UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No.: 0051705

MEMORANDUM

DATE: March 26, 2003

SUBJECT: Thiacloprid: Report of the Cancer Assessment Review Committee

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (HED) (7509C)

Jessica Kidwell

TO: John Doherty, Toxicologist
and
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The Cancer Assessment Review Committee met on January 29, 2003 to evaluate the carcinogenic potential of Thiacloprid. Attached please find the Final Cancer Assessment Document.

cc: R. Hill
J. Pletcher
Y. Woo

CANCER ASSESSMENT DOCUMENT

Evaluation of the Carcinogenic Potential of

Thiacloprid

PC Code 014019

Including Appendix 1:

Consideration of the Use of the Threshold Model for Thyroid Tumors

FINAL
MARCH 26, 2003

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: 03/26/03
John Doherty, Ph.D.

DOCUMENT PREPARATION:
Jessica Kidwell, Executive Secretary

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

Karl Baetcke

William Burnam

Marion Copley

Kit Farwell

Abdallah Khasawinah

Nancy McCarroll

Esther Rinde

Jess Rowland

Linda Taylor

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

John Pletcher, Consulting Pathologist See attached sheet

Lori Brunsman, Statistical Analysis

OTHER ATTENDEES: Pamela Hurley (HED/RAB2)

DATA PRESENTATION: John Doherty, Ph.D.

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EXECUTIVE SUMMARY

On January 29, 2003, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met to evaluate the carcinogenic potential of thiacloprid.

Dr. John Doherty of Reregistration Branch III of HED presented the background information including the experimental design and non-neoplastic and neoplastic results of the rat and mouse carcinogenicity studies. He also discussed the systemic toxicity, mutagenicity and metabolism/pharmacokinetics data. In addition, he presented the results of special studies submitted by the registrant to attempt to determine the mode of action for thyroid and uterine tumors in rats and ovary tumors in mice.

Thiacloprid was administered in the diet to groups of 60 male and 60 female Wistar Hsd Cpd:WU rats at concentrations of 0, 25, 50, 500, or 1000 ppm (0, 1.2, 2.5, 25.2, and 51.7 mg/kg/day, respectively, for males and 0, 1.6, 3.3, 33.5, and 69.1 mg/kg/day, respectively, for females) for up to 2 years. Thyroid hormones were evaluated at the same time as serum chemistry parameters, and liver homogenates were prepared from 10 rats/sex/dose at 0 and 25 ppm and 5 rats/sex/dose at 50, 500, and 1000 ppm for evaluation of phase I and phase II enzymes at 54 weeks.

Thiacloprid was administered in the diet 60 SPF-bred $B6C3F_1$ mice/sex/dose in the diet at concentrations of 0, 30, 1250, or 2500 (equivalent to 0, 5.7, 234.1, or 546.4 mg/kg bw/day for males and 0, 10.9, 475.3, or 872.5 mg/kg bw/day for females) for 2 years.

The CARC concluded that thiacloprid showed evidence of carcinogenicity based on the following:

The rat and mouse studies demonstrated that thiacloprid was associated with thyroid tumors in both sexes in rats, uterine tumors in rats, and ovarian tumors in mice.

Thyroid Tumors

- Male Wistar rats had a significant increasing trend ($p<0.01$), and significant differences in the pair-wise comparisons of the 500 ppm ($p<0.05$) and 1000 ppm ($p<0.01$) dose groups with the controls, for thyroid follicular cell adenomas. The incidence of thyroid follicular cell adenomas for males in the 500 ppm (10%) and 1000 ppm (17%) dose groups exceeded the historical control range (0 - 5.1%). The single incident (2%) in the 50 ppm dose group was within the historical control range. However, since there were no follicular cell adenomas in either the control or 25 ppm dose groups in this study, the single incidence at 50 ppm was considered to be treatment-related. Thus, the CARC considered the increase in thyroid follicular cell adenomas to be treatment-related in

males. No thyroid follicular cell carcinomas were observed at any dose.

- In female Wistar rats, thyroid follicular cell adenomas occurred in 0/50, 1/50, 1/50, 1/50 and 2/48 rats in the control group, 25, 50, 500 and 1000 ppm dose groups, respectively. The historical control incidence of thyroid follicular cell adenomas was 6 adenomas out of 707 rats for 14 studies. Six of the 14 studies had one incident each of thyroid follicular cell adenomas (range 0-2%); 8 of the 14 studies had no adenomas. The two follicular cell adenomas in the 1000 ppm dose group exceeded the historical control data for this tumor type. Although these data did not attain statistical significance for either a positive trend or pair-wise comparisons, the CARC considered the tumors to be possibly related to treatment. This possibility is supported by the fact that this same type of tumor was found to be statistically significant in males as well as in excess of the historical control range.

Uterine Tumors

- Wistar rats had a significant increasing trend, and significant differences in the pair-wise comparisons of the 500 ppm and 1000 ppm dose groups with the controls, for combined uterine adenomas, adenocarcinomas, and/or adenosquamous carcinomas, all at $p<0.01$. There was also a significant increasing trend ($p<0.01$), and significant differences in the pair-wise comparisons of the 500 ppm ($p<0.05$) and 1000 ppm ($p<0.01$) dose groups with the controls for uterine adenocarcinomas. For adenocarcinomas, the incidence in the concurrent control group (13%) was slightly higher than the historical control database range (0.0 to 12.0%), however, the 500 ppm (30%) and 1000 ppm (37%) incidence greatly exceeded the historical control range. The CARC agreed that, due to the rarity of uterine adenomas and the absence of adenomas in the control or 25 ppm dose groups, the incidence of adenomas in the 50 ppm (2%), 500 ppm (2%), and 1000 ppm (4%) dose groups were considered to be treatment-related even though these data did not attain statistical significance. Thus, the CARC considered the increase in adenomas, adenocarcinomas, and adenosquamous carcinomas of the uterus to be treatment-related.

