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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
7AND TOXIC SUBSTANCES

June 20, 2000

MEMORANDUM

SUBJECT:

Thiamethoxam - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

Sanjivani Diwan

PC Code - 060109

TO: Pamela Hurley, Toxicologist/Risk Assessor
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And

Dani Daniel, Product Manager
Insecticide/Rodenticide Branch
Registration Division (7505C)

The Cancer Assessment Review Committee met on April 5, 2000 to evaluate the carcinogenic potential of Thiamethoxam. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

014209

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
THIAMETHOXAM
P.C .Code: 60109

FINAL REPORT

20-JUNE, 2000

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION:

Pamela Hurley, Toxicologist

Gordon Cockell, Toxicologist, PMRA, Canada

DOCUMENT PREPARATION:

Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam

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Nancy McCarroll

Esther Rinde

Jess Rowland

Joycelyn Stewart

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John Pletcher, Pathology — See attached sheet

Lori Brunsman, Statistician

GUEST IN ATTENDANCE

Brenda Linke/ PMRA, Canada

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CONTENTS

EXECUTIVE SUMMARY

On April 5, 2000, the Cancer Assessment Review Committee (CARC) met to evaluate the carcinogenic potential of thiamethoxam. The evaluated studies included a 2-year combined chronic toxicity/carcinogenicity study in Tif:RAIf(SPF) Sprague-Dawley rats and an 18-month carcinogenicity study in Tif:MAG(SPF) mice.

In the chronic toxicity/carcinogenicity study, 50 rats/sex/dose received thiamethoxam at dietary levels of 0, 10, 30, 500 or 1,500 ppm for males (0, 0.41, 1.29, 21 or 63 mg/kg/day, respectively) and 0, 10, 30, 1,000 or 3,000 ppm for females (0, 0.48, 1.56, 50.3 or 155 mg/kg/day, respectively) for 24 months. In the carcinogenicity study in mice, thiamethoxam was administered in the diet to 50 mice/sex/group at 0, 5, 20, 500, 1,250 or 2500 ppm (0, 0.65, 2.63, 63.8, 162 or 354 mg/kg/day for males and 0, 0.89, 3.68, 87.6, 215 or 479 mg/kg/day for females, respectively) for 18 months.

The CARC concluded that

- **Under the conditions of the study, thiamethoxam was not carcinogenic to male and female rats.** The Committee determined that the animals could have tolerated higher doses because the dose levels tested were not supported by the results of a 90-day study and short-term studies which exhibited liver toxicity at higher doses. Therefore, the CARC concluded that higher doses should have been used to assess the carcinogenic potential of thiamethoxam.

- **Dietary administration of thiamethoxam was associated with increased incidence of liver tumors in both sexes of mice** because: 1) There was a significant increase by pair-wise comparison with the controls for hepatocellular adenomas and combined adenomas/carcinomas at 500 ppm in females (87.6 mg/kg/day) and at 1,250 and 2,500 ppm in males and females (162 and 354 mg/kg/day in males and 215 and 479 mg/kg/day in females, respectively) as well as carcinomas at 1,250 and 2,500 ppm in males (162 and 354 mg/kg/day, respectively) and at 2,500 ppm (479 mg/kg/day) in females. The incidence of carcinomas in females at 500 and 1,250 ppm, although not statistically significant, exceeded the concurrent and historical control values. In addition, there were significant positive trends for adenomas, carcinomas and combined adenomas/carcinomas in both sexes. The increases in these tumors in males at 500 ppm were considered to be biologically significant and were supported by a dose-related increase in the incidence and severity of non-neoplastic lesions. The incidences of adenomas and carcinomas at ≥500 ppm exceeded the historical control ranges (males: 10%-46% and 0%-24%, respectively; females: 0%-8% and 0%-2%, respectively). The CARC concluded that the liver tumors in male and female mice were treatment-related. Dosing at the highest dose was considered adequate and not excessive based on decreased body weight gains in both sexes and histopathological changes in the liver and kidney.

- Thiamethoxam was negative in both *in vitro and in vivo* mutagenicity assays. The structurally-related compound, imidacloprid, induces chromosomal aberrations only at toxic dose levels.

- The Mechanism of Toxicity Assessment Review Committee (MTARC) concluded that the available data are insufficient at this time to support the proposed non-linear mode of action for liver carcinogenicity of thiamethoxam.

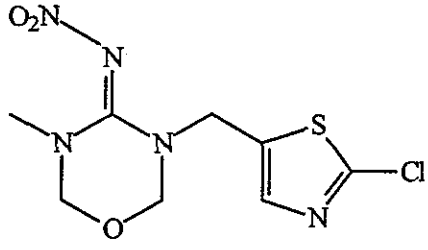
According to EPA's *Draft Cancer Risk Assessment Guidelines* (July, 1999), the CARC classified thiamethoxam as **"likely to be carcinogenic to humans "** by the oral route based on the occurrence of both benign and malignant hepatocellular tumors in both sexes of mice. Although no tumors were observed in rats, a hepatocarcinogenic response cannot be ruled out because the animals were not tested at higher dose levels. The CARC recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on the most potent of the liver tumor response observed in mice. This approach is supported by the lack of confirmation of the mode of action of thiamethoxam.

I. INTRODUCTION

On April 5, 2000, the Cancer Assessment Review Committee [CARC] met to discuss the carcinogenic potential of thiamethoxam. At this meeting, a chronic toxicity/carcinogenicity study in rats and a carcinogenicity study in mice were evaluated. Dr. Pamela Hurley from the Registration Action Branch 1 and Mr. Gordon Cockell, PMRA, Canada presented the results of the two studies, weight-of the evidence, mutagenicity studies and the mode of action data submitted by the registrant as well as structure-activity of related compounds.

II. BACKGROUND INFORMATION

Thiamethoxam, **PC Code 60109** (4H-1,3,5-oxadiazin-4-imine, 3-[(2-chloro-5-thiazoly)methyl]tetrahydro-5-methyl-N-nitro-; CAS No. 153719-23-4) is a new insecticide active ingredient. The molecular weight is 291.7 and the molecular formula is $C_8H_{10}ClN_5O_3S$. The chemical structure is as follows:



Thiamethoxam has activity against sucking and chewing insects. It is a broad spectrum insecticide which belongs to a new class of compounds, the neonicotinoids. While laboratory data indicate that thiamethoxam interferes with the nicotinic acetyl choline receptors of the insect's nervous system, the specific binding site/receptor(s) is unknown at this time. Although imidacloprid, also a neonicotinoid insecticide, is also known to interfere with nicotinic acetyl choline receptors, thiamethoxam appears to function at a different location. Thiamethoxam does not inhibit cholinesterase or interfere with sodium channels and, therefore, has a different mode of action than organophosphate, carbamate and pyrethroid insecticides. It is reported to have excellent acropetal translocation in the xylem and no basipetal movement in the phloem. Registration is being requested for foliar uses as well as treatment of canola and mustard seed. For seed treatment, the formulation will be applied to seed as a slurry or mist in commercial seed treatment facilities. Thiamethoxam will be both a food and nonfood-use chemical. There will be occupational exposure. There are no current residential uses; however, pet and residential uses (turf) will be proposed in the future.

III. EVALUATION OF CARCINOGENICITY STUDIES

A. Combined Chronic Toxicity/Carcinogenicity Study with Thiamethoxam in Tif: RAIf (SPF) Rats

Reference: Bachmann, M. (1998) 24-Month Carcinogenicity and Chronic Toxicity Study in Rats. Novartis Crop Protection AG Toxicology, Stein, Switzerland. Test No. 942110, July 27, 1998. Unpublished. **MRID 44718708.**

1. Experimental Design

In a combined chronic toxicity and carcinogenicity study, CGA 293343, 98.6% a.i. was administered to 80 Tif: RAIf (SPF) Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 10, 30, 500 or 1500 ppm (0, 0.41, 1.29, 21.0 or 63.0 mg/kg/day) in males and 0, 10, 30, 1000 or 3000 ppm (0, 0.48, 1.56, 50.3 or 155 mg/kg/day) in females for 24 months. The animals were divided into 4 groups per dose level, with 50/sex/group used in the main chronic toxicity and carcinogenicity study, 10/sex/group for haematology, clinical chemistry and urinalysis, 10/sex/group for haematology and 10/sex/group for interim sacrifice at 12 months.

2. Discussion of Tumor Data

The dietary administration of thiamethoxam up to 1500 ppm in males (63.0 mg/kg/day) and 3000 ppm in females (155 mg/kg/day) did not result in an overall treatment-related increase in tumor incidences in Tif: RAIf (SPF) Sprague-Dawley rats; however, foci of hepatocellular alteration were seen in high-dose females. Although no increases in tumor incidences were observed, it appears that higher doses would have been tolerated, hence there is residual uncertainty regarding what liver toxicity might have been observed if higher doses had been used in the study. It is quite possible that liver tumors would appear following treatment with higher doses in rats.

