There was one component in the irradiated incubations that exceeded the 10% of the total applied dose. It was identified as CGA 353042 and accounted for 65.8% at day 30. Other degradates identified were CGA 355190 (average maximum 4.1%), CGA 353968 (0.6%), CGA 322704 (3.0%), NOA 407475(2.3%) and methylurea (3.5%). Further minor degradates are proposed to be formed as a result of a multi-step hydrolysis via the isocynate form of the thiazolyl ring components and subsequent recyclization.

Table 1: Degradation pattern of ^{14}C- Guanidine -thiamethoxam in pH 5 buffered Solution with and without irradiation (Sparrow 1997c)

	Irradiated (in % of total dose)							Non-Irradiated (in % of total dose)		
Time (d)	Volatiles	CGA-293343	CGA 353042	CGA-355190	CGA 322704	"Zone O" (several minor components)	Balance	Volatiles	CGA-293343	Balance
0		96.33	0.22	0.32	1.04	0.00	101.08	-	95.62	101.46
0.17	0.02	85.28	2.70	0.39	0.92	1.33	99.73	0.01	96.02	100.21
0.33	0.06	72.86	6.14	0.58	1.24	1.99	99.46	0.01	92.84	100.21
0.46	0.11	70.18	8.90	1.05	2.11	4.71	99.27	0.00	98.93	100.37
0.58	0.09	69.53	9.62	1.17	1.55	5.03	99.97	0.00	97.89	99.96
1	0.10	71.34	9.14	0.94	1.48	4.12	99.17	0.00	94.92	100.07
3	0.45	26.24	29.64	2.57	2.20	10.21	100.02	0.00	91.49	100.50
5	0.81	17.44	35.29	2.91	2.47	13.76	100.08	0.00	96.26	101.31
7	0.82	13.98	39.98	3.37	2.49	11.15	102.33	0.00	91.97	100.49
14	1.46	1.16	54.14	4.08	2.81	14.13	105.18	0.01	94.57	100.48
21	1.35	0.48	58.22	3.34	2.65	13.77	104.56	0.02	91.12	100.54
30	1.55	0.45	65.75	3.32	2.94	13.85	109.93	0.00	93.73	100.00

Values are means of two replicates

Evaluation by Competent Authorities	
	7.1.1.1.2/04
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20/08/04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	
Remarks	