- In rats, dosing at the highest dose (1000 ppm) was considered by the CARC to be adequate, but not excessive, based on an overall decrease in body weight gain of 20% in females and 12% in males at 1000 ppm, no treatment-related effects on mortality, increased absolute/relative liver weight (19/31%) in males at 1000 ppm, and increases in several hepatic enzymes at ≥ 50 ppm, hepatic hypertrophy and cytoplasmic change at ≥ 50 ppm, and eventual vacuolation at the highest test dose. In addition, there was thyroid hypertrophy, cellular alteration and pigment formation and indications of acceleration of ocular degeneration in females. None of these effects were considered to be severely adverse.

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Ovarian Tumors

- Female B6C3F mice had a significant increasing trend in ovarian luteomas at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 1250 ppm and 2500 ppm dose groups with the controls for ovarian luteomas, both at $p < 0.05$. The incidence of luteomas in the 1250 ppm (10%) and 2500 ppm (12%) dose groups exceeded the historical control range (0-2%) for this type of tumor in this strain of mouse. The single incident at 30 ppm may also be related to treatment since this is a rare tumor type and the concurrent control group did not have this type of tumor. The CARC considered the increase in ovarian tumors to be treatment related.

- There was no treatment-related increase in any tumors in male B6C3F mice.

- In mice, dosing at the highest dose (2500 ppm) was considered by the CARC to be adequate, but not excessive, for carcinogenicity assessment. This was based on no treatment-related effects on survival, a 14% decrease in body weight gain for males (body weight was not affected in females), increases in relative liver weight and minimal hypertrophy, as well as enzymes (possibly including aromatase), and eventual hepatic fatty change and minimal necrosis in both sexes. Male mice had centrilobular hepatocellular degeneration at 1250 and 2500 ppm (severity was slight to minimal). There were also increases in vacuolation and atrophy of the X-zone of the adrenal in females and there was an increase in "eosinophilic luteinized cells" in the ovary to indicate possible disruption of the endocrine systems. None of these effects were considered to be severely adverse.

- There is no mutagenicity concern.

- Thiazopyr is a structural analogue to thiacloprid. Thiazopyr was classified as a "Group C - Possible Human Carcinogen", with a Margin of Exposure approach recommended. Thiazopyr has been demonstrated to cause thyroid tumors (male rats), but not uterine or ovarian tumors. There was no evidence of genotoxicity.

- There is no established mode of action at this time for the increases in thyroid and uterine tumors in rats and ovarian tumors in mice.

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July 1999), the CARC classified thiacloprid into the category **"Likely to be Carcinogenic to Humans"**. The Committee further recommended that a linear low-dose extrapolation approach for the quantification of human cancer risk be applied to the experimental animal tumor data and that quantifications of risk be estimated for male rat thyroid, rat uterine, and mouse ovarian tumors for thiacloprid. The data did not support a mode of action.

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I. INTRODUCTION

On January 29, 2003, the *Cancer Assessment Review Committee* (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met to evaluate the carcinogenic potential of thiacloprid.

II. BACKGROUND INFORMATION

Chemical: Thiacloprid

Chemical name: [3-(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene cyanamide.

Structure:

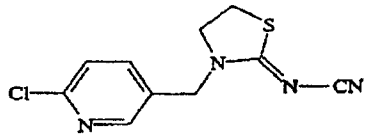

Figure 1

PC Code: 014019

CAS Number: 111988-49-9

Registered uses: Not currently registered. It is understood that thiacloprid is under consideration for registration on cotton and may result in residues in human food from cotton by- products such as cottonseed oil. Also there have been indications that in the future there will be applications for registration and tolerances for "tree nuts" and "vegetables."

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III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Reference: Bomhard, E., A. Popp, C. Ruhl-Fehlert (1998) YRC 2894: combined chronic toxicity carcinogenicity study in Wistar rats dietary administrationover 2 years. Institute of Toxicology, Bayer AG, Friedrich-Ebert-Strasse 217- 333, D-42096 Wuppertal, Germany. Laboratory Report No. 27480, May 13, 1998. MRID No. 44927712

A. Experimental Design

The rat combined chronic feeding/carcinogenicity study (MRID No. 44927712) with thiacloprid (96.8 to 97.2% purity, batch #290894) was conducted using Wistar Hsd Cpd.WU strain rats and the dietary dose levels were 0, 25, 50, 500 or 1000 ppm which corresponded to 0, 1.2, 2,5 25.2 and 51.7 mg/kg/day in males an d 0. 1.6, 3.3, 33.5 and 69.1 mg/kg/day in females. There were initially 60 rats/sex/group and 10 rats/sex/dose were sacrificed after one year for an interim sacrifice. The remaining rats received their diets for 2 years.

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality indicated a significant decreasing trend with increasing doses of thiacloprid in female rats. There were no statistically significant incremental changes in mortality with increasing doses of thiacloprid in male rats (Memo, L. Brunsman, 1/08/03, TXR No. 0051446).