3. Non-Neoplastic Lesions

At interim sacrifice, treatment-related findings include increased incidence of lymphocytic infiltration of the kidneys and chronic tubular lesions in males at 21.0 mg/kg/day and above. The incidence of basophilic proliferation of renal tubules, when considered in conjunction with the chronic tubular lesions indicates an increase in total regenerative tubular lesions in male kidneys at 21 mg/kg/day and above. Lymphocytic infiltration of the renal pelvis was observed more frequently in high-dose males, and a slight increase in the severity of hemosiderosis of the spleen was observed in high-dose females.

At terminal sacrifice, an increased incidence of foci of cellular alteration in the livers of high-dose females (mainly clear cell); lymphocytic infiltration in the kidneys of males (considered treatment-related at 1500 ppm by the study authors; only found to be an increasing trend by EPA statistician); and, chronic nephropathy in high-dose males. There was also an increasing trend in the incidence of chronic tubular lesions in the kidneys of females. Increasing trends were observed with the following lesions that were not considered by the study authors to be related to treatment: bronchiolo-alveolar hyperplasia (females), dilatation of the large intestine (females), inflammatory cell infiltration of the urinary bladder (females), chronic inflammation of the thymus (females), inflammation of the eyes with fibrosis (females), and hydrocephalus (males - secondary to pituitary adenoma).

TABLE 1: Non-neoplastic microscopic findings at terminal sacrifice

Dose (ppm)	0	10	30	500	1500	0	10	30	1000	3000
Organ/observation	**Males (n = 50)**					**Females (n = 50)**				
Liver - focus of cellular alteration	20	21	15	21	20	10**	21**	12	15	26**
Kidney - lymphocytic infiltration	10**	10	7	14	17	2	3	4	2	2
- chronic nephropathy	30**	35	32	37	42**	12	10	8	6	10
Kidney - chronic tubular lesion	10	10	9	6	4	14*	16	13	18	21

* $p < 0.05$; ** $p < 0.01$. All analyses used the Exact trend test (more precise than the Cochran-Armitage) and the Fisher's Exact test for pair-wise comparisons. Analyses conducted by EPA statistician.

4. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate by the Registrant based on the observed reduction in body weight gain among high-dose females and increase in incidence of chronic nephropathy in males. However, the CARC determined that the doses tested were not supported by the results of a 90-day study and short-term studies. In a 90-day dietary study, decrease in body weight gain (22%, 19% and 27% at 1,250, 2,500 and 5,000 ppm, respectively) was not dose-dependent. At ≥1,250 ppm, increases in liver and kidney weights were noted. In addition, increases in the incidence and occasionally the severity of microscopic lesions were observed in liver (hepatocellular hypertrophy and lymphohistiocytic infiltration), kidney (hyaline change in the tubular epithelium in males, chronic tubular lesions in both sexes and nephrocalcinosis in females), spleen (hemosiderosis and extramedulary haematopoiesis) and adrenal gland (fatty change). In addition, significant increase in relative liver, kidney, spleen, and adrenal weights was noted only in males at 5,000 ppm. The 28-day exploratory study reported adverse effects at ≥300 mg/kg/day consisting of increased liver weights, hepatocellular hypertrophy, increased incidence of renal pelvic dilatation and adrenocorticoid fatty change. At 1,000 mg/kg/day, decrease in body weight gain, increased levels of AST, ALP and GGT as well as enlarged liver were noted. In a 28-day range finding study, changes in the kidney

(hyaline change of renal tubular epithelium, focal calcification of the renal cortex, pelvic dilatation with epithelial hyperplasia, renal cyst, lymphohistiocytic infiltration, acute tubular lesions and tubular basophilic proliferation) and liver (increases in plasma cholesterol and AST, increased absolute and relative liver weights, and hepatocellular hypertrophy) were noted at ≥1000 ppm in one or both sexes. Thus, the evidence from the short-term studies in rats indicate hepatocellular hypertrophy at higher dose levels. The chronic study was not tested in that range either. These observations seem to indicate that if higher doses were used in the rat chronic toxicity and carcinogenicity study, liver pathology would have occurred. This is apparent from the liver histopathology among high-dose females in the chronic toxicity/carcinogenicity study in which an increase in the incidence of foci of cellular alteration was observed in females receiving 3,000 ppm (155 mg/kg/day). The most important consideration is the dose level selection in the rat chronic toxicity and carcinogenicity study. High-dose males were treated at daily doses that were lower than the LOAELs for liver toxicity in the short-term studies, and proliferative, pre-neoplastic lesions were observed in high-dose females. Mortality was not affected by treatment. If these animals had been tested at higher dose levels, tumors may have been induced. Therefore, the CARC concluded that the higher doses should have been used to assess the carcinogenic potential of thiamethoxam.

B. Carcinogenicity Study in Mice

Reference: Bachmann, M. (1998) 18-Month Oncogenicity Study in Mice. Novartis Crop Protection AG Toxicology, Stein, Switzerland. Test No. 942109, June 2, 1998. Unpublished. **MRID 44703326.**

1. Experimental Design

In a carcinogenicity study, CGA 293343 technical, 98.6% was administered to 50 mice/sex/dose in the diet, at dose levels of 0, 5, 20, 500, 1250 or 2500 ppm (0, 0.65/0.89, 2.63/3.68, 63.8/87.6, 162/215 or 354/479 mg/kg/day in males/females, respectively) for 78 weeks. An additional 10 mice/sex in the control and high dose were used for interim sacrifice after 9 months of treatment.

2. Discussion of Tumor Data

Prior to completion of the data evaluation record from the submitted study, a re-read of the liver tumors was conducted by a Pathology Working Group (PWG; MRID No. 44988301). Tables 2 and 3 reflect the statistical analyses of the results of the re-read. In male mice, the dietary administration of thiamethoxam resulted in significant increases in the pair-wise comparisons with the controls for hepatocellular adenomas and combined adenomas/ carcinomas ($p < 0.01$) at both 1250 ppm (162 mg/kg/day) and 2500 ppm (354

mg/kg/day). Significant increases in the pair-wise comparison with the controls for carcinomas were also observed ($p < 0.01$ at 2500 ppm (354 mg/kg/day) and $p < 0.05$ at 1250 ppm (162 mg/kg/day)).The incidence of adenomas and carcinomas exceeded the historical control ranges (10%-46% and 0%-24%, respectively). Significant increasing trends ($p < 0.01$) were observed for all three parameters: hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. **It should be noted that the Registrant's statistical analysis for male mice at 500 ppm using the same values, indicated pair-wise statistical significance ($p < 0.05$) for either adenomas, carcinomas or combined adenomas/carcinomas, using Peto's mortality prevalence test (Peto et al. 1980) (step down and closed testing procedure).** Female mice had significant differences in the pair-wise comparisons of the 2,500 ppm dose group with the controls, for hepatocellular adenomas, carcinomas, and combined adenomas/carcinomas, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 500 ($p < 0.05$) and 1,250 ($p < 0.01$) ppm dose groups with the controls for hepatocellular adenomas and combined adenomas/carcinomas. The incidences of adenomas and carcinomas exceeded the historical control ranges (0%-8.16% and 0%-2%, respectively). Significant increasing trends ($p < 0.01$) were observed for all three parameters: hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. The statistical analyses of the male and female mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of thiamethoxam in either sex.

Table 2. CGA 293343 (Thiamethoxam™) - Tif:MAGf(SPF) Mouse Study: 1999 Re-Read
Male Liver Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p values)

Dose (ppm)	0	5	20	500	1250	2500
Adenoma	11/50	5/50	10[a]/49	17/50	27/48	40/50
(%)	(22)	(10)	(20)	(34)	(56)	(80)
p =	0.000**	0.086	0.521	0.133	0.001**	0.000**
Carcinoma	1[b]/50	4/48	2/46	5/50	7/46	20/49
(%)	(2)	(8)	(4)	(10)	(15)	(41)
p =	0.000**	0.169	0.468	0.102	0.022*	0.000**
Combined	12/50	7[c]/50	12/49	19[d]/50	27[e]/48	45[f]/50
(%)	(24)	(14)	(24)	(38)	(56)	(90)
p =	0.000**	0.154	0.570	0.097	0.001**	0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 41 for adenomas and combined, and before week 53 for carcinomas.

[a]First adenoma observed at week 41, dose 20 ppm.

[b]First carcinoma observed at week 64, dose 0 ppm.

[c]Two animals in the 5 ppm dose group had both an adenoma and a carcinoma.

[d]Three animals in the 500 ppm dose group had both an adenoma and a carcinoma.

[e]Seven animals in the 1250 ppm dose group had both an adenoma and a carcinoma.

[f]Fifteen animals in the 2500 ppm dose group had both an adenoma and a carcinoma.
Note: Interim sacrifice animals are not included in this analysis. There were no liver adenomas or carcinomas observed in any interim sacrifice animals. The significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. CGA 293343 (Thiamethoxam™) - Tif:MAGf(SPF) Mouse Study: 1999 Re-Read

Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

Dose (ppm)	0	5	20	500	1250	2500
Adenoma (%) p =	0/48 (0) 0.000**	0/50 (0) 1.000	0/49 (0) 1.000	5[a]/44 (11) 0.022*	8/47 (17) 0.003**	31/49 (63) 0.000**
Carcinoma (%) p =	0/48 (0) 0.000**	0/50 (0) 1.000	0/49 (0) 1.000	0/44 (0) 1.000	2[b]/47 (4) 0.242	11/49 (22) 0.000**
Combined (%) p =	0/48 (0) 0.000**	0/50 (0) 1.000	0/49 (0) 1.000	5/44 (11) 0.022*	9[c]/47 (19) 0.001**	32[d]/49 (65) 0.000**

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First adenoma observed at week 79, dose 500 ppm.
[b]First carcinoma observed at week 58, dose 1250 ppm.
[c]One animal in the 1250 ppm dose group had both an adenoma and a carcinoma.
[d]Ten animals in the 2500 ppm dose group had both an adenoma and a carcinoma.
Note: Interim sacrifice animals are not included in this analysis. There were no liver adenomas or carcinomas observed in any interim sacrifice animals. Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control incidences for males are as follows: hepatocellular adenoma: mean = 25% with a range of 10-46% and hepatocellular carcinoma: mean = 12.2% with a range of 0-24%. For females, they are as follows: hepatocellular adenoma: mean = 2.9% with a range of 0-8% and hepatocellular carcinoma: mean = 0.3% with a range of 0-2%. For males, the incidence of hepatocellular adenomas is higher than the historical control range at ≥1250 ppm and the incidence of hepatocellular carcinomas is higher than the historical control range at 2500 ppm. For females, the incidence of adenomas is higher than the historical control range at ≥500 ppm and the incidence of carcinomas is higher than the range at ≥1250 ppm. Tables 4 and 5 contain historical control incidences of selected liver lesions. These data were taken from a submitted analysis on the mouse liver tumors from the Registrant (MRID 44988301, page 13).

3. Non-Neoplastic Lesions

At interim sacrifice, the livers of 10 control and 10 high-dose animals were examined

microscopically. There was an increase in the incidence of inflammatory cell infiltration, necrosis of single hepatocytes, Kupffer cell pigmentation and hepatocyte hypertrophy in males and females. The findings ranged in severity from minimal to moderate.

Table 4. Historical Control Incidences of Proliferative Liver Lesions in the Tif: MAGf (SFP) Male Mouse

Study No.[a]	Date of 1st Dose	HA[b]	HCC[c]	FOCA[d]	HB[e]	HN[f]	Livers Examined
881124	Feb 90	18	7	2	1	26	60
901462	Mar 91	17	8	0	0	25	70
891325	May 91	17	7	1	0	24	50
901482	May 92	10	5	2	1	16	50
921063	Jul 92	11	4	7	0	15	50
911122	Sep 92	10	6	0	0	16	50
922117	Nov 92	8	8	5	0	16	50
923177	Aug 93	5	0	1	0	5	50
923150	Sep 93	11	5	2	1	17	50
922815	Oct 93	11	6	4	3	20	53
943039*	Nov 94	23	9	8	1	26	50
941112*	Dec 94	9	12	4	0	18	50
951028*	Feb 96	21	6	2	1	24	50
Total		171	83	33	8	248	683
Mean & SD		25% ± 10%	12.2% ± 5.5%	5.6% ± 5.1%	1.2% ± 1.6%	36% ± 11%	
Minimum		5/50 (10%)	0/50 (0%)	0/50 (0%)	0/70 (0%)	5/50 (10%)	
Maximum		23/50 (46%)	12/50 (24%)	8/50 (16%)	3/53 (6%)	26/50 (52%)	

[a]For studies of 18 month duration
[b]Animals with at least one hepatocellular adenoma (HA).
[c]Animals with hepatocellular adenocarcinoma (HCC).
[d]Animals with focus of cellular alteration (FOCA).
[e]Animals with hepatoblastoma (HB)
[f]Animals with hepatocellular neoplasia (HA or HCC or HB)

*Diagnostic was performed using PathData system based on individual animal data.

Table 5. Historical Control Incidences of Proliferative Liver Lesions in the Tif: MAGf (SFP) Female Mouse

Study No.[a]	Date of 1st Dose	HA[b]	HCC[c]	FOCA[d]	HB[e]	HN[f]	Livers Examined
881124	Feb 90	1	0	0	0	1	60
901462	Mar 91	3	0	0	0	3	70
891325	May 91	0	0	1	0	0	50
901482	May 92	4	0	1	0	4	49
921063	Jul 92	3	0	0	0	3	50
911122	Sep 92	2	0	1	0	2	50
922117	Nov 92	2	1	2	0	3	50
923177	Aug 93	1	1	2	0	2	50
923150	Sep 93	1	0	1	0	1	50
922815	Oct 93	0	0	7	0	0	52
943039*	Nov 94	2	0	5	0	2	50
941112*	Dec 94	1	0	0	0	1	50
951028*	Feb 96	0	0	2	0	0	50
Total		20	2	22	0	22	681
Mean & SD		2.9% ± 2.5%	0.3% ± 0.8%	3.2% ± 4.1%	0.0% ± 0.0%	3.23% ± 2.57%	
Minimum		0/52 (0%)	0/70 (0%)	0/70 (0%)	0/70 (0%)	0/52 (0%)	
Maximum		4/49 (8%)	1/50 (2%)	7/52 (13%)	0/49 (0%)	4/49 (8%)	

[a]For studies of 18 month duration
[b]Animals with at least one hepatocellular adenoma (HA).
[c]Animals with hepatocellular adenocarcinoma (HCC).
[d]Animals with focus of cellular alteration (FOCA).
[e]Animals with hepatoblastoma (HB)
[f]Animals with hepatocellular neoplasia (HA or HCC or HB)
*Diagnostic was performed using PathData system based on individual animal data.

TABLE 6: Microscopic liver observations at interim sacrifice

Observation	Males		Females	
	Control	2500 ppm	Control	2500 ppm
Inflammatory cell infiltration	1	8	3	8
Single cell necrosis	0	10	0	9
Kupffer cell pigmentation	0	8	0	4
Hepatocellular hypertrophy	0	9	0	10
Focus of cellular change	0	1	0	1

At terminal sacrifice, a number of treatment-related decreased incidences of pathological findings were observed, including splenic white pulp hyperplasia in both sexes; lymphocytic infiltration in the salivary gland and cataract formation in the ocular lens in females; and chronic nephropathy, tubular atrophy of the testes, dilatation and inflammation of seminal vesicles, hyperplasia of the adrenal cortex and hyperplasia of pancreatic islet cells in males.

TABLE 7: Non-neoplastic microscopic pathology at terminal sacrifice

Dose (ppm)	0	5	20	500	1250	2500
Males (number examined = 50)						
Liver -focus of cellular alteration	7	4	4	11	22	32
- incidence of grade 4 foci	4	2	1	6	13	26
mean severity	3.0	3.3	3.0	3.5	3.5	3.8
- increased mitotic activity	0	0	0	1	10	8
- hyperplasia of Kupffer cells	0	0	1	0	0	10
- inflammatory cell infiltration	13	9	13	33	41	43
mean severity	1.5	2.6	2.0	2.2	2.8	2.7
- single cell necrosis	5	3	5	40	40	46
mean severity	1.6	2.0	2.0	2.5	3.3	3.3
- hepatocellular hypertrophy	8	11	6	41	40	45
mean severity	1.5	1.6	1.5	2.5	3.3	3.3
- deposition of pigment	2	2	3	13	33	44
Spleen - extramedullary haematopoiesis	18	17	23	27	23	36
Glandular stomach - epithelial hyperplasia	10	14	8	14	13	24

Dose (ppm)	0	5	20	500	1250	2500
Females (number examined = 50)						
Liver -focus of cellular alteration - incidence of grade 4 foci	2 1	2 1	2 1	2 0	14 7	37 26
- increased mitotic activity	1	1	0	4	5	4
- infiltration of inflammatory cells	18	20	20	24	33	45
- necrosis of single cells mean severity	3 2.0	2 2.5	5 2.0	18 2.2	36 2.7	46 3.2
- hepatocellular hypertrophy mean severity	3 2.0	2 1.5	3 1.0	19 2.2	39 2.8	45 3.3
- deposition of pigment	6	5	3	5	14	30
Spleen - extramedullary haematopoiesis	26	28	25	24	27	34
Glandular stomach - epithelial hyperplasia	7	8	5	7	6	20

Data obtained from pages 50-51 and 227-252 of the study report

As stated in the section on the rat chronic feeding/carcinogenicity study, the non-neoplastic changes that were statistically found to be the most closely associated with development of hepatocellular adenoma and/or carcinoma in the mouse include the following: pigmented cells, foci of cellular alteration, hepatocellular hypertrophy, inflammatory cell infiltration, and necrosis. Other observed changes to liver morphology include fatty change, increased mitotic activity, and hyperplasia of Kupffer cells. Some of these were observed at 500 ppm and above. It is important to note that particularly for females, there is no clear departure point between the onset of non-neoplastic effects and neoplastic effects, i.e., the LOAEL for systemic toxicity is the same dose at which the increase in tumor incidence occurred.