Tumor Analysis

This study indicated that thiacloprid was associated with increases in thyroid tumors in male and female rats and uterine tumors as indicated in Table 1 (thyroid) and Table 2 (uterus).

Male Thyroid Tumors: Male rats had a significant increasing trend, and a significant difference in the pair-wise comparison of the 1000 ppm dose group with the controls, for thyroid follicular cell adenomas, both at $p < 0.01$ (Memo, L. Brunsman, 1/08/03, TXR No. 0051446). There was also a significant difference in the pair-wise comparison of the 500 ppm dose group with the controls for thyroid follicular cell adenomas at $p<0.05$. The statistical analyses of the male rats were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons.

Table 1. Male Rat: Thyroid Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

Adenoma[#]	0/48	0/49	1/50	5/49	8[a]/48
(%)	(0)	(0)	(2)	(10)	(17)
p =	0.0000**	1.000	0.5102	0.0296*	0.0028**

[+] Number of tumor bearing animals/number of animals examined, excluding those that died before week 53.

[a] First adenoma noted at week 100 in the 1000 ppm dose group.

[#] No thyroid follicular cell carcinomas were reported.

Note: There were no thyroid follicular cell tumors 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 $p < 0.01$.

The Bayer Company provided historical control data for the occurrence of thyroid adenoma and adenocarcinoma for 14 studies with the Wistar strain rat from 1986 to 1997. The response in males and females is as follows:

Tumor	Total Examined	Incidence	% Range
Follicular cell adenoma - males	706	11	0.0 to 5.1%
Follicular cell adenoma - females	707	6	0.0 to 2%
Follicular cell adenocarcinoma - males	706	1	0.0 to 2%
Follicular cell adenocarcinoma - females	707	0	0%

Thus, the incidence of follicular cell adenoma for males in the 500 ppm (10%) and 1000 ppm (16.3%) exceeds the historical control range. The single incident (2%) in the 50 ppm dose group is within the historical control range of 0.0 to 5.1%. However, since there were no follicular cell adenomas in either the control or 25 ppm dose groups in this study, the single incident at 50 ppm may be treatment related.

Appendix 1 ("Consideration of the Use of the Threshold Model for thiacloprid") presents the rationale for attempting to establish the relationship between the induction of hepatic enzymes and the increases in thyroid hypertrophy and thyroid tumors.

Female thyroid tumors. There were 0/50, 1/50, 1/50, 1/50 and 2/48 incidents of thyroid follicular cell adenomas in the female control group, 25, 50, 500 and 1000 ppm dose groups, respectively. The historical control incidence of thyroid follicular cell adenomas was 6 adenomas out of 707 rats for 14 studies. Six of the 14 studies had one incident each of thyroid follicular cell adenomas (range 0-2%); 8 of the 14 studies had no adenomas. The single incident in each of the 25, 50 and 500 ppm dose groups was within the historical control range (0-2%). The two follicular cell adenomas in the 1000 ppm dose group exceeded the historical control range for this tumor type. These data did not attain statistical significance for either a positive trend or pair-wise comparisons (L. Brunsman, personal communication, 01/07/03).

Uterine Tumors: Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls, for uterine adenocarcinomas and combined adenomas, adenocarcinomas, and /or adenosquamous carcinomas all at $p<0.01$ (Memo, L. Brunsman, 1/08/03, TXR No. 0051446; Memo, L. Brunsman, 2/20/03, TXR No. 0051572). There were also significant differences in the pair-wise comparisons of the 500 ppm dose group with the controls for uterine adenocarcinomas ($p<0.05$) and combined adenomas, adenocarcinomas, and/or adenosquamous carcinomas ($p<0.01$). The statistical analyses of the female rats were based upon Peto's prevalence test (Table 2).

The Bayer Company provided historical control data for the occurrence of adenoma and adenocarcinoma in the uterus for 14 studies with the Wistar strain rat from 1986 to 1997. For adenocarcinomas, there were a total of 38 incidents in 709 rats examined, with a range of 0.0 to 12.0%. Thus, the concurrent control group (13% incidence) was slightly higher than the historical control data base range for adenocarcinomas. However, the 500 ppm (30%) and 1000 ppm (37%) incidence greatly exceeded the historical control database range.

For adenomas there was a total of 1 incident in 709 rats with a range of 0.0 to 2.0 %. This is considered a rare tumor and since the uterine adenoma incidence was 1, 1 and 2 in the 50, 500 and 1000 ppm dose groups respectively, with none in the control or 25 ppm dose groups, they were considered to be related to treatment.

In addition to the tumor types indicated in the above table, it was also noted that the following tumor types were found in the uterus of the 500 and 1000 ppm dose groups only:

-mixed Muellerian tumor (m) - one in each dose group.
-granular cell tumor (b) - one in the 1000 ppm dose group only.

(note: b - benign, m - malignant)

The incidence of these tumor types are being mentioned here as *possible* indication of some other effects of thiacloprid on the uterus.

Table 2: Rat: Uterine Tumor Rates[+] and Peto's Prevalence Test Results (p-values)

Adenomas (%) p =	0/43 (0) 0.0569	0/46 (0) -	1[a]/46 (2) 0.0705	1/46 (2%) 0.2071	2/48 (4) 0.1294
Adeno-carcinomas (%) p =	6/47 (13) 0.0000**	3/50 (6) -	3/47 (6) -	14/46 (30) 0.0125*	18[b]/49 (37) 0.0039**
Adenosquamous carcinomas (%) p =	0/43 (0) 0.0569	0/46 (0) -	1[a]/46 (2) 0.0705	1/46 (2) 0.2071	2[c]/48 (4) 0.1294
Combined (%) p =	6/47 (13) 0.0000**	3/50 (6) -	4/47 (9) -	16/46 (35) 0.0037**	21[d]/49 (43) 0.0008**

[+] Number of tumor bearing animals/number of animals examined, excluding those that died before observation of first tumor.

[a] First adenoma observed at week 72, dose 50 ppm.

[b] First adenocarcinoma observed at week 67, dose 1000 ppm.

[c] First adenosquamous carcinoma observed at week 96, dose 1000 ppm

[d] One animal in the 1000 ppm dose group had both an adenoma and an adenocarcinoma

Note: There were no uterine tumors 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 $p < 0.01$.

C. Non-Neoplastic Lesions in the thyroid, liver and pituitary

Table 3 shows the treatment-related incidence of non-neoplastic lesions in the liver, thyroid and pituitary. The significance of these non-neoplastic conditions is further discussed in Appendix I. Table 3 also includes body weight and liver weight data.

Table 3. Non-Neoplastic Lesions in male and female Wistar strain rats dosed with thiacloprid for two years.

Body Weight ♂ (as % of control)	-week	100 %	102 %	103 %	99%	96% *
53	-				98%	
terminal		100 %	105 %	104 %		91% **
Liver ♂	n = 50					
Hepatocellular cytoplasmic change		0	0	8**	41*	47**
Hepatocellular centrilobular hypertrophy		0	0	12**	*	49**
Mix eosinophilic/clear cell focus		1	2	5*	44*	22**
Hepatocellular vacuolation		8	14	8	*	24**
Hepatocellular adenoma (b) or carcinoma (m)		0	0	1b,	15*	0
Relative liver weight (as % of control)		100 %	98 %	1m 95%	* 12 0 106 %	131% **
Thyroid ♂	n = 50					
Colloid alteration		17	16	21	37*	41**
Follicular epithelial hypertrophy		12	10	22**	*	34**
Follicular cell hyperplasia		1	2	2	27*	3
Pigment		16	11	23	* 6 30* *	32**
Pituitary ♂	n = 50					
Cholesterol clefts		0	1	2	4*	4*
Body Weight ♀ (as % of control)	-week	100 %	105 %	100 %	89% **	84% **
53	-					
terminal		100 %	105 %	100 %	89% **	86% **

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Liver ♀ n = 50					
Hepatocellular cytoplasmic change	0	1	0	30*	34**
Hepatocellular centrilobular hypertrophy	0	1	0	*	36**
Mix eosinophilic/clear cell focus	2	1	3	30*	10**
Hepatocellular vacuolation	6	7	4	*	4
Hepatocellular adenoma (a) or carcinoma (b)	0	0	2b	6	0
Relative liver weight (as % of control)	100 %	89 %	92%	3 0 100 %	116% **
Eyes ♀ n = 50					
Retinal atrophy	15	20	24*	25*	32**
Lens degeneration	9	18	16	20* *	30**
Thyroid ♀ [n]	[50]	[50]	[50]	[50]	[48]
Colloid alteration	6	2	5	17*	28**
Follicular epithelial hypertrophy	6	2	6	*	23**
Follicular epithelial hyperplasia	0	0	1	16*	3*
Pigment	0	0	2	* 1 4*	4*

Table 3 shows that the liver and thyroid are affected by thiacloprid treatment. The eye of females was also affected to indicate that there is an increase in the frequency of degenerative changes associated with age. The pituitary was not clearly affected since the significance of cholesterol clefts is not certain and only a few animals were affected.

There were no non-neoplastic lesions in the uterus to indicate preneoplastic conditions or other effects of thiacloprid on this organ. There was only a single incident of ovarian luteoma in the control group (this is being mentioned because thiacloprid was indicated as causing luteoma in mice).

D. Adequacy of the Dosing for Assessment of Carcinogenicity

In rats, dosing at the highest dose (1000 ppm) was considered by the CARC to be adequate, but not excessive, for carcinogenicity assessment. This was based on the following effects, which were not considered to be severely adverse:

1) an overall decrease in body weight gain of 20% in females and 12% in males at 1000 ppm, and 16% in females at 500 ppm;

2) no treatment-related effects on mortality;

3) increased absolute/relative liver weight (19/31%) in males at 1000 ppm, increases in several hepatic enzymes at ≥50 ppm, hepatic hypertrophy and cytoplasmic change at ≥50 ppm, and eventual vacuolation at the highest test dose;

4) thyroid hypertrophy, cellular alteration and pigment formation in both sexes and indications of acceleration of ocular degeneration in females.

In addition, tumors (thyroid and uterus) were also seen at the next to highest dose of 500 ppm.

2. Carcinogenicity Study in Mice

References: Wirnitzer, U, Geiss, V. (1998) YRC 2894: Oncogenicity study in B6C3F$_1$ mice (administration in feed over 2 years). Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-233, D-42096 Wuppertal, Germany. Bayer Report No. 27247, Study No. T9059195, February 26, 1998. MRID No. 44927710

Wirnitzer, U. (1998) YRC 2894: Oncogenicity study in B6C3F$_1$ mice (administration in feed over 2 years). Supplemental submission to AC No. 108358. Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-233, D-42096 Wuppertal, Germany. Bayer Report No. 27247 A, Study No. T9059195, August 18, 1998. MRID No. 44927711

A. Experimental Design

The mouse carcinogenicity study (MRID No. 44927711) with thiacloprid (96.8 to 97.2% purity, batch #290894) was conducted using 50 mice/sex/dose group SPF-bred B6C3F strain mice. The dietary dose levels were 0, 30, 1250 or 2500 ppm ppm, which corresponded to 0, 0.5.7, 234.1 and 546.4 mg/kg/day for males and 0. 10.9, 475.3 and 872.5 m/kg/day for females. A satellite group of 10 mice/sex in the control and high dose group were sacrificed after one year for an interim sacrifice. The remaining mice received their diets for 24 months.