4. Adequacy of Dosing for Assessment of Carcinogenicity

The dosing was considered by the CARC to be adequate and not excessive based on the observed reduction in body weight gain in high-dose males (18%) and females (14%) and increased incidence of non-neoplastic and neoplastic microscopic changes in the liver at ≥ 500 ppm in both sexes.

IV. TOXICOLOGY

1. Metabolism

In metabolism studies in rats, thiamethoxam was absorbed rapidly and extensively, and was widely distributed to the tissues. Blood concentrations peaked within 4 hours of dosing, followed by very rapid elimination. The highest tissue concentrations were detected in skeletal muscle within 8 hours of dosing, and accounted for 10-15% of the administered dose. Half life times of elimination from

the tissues ranged from 2-6 hours. Tissue residues after 7 days were extremely low. The majority of the administered dose was excreted in the urine (84-95%), with relatively small amounts excreted in the bile and the faeces (2.5-6%) within 24 hours. Although there were a large number of metabolites, only three accounted for up to 2% or more of the administered dose. The unchanged parent compound was the predominant constituent, accounting for 69-83% of the administered dose (**MRID Nos. 44703532, 44703533, 44710408).**

2. **Mutagenicity:**

Thiamethoxam was tested in gene mutation assays in bacteria and mammalian cells, in *in vivo* and *in vitro* cytogenetics assays, and in an unscheduled DNA synthesis (UDS) assay. All of the assays were acceptable for regulatory purposes and thiamethoxam did not test positive in any of the test systems.

In a reverse gene mutation assay in *S. typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 of and *E. coli* WP2 uvrA were exposed to thiamethoxam (98.6%) at concentrations up to 5000 μg/plate. There was no increase in the number of revertants at any of the concentrations tested, either in the presence or absence of exogenous metabolic activation. There was no evidence of cytotoxicity up to the limit dose. The study satisfies the requirements for a FIFRA Test Guidelines 84-2 for a bacterial gene mutation assay**(MRID 44710404).**

In a reverse gene mutation assay in bacteria, strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 of *S. typhimurium* were exposed to thiamethoxam (98.6%) at concentrations up to 5000 μg/plate. A series of six experiments were conducted, using different induction systems to generate the S9 fraction: non-induced mouse liver, Aroclor 1254 induced mouse liver, or thiamethoxam induced mouse liver, following dietary administration of thiamethoxam for 14 days at concentrations up to 2500 ppm. There was no increase in the number of revertants at any of the concentrations tested, in the presence of any of the exogenous metabolic activation systems. The study satisfies the requirements for a FIFRA Test Guidelines 84-2 for a bacterial gene mutation assay **(MRID 44968301).**

In a mammalian cell gene mutation assay at the *hgprt* locus, Chinese hamster V79 cells cultured *in vitro* were exposed to CGA 293343 (98.6% a.i.) in dimethylsulfoxide at concentrations up to 3330 μg/ml (solubility limit) in the presence of mammalian metabolic activation and 1665 μg/ml in the absence of mammalian metabolic activation. No significant increase in mutant frequency was observed at any concentration of CGA 293343 in either the original or the confirmatory trials. The study satisfies the requirements for a FIFRA Test

Guidelines 84-2 for a mammalian cell gene mutation assay **(MRID 44710405).**

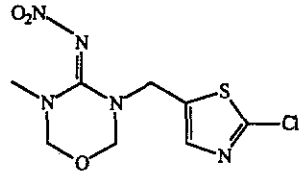
In a mammalian cell cytogenetics assay, CHO cell cultures were exposed to CGA 293343 technical in dimethylsulfoxide at concentrations up to 1135 μg/ml (original experiment), 2270 μg/ml (confirmatory experiment) for a 21 hour treatment time; up to 1702.5 μg/ml for a 45 hour treatment time in the absence of metabolic activation; up to 4540 μg/ml (original experiment) or 4540 μg/ml (confirmatory experiment) for a 3 hour treatment time with 18 hour recovery; and up to 4540 μg/ml for a 3 hour treatment with 42 hour recovery in the presence of metabolic activation. It was tested up to cytotoxic or solubility limit concentrations with no evidence of increased chromosome aberrations over background. The study satisfies the requirements for a FIFRA Test Guidelines 84-2 for an *in vitro* cytogenetic assay **(MRID 44710403).**

In an *in vivo* mouse bone marrow micronucleus assay, groups of Tif: MAGf[SPF] mice, 5/sex/dose/sacrifice time were treated by oral gavage with CGA 293343 (98.6% a.i.) at doses up to 1000 (all males and 16-hour females) and 1250 (24- and 48-hour females) mg/kg bw. An additional 3 rats per sex were assigned to the high-dose group as reserve animals. Bone marrow cells were harvested at 16, 24 and 48 hours post-treatment. The vehicle was bidistilled water and a positive control group of 5 males and 5 females received a single oral dose (64 mg/kg bw) of cyclophosphamide and were sacrificed 24 hours later. In the 1250 mg/kg bw group, 7/13 females were found dead within 5 hours post-treatment and reduced locomotor activity was noted for 3/5 males and 1/5 females in the 625 mg/kg bw group and for 10/18 males and 1/5 females in the 1000 mg/kg bw group. The only other clinical finding was ataxia, noted in 2/18 males and 1/5 females in the 1000 mg/kg bw group. There was no evidence of target cell cytotoxicity. There was also no increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow for males or females after any treatment time. CGA 293343 is, therefore, not considered to be a clastogenic in this test system. The study satisfies the requirements for a FIFRA Test Guidelines 84-2 for an *in vivo* cytogenetic assay **(MRID 44710406).**

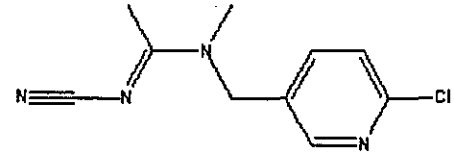
In an UDS assay, primary rat hepatocyte cultures were exposed to CGA 293343 (98.6% a.i.) in dimethylsulfoxide at concentrations of 0, 13.01, 52.04, 208.13, 416.25, 832.5 or 1665 μg/ml for 16-18 hours. CGA 293343 was tested up to precipitating concentrations. There was no evidence that UDS was induced, as determined by radioactive tracer procedures (nuclear silver grain counts) The study satisfies the requirements for a FIFRA Test Guidelines 84-2 for a UDS assay **(MRID 44710407).**

3. Structure-Activity Relationship

Thiamethoxam belongs to a new class of compounds, the neonicotinoids. Related compounds are identified in three subclasses of the neonicotinoids. At the present time, no other compounds are identified in the same subclass as thiamethoxam. Three compounds are currently found in the chloronicotinyl subclass: imidacloprid, nitenpyram and acetamiprid. Nithiazin, another structurally related chemical has been placed in the nitromethylene subclass. Imidacloprid tested negative in all of the mutagenicity studies except for the *in vitro* chromosomal aberration assay (MRID 42256345), where it tested positive at 500 μg/mL (without S9) and 1300 μg/mL (with S9); however, both doses were considered to be toxic. It also tested negative in carcinogenicity studies in rats and mice. Acetamiprid is a new chemical and the toxicity data have not yet been evaluated. With the exception of chromsomal aberration assay, the results of all other mutagenicity studies indicate that acetamiprid is not mutagenic under the conditions of the studies. The Agency has no data on the other compounds in this chemical class.



Thiamethoxam



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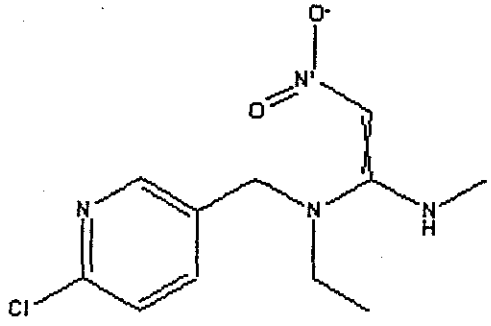
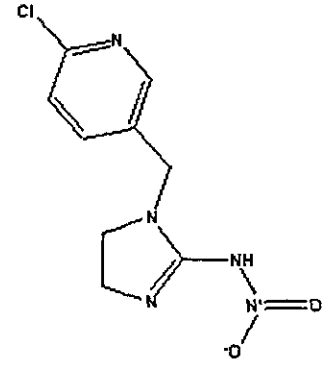


Nitenpyram



Imidacloprid

Subchronic, and Chronic Toxicity

Subchronic Toxicity

Rat

In short-term toxicity studies in rats, the primary target organs were identified as kidney and liver. Males were more sensitive to effects on the kidney than females. Liver toxicity was observed at higher doses, manifested as hepatocellular hypertrophy, increased liver weights and associated changes in clinical biochemical parameters (including increased cholesterol levels and activity of certain liver enzymes). It was postulated that the observed hyaline change was due to the accumulation of α-2-u globulin in the proximal convoluted tubules, a protein which is unique to male rats. The observed kidney pathology is consistent with α-2-u globulin mediated effects, however no data are available to confirm that α-2-u globulin is present in the lesions, nor that it is the causative agent leading to the development of the observed kidney lesions. It should also be noted that the same hyaline change, consisting of eosinophilic droplets within the cytoplasm of the proximal convoluted tubules, was observed in one female of the F1 generation in the 2-generation rat reproduction study. In addition, other kidney toxicity was observed in female rats, consisting of chronic tubular lesions and nephrocalcinosis.