B. Discussion of Tumor Data

Survival Analyses

There were no statistically significant incremental changes in mortality with increasing doses of thiacloprid in male or female mice (Memo, L. Brunsman, 1/08/03, TXR No. 0051446; Memo, L. Brunsman, 2/20/03, TXR No. 0051572). A corrected female mortality table (Table 4) is included in this report. It replaces the female mortality table which is in the 1/08/03 qualitative memo by L. Brunsman (TXR No. 0051446).

Table 4: Thiacloprid - B6C3F1 Mouse Study
Female Mortality Rates[+] and Cox or Generalized K/W Test Results
(Provided by L. Brunsman, Statistician, 2/13/03)

[illegible]					
0	1/50 (2)	0/49 (0)	0/49 (0)	14/49 (29)	15/50 (30)
30	2/50 (4)	0/48 (0)	4/48 (8)	15/44 (34)	21/50 (42)
1250	0/50 (0)	1/50 (2)	8/49 (16)	10/41 (24)	19/50 (38)
2500	1/50 (2)	0/49 (0)	3/49 (6)	16/46 (35)	20/50 (40)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 107.
( )Percent.

Note: Interim sacrifice animals were not provided in the electronic tumor file submission.
Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analyses

There were no compound-related tumors observed in male mice.

Ovarian Tumors: Female mice had a significant increasing trend in ovarian luteomas at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 1250 ppm and 2500 ppm dose groups with the controls for ovarian luteomas, both at $p < 0.05$. The statistical analyses of the female mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons (Memo, L. Brunsman, 1/08/03, TXR No. 0051446). The treatment-related increases in ovarian luteomas are shown in Table 5.

Table 5. Mouse: Ovary Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p-values)

Luteomas (%) p =	0/49 (0) 0.0038**	1/47 (2) 0.4896	5/49 (10) 0.0281*	6[a]/49 (12) 0.0133*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

[a] First luteoma observed at week 53, dose 2500 ppm

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$

The Bayer Company provided historical control data for the incidence of luteomas in the strain B6C3F1 mouse strain (all from the same breeder) from five studies of 24 to 25 months. There was only one incident among 250 mice for a range of 0.0 to 2.0 %. Thus, the tumor incidence in the 1250 ppm (10%) and 2500 ppm (12%) dose groups are well over the historical control range for this type of tumor in this strain of mouse. The single incident at 30 ppm may also be related to treatment since this is a rare tumor type and the concurrent control group did not have this type of tumor.

C. Non-Neoplastic Lesions

The non-neoplastic lesions observed in the liver and adrenal and ovary of both sexes of mice are presented in Table 6.

Table. 6. Non-Neoplastic Lesions of the Liver, Adrenal and Ovary in Mice Fed Thiacloprid in the Mouse Carcinogenicity Study.

Body Weight at week 53 (a)				
Males	100%	98.6%	98.6%	92.9%**
Females	100%	100%	97.6%	100%
(a-similar effect at week 105)				
Liver- ♂ [n = 50]	0	0	46**	49**
hepatocellular hypertrophy	3	4	15**	21**
centrilobular fatty change	5	3	6	31**
focal hepatocellular necrosis	1	0	5*	16**
centrilobular hepatocellular degeneration	100%	94%	108.4%**	116.7%**
relative liver weight (terminal)				
Liver- ♀ [n]	[49]	[49]	[50]	[50]
hepatocellular hypertrophy	0	0	2	3*
centrilobular fatty change	2	3	3	7*
focal hepatocellular necrosis	15	17	17	25*
relative liver weight (terminal)	100%	107.4%	108.8%*	122.5%**
Adrenal - ♀ [n]	[49]	[48]	[50]	[50]
X-zone vacuolization	33(1.1)	36(1.1)	48(2.0)**	50(2.1)**
X-zone atrophy	48(5.0)	48(4.9)	44(4.5)	46(4.4)
Ovary [n]	[47]	[48]	[49]	[47]
Eosinophilic luteinized cells	3(1.7)	0	5(2.0)	8(2.4)**

The number is ( ) is the mean severity score based on 1, slight, 2, minimal, 3 moderate, 4 marked, and 5 massive.

In addition to the non-neoplastic conditions noted in the ovary in the cancer study, there were also certain conditions in the ovary reported in the subchronic studies (MRID Nos. 44927633, 44927634) as indicated in Table 6A.

Table 6A. Non-neoplastic Conditions in the Mouse in the Subchronic Studies

Ovary					
Activated interstitial glands (n=10)	0	0	0	2	10**
Advanced corpora lutea graded 1 or 2 (n=10)	2	1	1	6	10**
Mean severity grade score	2.8	2.9	2.9	2.3	1.0
Liver					
Rel. Weight - males	100%	96.6%	97.5%	109.3%**	
- females	138.9%**				
Hepatocellular Hypertrophy (a)	100%	99%	100%	109.9%**	
-males	142.1%**				
-females					
	0	2(1.0)	6(1.2)**	9(2.1)**	
	10(4.0)**				
	0	0	1(1.0)	10(1.2)**	
	10(2.6)**				

** $p < 0.01$.

(a) Data are incidents out of 10 mice examined and the degree of severity is in () with 4 being maximum severity.

Table 6A shows that definitely at 6250 ppm there is some effect of thiacloprid on the ovary as indicated by there being more activated interstitial glands and more mice having advanced corpora lutea graded 1 or 2. Since the control, 50 ppm and 250 ppm groups all had mean severity scores of 2.8 or 2.9, there was a lowering of the severity score for "advanced corpora lutea" as the dose of thiacloprid increased to 1250 ppm and 6250 ppm. Table 6A also shows that the liver was affected in both males and females.

There were 0, 1, 1 and 1 incidents of adenocarcinomas in the uterus (or 2%) in the mice in this study. This is being mentioned because the rat study demonstrated statistically significant increases in this tumor type. It is noted that only the mice dosed with thiacloprid had the tumor but none in the control group did. Data provided in a company data base by the Charles River Breeding Laboratory (February, 1989) indicates that adenocarcinomas occur in the B6C3F1/CrlBR strain of mouse at a frequency of 8 out of 1361 mice (or 0.6%) and has a range of 0 to 3.5%. [Note: The Charles River Company was not the supplier for the mice in this study with thiacloprid which were supplied by the A/S Bomholtgard Company in Denmark. Thus, there may be some line within the strain difference in the frequency of this tumor type).

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There were no thyroid tumors in male mice. There were 1 incident each in the 20 and 1250 dose groups for follicular cell adenoma and one incident of c-cell adenoma in the 2500 ppm dose group females. The incidence of thyroid tumors is being mentioned because the male rat was demonstrated to have statistically significant increases in thyroid tumors.

D. Adequacy of Dosing for Assessment of Carcinogenicity

In mice, dosing at the highest dose (2500 ppm) was considered by the CARC to be adequate, but not excessive, for carcinogenicity assessment . This was based on the following effects, none of which were considered to be severely adverse:

1) no treatment-related effects on survival;

2) a 14% decrease in body weight gain for males; body weight was not affected in females;

3) increases in relative liver weight and hypertrophy (severity was slight to minimal), as well as enzymes (possibly including aromatase) and eventual hepatic fatty change and necrosis (severity was slight to minimal) in both sexes; male mice had centrilobular hepatocellular degeneration at 1250 and 2500 ppm (severity was slight to minimal);

4) increases in vacuolation and atrophy of the X-zone of the adrenal in females and there was an increase in "eosinophilic luteinized cells" in the ovary to indicate possible disruption of the endocrine systems.

IV. TOXICOLOGY

1. Metabolism

The metabolism and pharmacokinetics studies demonstrated that thiacloprid is rapidly absorbed (maximum plasma concentration within 1 to 4 hours depending on the dose). There was no specific retention in tissues (i.e. no evidence of bioaccumulation in the thyroid, uterus or ovary). The pathways of degradation (hydroxylation, opening of the thiazolidine ring, formation of an oxazole ring, oxidation and methylation and oxidative cleavage of the methylene bridge and conjugation of some of the metabolites) was established. The pharmacokinetic data in rats also demonstrated that the degradation of thiacloprid may be saturated in females at 1000 ppm.

2. Mutagenicity

The mutagenicity studies with thiacloprid and its metabolites did not indicate a pattern of positive responses such that there is a mutagenicity/genetic toxicity concern for thiacloprid. The available studies are Acceptable and satisfy the 1991 Guideline requirements for mutagenicity. A summary of the mutagenicity/genetic toxicity database is presented in the following table.

Study	Results
Bacterial systems (*Salmonella and Escherichia)* mammalian activation gene mutation assay. Nihon Bayer (Japan), Study # 95A011, August 21, 1995. MRID #44927643.	Neither in the presence or absence of S9 mix were there indications of increased revertants at concentrations up to and including 5000 µg/plate.
Bacterial systems (*Salmonella and Escherichia)* mammalian activation gene mutation assay. Bayer AG, Study # T4049371, February 13, 1995. MRID #45307401.	No evidence of increased revertants at dose levels up to and including 5000 µg/plate in presence or absence of S9.
Bacterial systems (*Salmonella and Escherichia)* mammalian activation gene mutation assay. Bayer AG, Study # T5054097, December 9, 1994. MRID #45307402.	No evidence of increased revertants at dose levels up to and including 5000 µg/plate in presence or absence of S9.
Bacterial systems (*Salmonella and Escherichia)* mammalian activation gene mutation assay. Bayer AG, Study # T 1053977 October 31, 1995. MRID #45307402.	*Study with metabolite KKO 2254.* No evidence of increased revertants at dose levels up to and including 5000 µg/plate in presence or absence of S9.
Bacterial systems (*Salmonella and Escherichia)* mammalian activation gene mutation assay. Bayer AG, Study # T 8053974, October 26, 1995. MRID #45307406.	*Study with metabolite WAK 6999.* No evidence of increased revertants at dose levels up to and including 5000 µg/plate in presence or absence of S9.
Bacterial DNA damage/repair in *Bacillus subtilis.* Nihon Bayer (Japan), Study # 97220, No date provided. MRID No.: 45344001.	No growth inhibition (differential zones) observed up to 6660 µg/disc in presence or absence of S9 mix.
Mammalian cells in culture gene mutation assay in V79 (CHO). Bayer AG, Study # T7054080, June 11, 1996. MRID # 44927739.	No evidence of increases in mutant frequency at dose levels up to and including an insoluble level (500 µg/mL) in The presence or absence of S9 mix.