In a 28-day range-finding dietary toxicity study, CGA 293343 (98.60%) was administered to 5 Sprague-Dawley Tif:RAIf (SPF) rats/sex/dose in the diet at dose levels of 0, 100, 1000, 2500 or 10000 ppm (0, 8.04/8.69, 81.7/89.3, 199/211, 711/763 mg/kg bw/day for males/females). Treatment with CGA 293343 resulted in decreased food intake and body weight gain in males treated at 10000 ppm. Treatment had no effect on mortality, haematology or urinalysis. Clinical biochemistry investigations revealed treatment-related increases in plasma cholesterol and aspartate aminotransferase at 10000 ppm. Increased absolute and relative liver weights were recorded at 10000 ppm and histopathological examination of liver revealed hepatocellular hypertrophy at 2500 and 10000 ppm. Histopathological lesions in the kidney were observed at doses of 1000 ppm and above, and included hyaline change of renal tubular epithelium, focal calcification of the renal cortex, pelvic dilatation with epithelial hyperplasia, renal cyst, lymphohistiocytic infiltration, acute tubular lesions and tubular basophilic proliferation. Fatty change of the adrenal cortex was observed in both sexes at 10000 ppm and hypertrophy of thyroid follicular epithelium was observed in males at 2500 and in both sexes at 10000 ppm. Treatment with CGA 293343 in the diet for 4

weeks resulted in a **NOAEL of 100 ppm**, based on the incidence of microscopic lesions of the kidney. **This dose is equal to a mean daily intake of 8.0 mg/kg body weight in males and 8.7 mg/kg body weight in females. The LOAEL is 1000 ppm, equal to 82 and 89 mg/kg bw/day in males and females, respectively**. This subchronic toxicity study is classified as supplementary and does not satisfy the guideline requirement for a subchronic oral study; OECD No. 407 in the rat. It was performed for range-finding purposes only **(MRID 44703322).**

In a 28-day exploratory toxicity study, CGA 293343 (98.60%) was administered by gavage, 5 times/week for 4 weeks, to 5 male Sprague-Dawley Tif:RAIf (SPF) rats/group at dose levels of 0, 100, 300 or 1000 mg/kg bw/day. Treatment with CGA 293343 resulted in significantly reduced body weight gain at 1000 mg/kg bw/day. Treatment had no effect on mortality and hematology. Increased incidence of skin lesions in the neck region, decreased plasma protein levels and higher activities of aspartate aminotransferase,alkaline phosphatase and gamma-glutamyltranspeptidase were observed at 1000 mg/kg bw/day. Slightly reduced plasma glucose levels and increased absolute and relative liver weights were observed at 300 mg/kg bw/day and above. Reduced absolute and relative thymus weights, enlarged liver, dilated renal pelvis and increased incidence of scab formation were observed at 1000 mg/kg bw/day. Minimal to moderate hepatocellular hypertrophy associated with pronounced nuclear alterations, increased incidence of renal pelvic dilatation and increased incidence and severity of adrenocortical fatty change were observed at 300 mg/kg bw/day and above. Minimal to moderate renal tubular hyaline change was observed at 100 and 300 mg/kg bw/day. **No NOAEL or LOAEL was derived from this study. It was conducted for range-finding purposes only**. This subchronic toxicity study is classified as supplementary and does not satisfy the guideline requirement for a subchronic oral study; OECD No. 407 in the rat. It was performed for range-finding purposes only **(MRID 44718701).**

In a 3-month dietary toxicity study, CGA 293343 (98.4%) was administered to 10 Sprague-Dawley Tif:RAIf (SPF) rats/sex/dose in the diet at dose levels of 0, 25, 250, 1250, 2500 or 5000 ppm (0, 1.743/1.879, 17.64/19.19, 84.9/92.5, 167.8/182.1 or 328.8/359.1 mg/kg/day for males/females). Treatment with CGA 293343 tech. did not affect the appearance and behaviour of the animals, mortality, food consumption, ophthalmology, hematology, urinalysis or gross pathology. Treatment resulted in reduced body weight and body weight gain in males at ≥1, 250 ppm, changes in clinical biochemical parameters including increases in creatinine, urea, cholesterol, and phosphate in males, decreased plasma glucose in males and marginal decreases in sodium and chloride levels in males and females at ≥1, 250 ppm. Increased liver, kidney, adrenal, heart and spleen weights relative to body weight were observed in high dose males, and decreased absolute heart and thymus weights were observed in

high dose females. At ≥1,250 ppm, increases in the incidence and occasionally the severity of microscopic lesions were observed in liver (including hepatocellular hypertrophy and lymphohistiocytic infiltration), kidney (including hyaline change in tubular epithelium in males, chronic tubular lesions in both sexes and nephrocalcinosis in females), spleen (hemosiderosis and extramedullary haematopoiesis) and adrenal gland (fatty change). Treatment with CGA 293343 in the diet for 3 months resulted in a **NOAEL of 25 ppm for males and 1250 ppm for females**, equal to mean daily intakes of **1.7 and 92.5 mg/kg body weight in males and females, respectively,** based on histopathological changes observed in kidney (increased incidence of hyaline change in renal tubular epithelium and chronic tubular lesions in males at 250 ppm and increased incidence of chronic tubular lesions and severity of nephrocalcinosis in females at 2500 ppm). **The LOAEL is 250 ppm for males and 2500 ppm for females, equal to 17.6 and 182.1 mg/kg bw/day for males and females, respectively.** This subchronic toxicity study is classified as acceptable and satisfies the guideline requirement for a subchronic oral study; OPPTS 87-3100 [§82-1]; OECD No. 408; 87/302 EEC, B.26 in the rat **(MRID 44718703).**

Dog

In the dog, the main target organs appear to be the testis and the haematopoietic system. In the 3-month study, the high dose initially caused severely decreased food consumption and concomitant body weight loss, necessitating cessation of treatment for 7 days and resumption at a lower dose. Animals in this group had decreased testis weights, reduced spermatogenesis and minimal to moderate occurrence of spermatic giant cells in the testes. In addition, decreased ovary weights associated with delayed maturation of the ovaries was observed at this dose. Slight microcytic anemia as well as leukopenia were also observed in animals receiving the higher doses.

In a subchronic toxicity study thiamethoxam, (98.4% a.i.) was administered to 2 Beagle dogs/sex/dose in the diet at dose levels of 0, 300, 1000 and 3000 ppm (0, 10.0/10.7, 31.6/32.6 and 47.7/43.0 mg/kg/day in males and females, respectively) for 28 days. Treatment with CGA 293343 had no effect on mortality, ophthalmology, urinalysis or gross pathology. Treatment-related effects were restricted to the top dose group and included reduced food intake and body weight loss; leucopenia and increased haematocrit, haemoglobin and erythrocyte count; increased plasma urea and creatinine; reduced thymus weight in males and females, increased thyroid weight in males and reduced brain weight in females; and, histopathological changes in liver, thymus and spleen. No treatment-related effects were observed at 300 or 1000 ppm. **The LOAEL is 3000 ppm, based on the above findings. The NOAEL**

is 1000 ppm. No treatment-related clinical findings occurred during this study. This subchronic toxicity study is classified as supplementary because it was performed for range-finding and palatability purposes. It does not satisfy the guideline requirement for a subchronic oral study (82-1); OECD 409 in the dog (**MRID 44703324**).

In a subchronic toxicity study thiamethoxam, (98.6% a.i.) was administered to 4 Beagle dogs/sex/dose in the diet at dose levels of 0, 50, 250, 1000 and 2500/2000 ppm (0, 1.58/1.80, 8.23/9.27, 32.0/33.9 and 54.8/50.5 mg/kg bw/day in males and females, respectively) for 3 months. Treatment with CGA 293343 had no effect on appearance and behaviour, mortality, ophthalmology, urinalysis and gross pathology. Treatment at 2500 ppm resulted in marked reduction in food consumption and body weight loss during the first two weeks of the study. This necessitated the reduction of the dose to 2000 ppm. Continued body weight loss prompted the investigators to feed high-dose animals control diets for study days 19-25. Treatment resumed at 2000 ppm for the remainder of the study. Reduced food consumption and body weight gain was apparent in high-dose animals for the remainder of the study. High-dose females had a slight microcytic anemia, as well as leucopenia characterized by reductions in neutrophils, lymphocytes and monocytes. High-dose males had reduced monocyte counts, slightly lower MCH and slightly increased HDW. Slightly prolonged prothrombin times were observed in males and females at 1000 and 2500/2000 ppm. Slight changes in clinical chemistry parameters were observed at 1000 and 2500/2000 ppm, including reduction in plasma albumin and A/G ratio in both sexes, reduced calcium in females and reduced cholesterol and phospholipid in males. Decreased ALT was observed at 1000 and 2500/2000 ppm in males and females. The decreases in ALT were considered to be biologically relevant and were thought to be caused by either thiamethoxam-induced interference with or depression of in vivo concentrations of pyridoxal phosphate (a cofactor necessary for ALT activity) or suppression of ALT synthesis. Decreased testis and ovary weights were associated with histopathological evidence of delayed maturation in ovaries and reduction in spermatogenesis, accompanied by minimal to moderate occurrence of spermatic giant cells in testes. **The NOAEL was 250 ppm, equal to 8.2 mg/kg bw/day in males and 9.3 mg/kg bw/day in females, based on hematology and clinical chemistry findings at 1000 ppm and above. The LOAEL was 1000 ppm, equal to 32 mg/kg bw/day in males and 34 mg/kg bw/day in females.** This subchronic toxicity study is classified as acceptable and it satisfies the guideline requirement for a subchronic oral study (82-1); OECD 409 in the dog (**MRID 44718702**).