Study	Results
In vitro mammalian chromosome aberration in Chinese hamster V79 cells. Bayer AG, Study No.: T5054079, November 23, 1995. MRID # 44927642.	No increases in number of aberrant metaphases at dose levels up to and including a cytotoxic dose (750 µg/mL) in the presence or absence of S9.
In vivo mammalian cytogenetic- micronucleus assay in mice. Bayer AG, Study # T0059051, November 23, 1995. MRID # 44927641.	No indication of clastogenic effect in bone marrow following the administration of a single intraperitoneal dose (60 mg/kg). Death and other signs of toxicity were seen at this level.
Unscheduled DNA synthesis in rat primary hepatocytes. Bayer AG, Study #T8054081, September 10, 1996. MRID # 44927738.	No evidence of unscheduled DNA repair at dose levels up to and including a cytotoxic concentration (500 µg/mL).

3. Structure-Activity Relationship

Thiacloprid belongs to a newer class of insecticides that affect the nicotinic receptor. Imidacloprid also belongs to this class of insecticides, but neither the rat or mouse cancer studies demonstrated thyroid, uterine or ovary tumors with this chemical.

Thiacloprid has an imino*thiazol*idine ring structure that is also found in thiazopyr (PC code 129100) (methyl 2-difluoromethyl-4-isobutyl-5-(4.5-dihydro-2-*thiazolyl*)-6-trifluoromethyl-3-pyridinecarboxylate, refer to Figure below). Both chemicals also have a pyridine ring.

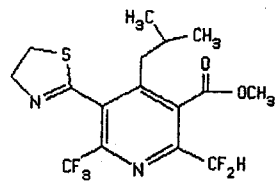

Figure 3
Thiazopyr

Thiazopyr was classified as a "Group C - Possible Human Carcinogen" (Memo, P. Hurley and E. Rinde, 5/25/94, TXR No. 0050215). Thiazopyr has been demonstrated to cause thyroid tumors (male rats), but not uterine or ovarian tumors. It also causes benign kidney tumors (rats), a rare tumor type. There was no evidence of genotoxicity.

4. Subchronic and Chronic Toxicity

Several subacute or subchronic studies were conducted to assess the hypothesis that the thyroid tumors are related to increased hepatic enzyme activity and data from these studies are

presented in Appendix 1. Appendix 1 also shows selected data indicating *apparent* increases in aromatase.

The Executive Summaries for the subchronic and chronic data on thiacloprid are presented below.

Subchronic Toxicity

EXECUTIVE SUMMARY: In a 14-week toxicity study (1994, 1995 and 1998, MRID 44927633, 44927634) YRC 2894 (98.6-98,7% a.i., batch # NLL 3351-13) was administered to 10 SPF-bred B6C3F mice/sex/dose in the diet at concentrations of 0, 50, 250, 1250 or 6250 ppm (equivalent to 0, 19.9, 102.6, 542.4, or 2819.9 mg/kg bw/day for males and 0, 27.2, 139.1, 704.3, 3351.0 mg/kg bw/day for females).

The incidence and severity of fatty vacuolation and hypertrophy of the **adrenal X zone** was increased in *all* treated females (incidence: control, 0/10; treated, 6-10/10). Incidences of **hepatocellular hypertrophy** were increased in males at 50 ppm (2/10, grade 1.0), 250 ppm (6/10, grade 1.2), 1250 ppm (9/10, grade 2.1), and at 6250 ppm (10/10, grade 4.0), and in females at 250 ppm (1, grade 1.0), 1250 ppm (10/10, grade 1.2) and 6250 ppm (10/10, grade 2.6) with none in the controls or 50 ppm females. Hepatic N-demethylase was increased in males at 250 ppm and higher, and in females at 1250 and 6250 ppm, and hepatic cytochrome P-450 was increased at 1250 and 6250 ppm in both sexes. The **body weight** gains of males at 6250 ppm were decreased throughout the study (57% of control at treatment week 14), and the overall food efficiency was decreased by about 47%. Food consumption in males was slightly increased at 6250 ppm, but water consumption was decreased by up to 30% (week 13). Absolute and relative (to body) **liver weights** were increased in males by 19% and 39%, respectively, at 6250 ppm and relative liver weight was increased by 9% at 1250 ppm ($p < 0.01$). Absolute and relative liver weights were increased in females by 10% and 12%, respectively, at 6250 ppm and by 8% and 10% at 1250 ppm ($p < 0.05, 0.01$). Decreases in serum **cholesterol** and **bilirubin** were seen in both sexes especially at 6250 ppm. Decreased absolute and relative (to body) **kidney weights** were seen in males at 6250 ppm (20% and 7%, respectively) accompanied by decreased incidence of proximal tubule vacuoles (control, 10/10; 6250 ppm, 1/10, $p < 0.01$). The numbers of advanced corpora lutea with eosinophilic cells were decreased, and the incidences of activated interstitial glands were increased in the **ovaries** at 1250 and 6250 ppm. **The LOAEL is < 50 ppm (27.2 mg/kg/day) in females adrenal X-zone changes in females. The LOAEL is 1250 ppm (542.4 mg/kg/day) in males based on liver effects (weight and hypertrophy). The NOAEL is 250 ppm (102.6 mg/kg/day) in males.** Liver hypertrophy was evident in all treated male groups but was of minimal severity at 50 ppm.