Mouse

In mice, the primary target organ was the liver, and males were more sensitive to the

liver pathology than females. Liver pathology was manifest as hepatocellular hypertrophy, necrosis of single hepatocytes, lymphocytic infiltration and Kupffer cell pigmentation. Subchronic administration of high doses resulted in decreased ovarian weights and ovarian atrophy.

In a 3-month dietary range finding toxicity study, CGA 293343 (98.4%) was administered to 10 Tif: MAGf (SPF) mice/sex/dose in the diet at dose levels of 0, 10, 100, 1250, 3500 or 7000 ppm (0, 1.41/2.01, 14.3/19.2, 176/231, 543/626 or 1335/1163 mg/kg bw/day for males/females). Treatment with CGA 293343 had no effect on mortality or gross pathology. Treatment resulted in significantly reduced body weight and body weight gain in high-dose males and reduced body weight gain in high dose females. A slight reduction in erythrocytes, hemoglobin and hematocrit was observed in high dose males, accompanied by increased MCV and slightly increased MCH. Changes in absolute and relative organ weights at the top dose were attributed to reduced body weight development. In addition to the changes at the high dose, absolute and relative kidney weights were reduced in males and liver weights were increased in females at 1250 and 3500 ppm. Ovary and spleen weights were reduced in females at 3500 ppm. Microscopic liver lesions were observed in both sexes, including hepatocyte hypertrophy and necrosis, pigmentation of Kupffer cells and lymphocytic infiltration. Ovarian atrophy was increased in females receiving 3500 and 7000 ppm. **The NOAEL from this study is 10 ppm for males and 100 ppm for females, equal to 1.4 and 19.2 mg/kg bw/day in males and females, respectively**, based on the increased incidence of hepatocyte hypertrophy at 100 ppm in males and at 1250 ppm in females. **The LOAEL is 100 ppm in males and 1250 ppm in females, equal to 14.3 and 231 mg/kg bw/day in males and females, respectively**. This subchronic toxicity study is classified as acceptable and satisfies the guideline requirement for a subchronic oral study; OPPTS 87-3100 [§82-1]; OECD No. 408; 87/302 EEC, B.26 in the mouse (**MRID 44703323**).

Chronic Toxicity

Rat

After chronic administration of thiamethoxam in rats, systemic toxicity was observed in males and females, manifest as chronic nephropathy and lymphocytic infiltration in the kidneys of males and decreased body weight gain, chronic tubular lesions in the kidneys and foci of cellular alteration in the liver of females. Body weight was unaffected in males, leading to questions on the adequacy of the high dose. However, the dose selection was based on the observed reduction in body weight gain (approximately 20% at 1000 ppm) in the subchronic toxicity study.

Refer to the combined chronic toxicity and carcinogenicity study on page 2 of this report. Treatment with CGA 293343 had no effect on appearance and behaviour, mortality, food consumption, ophthalmology, hematology, clinical chemistry and urinalysis. Body weight gain was reduced in high-dose females during the first half of the study and water consumption was slightly increased in high-dose males. At the interim sacrifice, there were no differences observed in organ weights and gross pathology between control and treated animals. Microscopic kidney lesions were observed in males treated at 500 ppm and above. A slight increase in the severity of hemosiderosis of the spleen was observed in high-dose females. At terminal sacrifice, there were no toxicologically significant changes in organ weights or gross pathology. As stated previously, microscopic examination revealed liver and kidney lesions. **The LOAEL for systemic toxicity was 1500 ppm in males, equal to 63 mg/kg bw/day**, based on histopathologic changes in the kidneys. **The NOAEL in males was 500 ppm, equal to 21 mg/kg bw/day,** based on the presence of regenerative kidney lesions at interim sacrifice that were not observed at terminal sacrifice. **The LOAEL for systemic toxicity was 3000 ppm in females, equal to 155 mg/kg bw/day**, based on the observed reduction in body weight gain and the incidence of foci of cellular alteration in the livers and chronic tubular lesions in the kidneys. **The NOAEL in females was 1000 ppm, equal to 50 mg/kg bw/day**. This combined chronic toxicity/carcinogenicitystudy in the rat is acceptable and satisfies the guideline requirement for a combined chronic toxicity and carcinogenicity study (83-2); OECD 453 in the rat (**MRID 44718708**).

Dog

In the dog, again the main target organ appeared to be the testis. Atrophy of the seminiferous tubules and decreased testis weight were observed after 12 months of treatment. Hematological parameters (primarily prolonged prothrombin times) were affected at higher doses.

In a chronic toxicity study thiamethoxam, (98.6% a.i.) was administered to 4 Beagle dogs/sex/dose in the diet at dose levels of 0, 25, 150, 750 and 1500 ppm (0, 0.70/0.79, 4.05/4.49, 21.0/24.6 and 42.0/45.1 mg/kg bw/day in males and females, respectively) for 52 weeks. Treatment with CGA 293343 had no effect on appearance and behaviour, mortality, ophthalmology, urinalysis or gross pathology. Treatment at 750 ppm and above resulted in a transient reduction in food consumption in females with weight loss at 1500 ppm, increased creatinine in both sexes, occasionally accompanied by increased urea levels, and atrophy of seminiferous tubules in two males at 750 and 1500 ppm. Decreased ALT was observed in males at 750 and 1500 ppm at all sampling intervals and in females at 1500 ppm at week 13. The decreases in ALT were considered to be biologically

relevant and were thought to be caused by either thiamethoxam-induced interference with or depression of in vivo concentrations of pyridoxal phosphate (a cofactor necessary for ALT activity) or suppression of ALT synthesis. Decreased testis weight was observed in two males at 1500 ppm. Slightly lower prothrombin activity was observed in high-dose males and slightly lower albumin levels were observed in high-dose females. **The NOAEL was 150 ppm, equal to 4.1 mg/kg bw/day in males and 4.5 mg/kg bw/day in females, based on observed changes in clinical chemistry parameters (increased creatinine and urea) in both sexes as well as the incidence of atrophy of seminiferous tubules in males at 750 ppm and above. The LOAEL was 750 ppm, equal to 21 mg/kg bw/day in males and 25 mg/kg bw/day in females.** This chronic toxicity study in the dog is acceptable and satisfies the guideline requirement for a 1-year oral toxicity study in dogs (82-1), OECD 452 (**MRID 44718704**).

Mouse

In mice, again the primary target organ was the liver, and males were more sensitive to the liver pathology than females. In the chronic studies, liver pathology was manifest as listed in the nonneoplastic lesion section on pages 8 and 9 of this report.

Refer to the mouse carcinogenicity study on page 4 of this report. Treatment with CGA 293343 had no effect on survival or appearance and behaviour, with the exception of a slight increase in the incidence of distended abdomen in the top two dose groups. This was usually associated with adverse liver pathology. Decreased body weight gain was observed in high-dose males and females with no effect on food consumption. There was no effect on hematological parameters. As stated previously, multiple liver effects were observed at doses of 500 ppm and above. **The systemic LOAEL is 500 ppm, equal to 63.8 and 87.6 mg/kg bw/day in males and females, respectively,** based on increased incidence of microscopic pathology observations in the liver at 500 ppm and above in both sexes. **The systemic NOAEL is 20 ppm, equal to 2.63 and 3.68 mg/kg bw/day in males and females, respectively**. Dosing was considered adequate based on the observed reduction in body weight gain in high-dose males and females. This carcinogenicity study in the mouse is acceptable, and satisfies the guideline requirement for a carcinogenicity study (83-2); OECD 451 in mice (**MRID 44703326**).

5. Mode of Action Studies

Mode of action studies were conducted in mice and rats; however, the assay to investigate liver biochemical parameters was performed in mice only. In addition, different hepatic cell proliferation assays were conducted in mice and rats. Thus,

direct comparisons of the rat and mouse data are difficult. For details refer to MTARC report (2000).

In a special study conducted to assess hepatic cell proliferation in mice, dietary administration of CGA 293343 at doses of 100, 500 or 2500 ppm to male and female Tif:MAGf (SPF) mice for 3, 7, 13, 27 or 59 days had no effect on appearance and behaviour, mortality, body weight or food consumption. Absolute and relative liver weights were increased in males and females at 2500 ppm. At necropsy, speckled liver was observed in almost all animals treated at 2500 ppm. Microscopically, this correlated with hepatocellular glycogenesis/fatty change. Hepatocellular necrosis, apoptosis and pigmentation were also observed in animals treated at 2500 ppm for 59 days. Evidence of proliferative activity in hepatocytes was observed in high-dose animals at most time points, as well as occasionally in animals treated at 500 ppm **(MRID 44703405)**.

In a special study conducted to assess liver biochemistry, dietary administration of CGA 293343 at doses of 100, 500 and 2500 ppm to male and female Tif:MAGf (SPF) mice for 14 days had no effect on appearance and behaviour, mortality, body weight, food consumption and gross pathology. Treatment resulted in marginal to slight increases in absolute and relative liver weights, a slight increase in the microsomal protein content of the livers of females at 2500 ppm, moderate increases in the cytochrome P450 content of males and females at 2500 ppm, slight to moderate increases in the activity of several microsomal enzymes at 2500 ppm with some slight increases also observed at 100 and/or 500 ppm, as well as slight to moderate induction of cytosolic glutathione S-transferase activity at 2500 ppm. Treatment did not affect peroxisomal fatty acid ß-oxidation **(MRID 44703406)**.

In a special study to assess replicative DNA synthesis, CGA 293343 was administered to groups of 5 male rats at doses of 0, 100, 1000, 2500 and 10000 ppm for 28 days. Immunohistochemical staining of liver sections from control and high-dose animals for proliferating cell nuclear antigen gave no indication for a treatment-related increase in the fraction of DNA synthesizing hepatocytes in S-phase. CGA 293343 did not stimulate hepatocyte cell proliferation in male rats upon dietary administration at 10000 ppm for 28 days **(MRID 44703407)**.

On April 3, 2000, the Health Effects Division's Mechanism of Toxicity Assessment Review Committee (MTARC) met to discuss mode of action on liver carcinogenicity of thiamethoxam.

The MTARC reviewed the data from the submitted studies by the Registrant. The Registrant's proposed mechanism of action is based on a progression of non-linear,

dose-related hepatotoxic effects that ultimately lead to the development of liver tumors in thiamethoxam-treated mice. The hepatotoxic effects progress from metabolic induction, hepatocellular apoptosis and necrosis, regenerative hepatocyte proliferation, and then to clonal expansion of aberrant cells. "Oxidative metabolic pathways may produce tissue-reactive metabolites that lead to tissue injury and cell death. This disruption in cellular homeostasis is persistent and results in higher cell turnover and regenerative cell proliferation. The persistent cell proliferation leads to higher probabilities of spontaneous cell mutation and subsequent tumors..." (Weber et. al: MRID 44988301). Since no treatment-related liver tumors were observed in rats, it is also proposed that the liver tumors are species specific, related to discrete dose- and time-related physiological changes seen only in mouse livers. The studies did not address the other potential modes of action (e.g. arylation). The Registrant's proposal made a statement on oxidative metabolism but did not provide supporting evidence. There was no information on the reversibility of the observed findings upon cessation of treatment. The doses selected for the special studies did not match (dose to dose) those in the carcinogenicity study (i.e. effects at 1250 ppm are not assessed). Thus, the dose spread in the special studies made it difficult to assess the dose-response. According to the special studies, thiamethoxam appears to be a weak enzyme inducer, even at the top dose level tested. Therefore, enzyme induction (the first proposed key step) was not clearly linked to the toxicity, proliferation or tumors. Only one dose was tested in the mouse metabolism study and there was a lack of comparative pharmocokinetic data between the rat and the mouse. The pharmacokinetics of thiamethoxam was not well characterized in the mouse (i.e. dose-response, half-life, etc.). There was no information on the relationship of enzyme induction to toxicity/apoptosis/proliferation/tumors which would establish the sequence of events associated with liver tumorigenesis.

The MTARC concluded that, although there appears to be an association between tumor induction and the toxicity profile (temporal as well as dose-response) of thiamethoxam, the available data are insufficient at this time to support the proposed non-linear mode of action for liver carcinogenicity of thiamethoxam.

Table 8 compares the mechanistic data to the tumor incidence observed in the mouse liver.

Table 8. A Comparison of the Mechanistic Data to Mouse Liver Tumor Incidence

Dose	Control	5 ppm	20 ppm	100 ppm	500 ppm	1250 ppm	2500 ppm
Males							
Proliferation	-	-	-	None	Weak	-	Statistically significant (3 days)
Enzyme induction	-	-	-	None	Very weak	-	Slight to moderate
Tumor incidence[1,2] (hepatic neoplasia)	12/50 (24%)	7/50 (14%)	12/49 (24%)	-	19/50 (38%)	27/48 (56%)	45/50 (90%)
Historical incidence: mean (range) hepatocellular adenoma 25% (10-46%) hepatocellular adenocarcinoma 12.2% (0-24%)							
Females							
Proliferation	-	-	-	Very weak	Weak	-	Statistically significant (7 days)
Enzyme induction	-	-	-	Very weak	Some	-	Slight to moderate
Tumor incidence (hepatic neoplasia)	0/48 (0%)	0/50 (0%)	0/49 (0%)	-	5/44 (11%)	9/47 (19%)	32/49 (65%)
Historical incidence: mean (range) hepatocellular adenoma 2.9% (0-8%) hepatocellular adenocarcinoma 0.3% (0-2%)							

[1]Hepatic neoplasia = combined hepatocellular adenoma and adenocarcinoma.
[2]Tumor data taken from Brunsman (2000) statistical analyses.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity:

- The CARC concluded that under the conditions of the study, thiamethoxam was not carcinogenic in male and female Sprague-Dawley rats.

  Dietary administration of thiamethoxam did not result in an overall treatment-related increase in tumor incidences in male and female rats. The Committee determined that the animals could have tolerated higher doses because the dose levels tested were not supported by the results of a 90-day study and short-term studies which exhibited liver toxicity at higher doses. Therefore, the CARC concluded that higher doses should have been used to assess the carcinogenic potential of thiamethoxam.

- Thiamethoxam induced hepatocellular tumors in male and female mice. There were significant ($p<0.01$) differences in the pair-wise comparisons of the high dose group with the controls for hepatocellular adenomas and combined adenomas/carcinomas in females at 500 ppm (5/44, 11%, $p<0.05$ vs 0/48, 0% in controls) and in males and females at 1,250 and 2,500 ppm (males/adenomas: 27/48, 56% and 40/50, 80% , $p<0.01$, respectively, vs 11/50, 22% in controls; combined: 27/48, 56% and 45/50, 90%, $p<0.01$, respectively, vs 12/50, 24% in controls; females/adenomas: 8/47, 17% and 31/49, 63% , $p<0.01$, respectively, vs 0/48, 0% in controls; combined: 9/47, 19% and 32/49, 65%, $p<0.01$, respectively, vs 0/48, 0% in controls) and carcinomas in males at 1,250 and 2,500 ppm (7/46, 15%, $p<0.05$ and 20/49, 41%, $p<0.01$, respectively vs 1/50, 2% in controls) and in females at 2,500 ppm (11/49, 22%, $p<0.01$ vs 0/48, 0% in controls). The incidence of carcinomas in females at 1,250 ppm, although not statistically significant, exceed the concurrent and historical control values. Significant ($p<0.01$) increasing trends were also evident for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. The occurrence of tumors was preceded by non-neoplastic changes in the liver. The incidence of adenomas and carcinomas exceeded the historical control range (males: 10%-46% and 0%-24%, respectively; females: 0%-8.16% and 0%-2%, respectively). The CARC therefore, considered these tumors to be treatment-related.

In conclusion, the assertion made by the Registrant that the carcinogenic response is species-specific was not supported by the data.

2. Mutagenicity

- Thiamethoxam was negative in both *in vitro* and *in vivo* mutagenicity assays. The CARC concluded that the submitted studies fulfill the guideline requirements.

3. Structure Activity Relationship

- A structurally-related compound, imidacloprid, induces chromosomal aberrations at very toxic dose levels. It also tested negative in carcinogenicity studies in rats and mice. Acetamiprid is a new chemical and the toxicity data have not yet been evaluated. The results of mutagenicity studies (with the exception of chromosomal aberrations assay) indicate that acetamiprid is not mutagenic under the conditions of the studies.

4. Mode of Action

- The MTARC concluded that the available data are insufficient at this time to support the proposed non-linear mode of action for liver carcinogenicity of thiamethoxam.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (EPA, 1999), the Committee classified thiamethoxam as **"likely to be carcinogenic to humans"** by the oral route based on the following weight-of-the-evidence considerations:

1. Liver tumors were seen in both sexes of mice including both benign and malignant liver tumors.

2. In the absence of data in rats at high doses there is residual uncertainty regarding the carcinogenic potential in rats.

3. The relevance of the observed tumors to human exposure cannot be discounted.

4. The available data do not support a non-linear mode of action for liver carcinogenicity of thiamethoxam.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose (Q_1*) extrapolation approach for the quantification of human cancer risk based on the most potent of the liver tumors in mice. This approach is supported by the lack of confirmation of the mode of action of thiamethoxam.

VIII. BIBLIOGRAPHY

MRID No.	CITATION
44718708	Bachmann, M. (1998) 24-Month Carcinogenicity and Chronic Toxicity Study in Rats. Novartis Crop Protection AG Toxicology, Stein, Switzerland. Test No. 942110, July 27, 1998. Unpublished.
44703326	Bachmann, M. (1998) 18-Month Carcinogenicity Study in Mice. Novartis Crop Protection AG Toxicology, Stein, Switzerland. Test No. 942109, June 2, 1998. Unpublished.
44703532	Müller T. and Stampf P. (1996) Absorption, Distribution and Excretion of [Thiazol-2-^{14}C] and [Oxadiazin-4-^{14}C] CGA 293343 in the rat. Ciba-Geigy Limited, Animal Metabolism, Basel, Switzerland. Project 027AM01, August 15, 1996. Unpublished.
44703533	Thanei P. (1998) The Metabolism of [Thiazol-2-^{14}C] and [Oxadiazin-4-^{14}C] CGA 293343 in the rat. Ciba-Geigy Limited, Animal Metabolism, Basel, Switzerland. Project 027AM02, September 18, 1998. Unpublished.
44710408	Mewes K.E. (1998) The Metabolism of [Thiazol-2-^{14}C] CGA 293343 after Multiple Oral Administration to Mice. Novartis Crop Protection AG, Animal Metabolism, Basel, Switzerland. Project 027AM09, November 3, 1998. Unpublished.
44710404	Hertner, T. (1995) Salmonella and Escherichia / mammalian-microsome mutagenicity test. Genetic Toxicology, CIBA-GEIGY Limited, Basel, Switzerland. Test No. 952014, November 2, 1995. Unpublished.
44968301	Deparade, E. (1999) Salmonella / mammalian-microsome mutagenicity test. Genetic Toxicology, Novartis Crop Protection AG, Basel, Switzerland. Test No. 992020, October 21, 1999. Unpublished.
44710405	Ogorek, B. (1996) Gene mutation test with Chinese Hamster cells V79. Genetic Toxicology, CIBA-GEIGY Limited, Basel, Switzerland. Test No. 952015, January 12, 1996. Unpublished.
44710403	Zeugin, S. (1996) Cytogenetic test on Chinese Hamster cells *in vitro*. Genetic Toxicology, CIBA-GEIGY Limited, Basel, Switzerland. Test No. 952016, June 18, 1996. Unpublished.

44710406 Hertner, Th. (1995). Micronucleus Test, Mouse (OECD Conform). Genetic Toxicology, Ciba-Geigy Ltd., Basle, Switzerland, Study no. 952018. December 15, 1995. Unpublished.

44710407 Ogorek, B. (1996) Autoradiographic DNA Repair Test on Rat Hepatocytes *in vitro*. Genetic Toxicology, CIBA-GEIGY Limited, Basel, Switzerland. Test No. 952017, January 29, 1996. Unpublished.

44703314 Oda, S. (1996). An Acute Oral Toxicity Study of CGA 293343 Tech. in Rats. Gotemba laboratory, Bozo Research Centre Inc., Tokyo. Study No. B-3120, CIBA Project No. 942111. May 23, 1996. Unpublished.

44703315 Oda, S. (1996). An Acute Oral Toxicity Study of CGA 293343 Tech. in Mice. Gotemba laboratory, Bozo Research Centre Inc., Tokyo. Study No. B-3122, CIBA Project No. 952058. May 23, 1996. Unpublished.

44703316 Oda, S. (1996). An Acute Dermal Toxicity Study of CGA 293343 Tech. in Rats. Gotemba laboratory, Bozo Research Centre Inc., Tokyo. Study No. B-3121, CIBA Project No. 942112. May 23, 1996. Unpublished.

44703317 Shutoh, Y. (1996). CGA293343 Tech: Acute Inhalation Toxicity Study in Rats. The Institute of Environmental Toxicology, Tokyo. Study No. IET 95-0120, CIBA Project No. 942122, August 14, 1996. Unpublished

44703318 Shibata, R. (1996). A Primary Eye Irritation Study of CGA 293343 Tech. in Rabbits. Kannami laboratory, Bozo Research Centre Inc., Tokyo. Study No. B-3123, CIBA Project No. 942114. May 31, 1996. Unpublished.

44703319 Shibata, R. (1996). A Primary Skin Irritation Study of CGA 293343 Tech. in Rabbits. Kannami laboratory, Bozo Research Centre Inc., Tokyo. Study No. B-3124, CIBA Project No. 942113. May 31, 1996. Unpublished.

44710401 Winkler, G. (1995). Skin Sensitization Test in the Guinea Pig Maximisation Test. Ciba-Geigy Limited Short-term Toxicology, Stein, Switzerland. CIBA Project No. 942115, December 21, 1995. Unpublished.

44703320 Minnema, Daniel J., (1997). Acute Neurotoxicity Study of Orally Administered CGA-293343 TECH in Rats. Covance Laboratories Inc., Vienna, Virginia, Study no. Covance 6117-364. September 23, 1997. Unpublished.

44703322 Bachmann M. (1995) 28-Days Range Finding Study in Rats (Administration in

Food). Ciba-Geigy Limited Short/Long-term Toxicology, Stein, Switzerland. CIBA Project No. 942088, May 5, 1995. Unpublished.

44718701 Bachmann M. (1994) 28-Day Exploratory Toxicity Study in Male Rats (Gavage). Ciba-Geigy Limited Short/Long-term Toxicology, Stein, Switzerland. CIBA Project No. 932107, April 25, 1994. Unpublished.

44718703 Bachmann M. (1996) 3-Month Oral Toxicity Study in Rats (Administration in Food). Ciba-Geigy Limited Short/Long-term Toxicology, Stein, Switzerland. CIBA Project No. 942089, January 23, 1996. Unpublished.

44703324 Altmann, B. (1996) 28-Day Range Finding Toxicity Study in Beagle Dogs. Ciba-Geigy Limited Short/Long-term Toxicology, Stein, Switzerland. CIBA Project No. 942106, June 19, 1996. Unpublished.

44718702 Altmann, B. (1996) 3-Month Subchronic Toxicity Study in Beagle Dogs. Ciba-Geigy Limited Short/Long-term Toxicology, Stein, Switzerland. CIBA Project No. 942107, October 15, 1996. Unpublished.

44703323 Bachmann M. (1996) 3-Month Range Finding Toxicity Study in Mice (Administration in Food). Ciba-Geigy Limited Short/Long-term Toxicology, Stein, Switzerland. CIBA Project No. 942105, August 13, 1996. Unpublished.

44718704 Altmann, B. (1998) 12-Month Chronic Dietary Toxicity Study in Beagle Dogs. Novartis Crop Protection AG Toxicology, Stein, Switzerland. Test No. 942108, July 22, 1998. Unpublished.

44703405 Persohn, E. (1995) CGA 293343: Assessment of replicative DNA synthesis in the course of a 28-day oral (feeding) toxicity study in male rats. Ciba-Geigy Limited, Toxicology Services/Cell Biology, Basel, Switzerland. Report No. CB 94/47, February 27, 1995. Unpublished.

44703406 Weber, E. (1998) CGA 293343 tech. Assessment of hepatic cell proliferation in mice. Novartis Crop Protection AG, Toxicology/Cell Biology, Stein, Switzerland. Report No. CB 98/12, September 24, 1998. Unpublished.

44703407 Trendelenburg, C. (1998) CGA 293343 tech. Effects on biochemical parameters in the liver following administration to male and female mice. Novartis Crop Protection AG, Cell Biology, Stein, Switzerland. Report No. CB 98/11, September 15, 1998. Unpublished.

44988301 Weber, E., Waechter, F., Skripsky, T., Thanei, P., Pastoor, T., Peffer, R., Stevens, J. (1999) Liver tumor formation in mice by thiamethoxam (CGA-293343) - implications for human risk assessment. Novartis Crop Protection, AG, Basel, Switzerland and Novartis Crop Protection, Inc. Greensboro, NC. Laboratory Study IdentificationNo. 1199-99, December 7, 1999. Unpublished.

------- Brunsman, L.L. (2000). CGA 293343 (Thiamethoxam) Qualitative Risk Assessment Based on 1999 Re-read of Tif:MAGf(SPF) Mouse Dietary Study. Memorandum by Lori L. Brunsman , Science Analysis Branch to Pamela Hurley, RAB2, Health Effects Division (7509C), Office of Pesticide Programs, U.S. EPA., dated 2/15/2000.

------- Brunsman, L.L. (2000). CGA 293343 (Thiamethoxam) Qualitative Risk Assessment Based on 1999 Re-read of Tif:MAGf(SPF) Mouse Dietary Study using mg/kg b.w.$^{3/4}$s Cross Species Scaling factor . Memorandum by Lori L. Brunsman , Science Analysis Branch to Pamela Hurley, RAB2, Health Effects Division (7509C), Office of Pesticide Programs, U.S. EPA., dated 2/16/2000.

------- MTARC. (2000). A Memorandum by pamela Hurley, Pamela Hurley, RAB2, Health Effects Division (7509C) to Karl Baetcke, Chairman, MTARC, Health Effects Division (7509C), Office of Pesticide Programs, U.S. EPA., dated 5/1/2000.