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in mice. Although a NOAEL was not

determined for females, the adverse nature of the adrenal X zone vacuolation is uncertain.

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (1997, MRID 44927714) Thiacloprid (98.6% a.i., batch # NLL 3351-13) was administered to 10 SPF-bred Wistar rats/sex/dose in the diet at dose levels of 0, 25, 100, 400, or 1600 ppm (equivalent to 0, 1.9, 7.3, 28.6, and 123.2 mg/kg bw/day in males and 0, 2.0, 7.6, 35.6, and 160.6 mg/kg bw/day in females). Recovery groups (0 and 1600 ppm) with the same number of rats were observed for an additional five weeks without treatment.

No animals died during the study. Moderate hepatocellular hypertrophy associated with a fine granular to vesicular structure of the perinuclear cytoplasm was seen in 9/10 males and 2/10 females of the 400 ppm group and in all 1600 ppm animals. After the recovery period, minimal hepatocellular hypertrophy without cytoplasmic change was seen in 3/10 of the 1600 ppm males. There was no evidence of cell proliferation in the liver. Body weights of the 1600 ppm groups were significantly lower than those of controls throughout the study, and overall body weight gains were decreased by 22-24%. There were no treatment-related food consumption, ophthalmology, or hematology findings. At the end of treatment, serum cholesterol and total protein were increased up to 84% and 11%, respectively, but returned to normal levels following the recovery period. Hepatic enzyme activity activities were elevated in the 400 (especially males) and 1600 ppm groups, but returned to normal levels following the recovery period. Absolute liver weights were increased by up to 21% in the 1600 ppm groups. Absolute thyroid weight increased 67% in 1600 ppm males, but no microscopic thyroid changes were seen. There were no significant differences in liver or thyroid weights of the control or treated groups after the recovery period. **The LOAEL is 400 ppm (28.6 mg/kg/day in males, 35.6 g/kg/day in females) based on decreased body weight throughout the treatment period. The NOAEL is 100 ppm 7.3 mg/kg/day in males, 7.6 mg/kg/day in females).**

This study is **Acceptable (Guideline)** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats.

Chronic toxicity

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (1998, MRID 44927712), YRC 2894 (thiacloprid) (96.8-97.2% a.i., batch # 290894) was administered in the feed to groups of 60 male and 60 female Wistar Hsd Cpd:WU rats at concentrations of 0, 25, 50, 500, or 1000 ppm (0, 1.2, 2.5, 25.2, and 51.7 mg/kg/day, respectively, for males and 0, 1.6, 3.3, 33.5, and 69.1 mg/kg/day, respectively, for females) for up to 2 years. Groups of 10 male and 10 females per dose were sacrificed at 12 months for interim evaluations. Thyroid hormones were evaluated at the same time as serum chemistry parameters, and liver homogenates were prepared from 10 rats/sex/dose at 0 and 25 ppm and 5 rats/sex/dose at 50, 500, and 1000 ppm for evaluation of phase I and phase II enzymes at 54

weeks.

No treatment-related effects occurred on clinical signs of toxicity, mortality rates, hematologic, clinical chemistry, urinalysis parameters, or serum thyroid hormone levels in male or female rats receiving any dose. Male rats in the 1000-ppm group weighed 12% ($p<0.01$) less than controls during weeks 1 and 2 and 4-11% ($p<0.01$ or <0.05) for most time points thereafter, gained 43% less weight than controls during week 1, gained 12% less over the entire study, and consumed 20% less food than controls during week 1 and a similar amount averaged over the entire study. Overall food efficiency was reduced by only 9%. Females in the 1000-ppm group weighed 5-21% ($p<0.01$ or <0.05) less than controls during the study, gained 20% less weight, consumed 8% less food, and had an overall food efficiency value 12% less than controls. The 500-ppm female group weighed 4-15% less than controls during most of the study, gained 16% less weight, consumed 8% less food, and had an overall food efficiency value 9% less than that of controls. No treatment-related effects on these parameters were observed in males at 25-500 ppm and in females at 25 and 50 ppm. The only treatment-related finding reported during the ophthalmoscopic examination was an increased incidence of cortical lens abnormalities (waterclefts and opacity) in females receiving the 1000-ppm diet.

In male and female rats, the activities of the phase I enzymes, ethoxycoumarin deethylase (ECOD), aldrin epoxidase (ALD), and epoxide hydrolase (EH) (females only), and phase II enzymes, glutathione-S-transferase (GS-T) and UDP-glucuronyl transferase (GLU-T), in liver homogenates were significantly increased at ≥500 ppm. ECOD and ALD activities in males and EH activity in females also were increased at 50 ppm. EH activity was significantly increased in males only at 1000 ppm. No effect was observed on 7-ethoxyresorufin deethylase (EROD) activity.

The only treatment-related organ weight change was increased absolute and relative liver weight in 1000-ppm males at study termination. Treatment-related pathologic lesions occurred in the liver, thyroid gland, and sciatic nerve in males and females; pituitary gland in males; and spinal cord, mesenteric lymph node, skeletal muscle, and eyes in females. Treatment-related lesions at ≥50 ppm included hepatocellular cytoplasmic change (eosinophilic cytoplasm with basophilic strands), hepatocyte centrilobular hypertrophy, and follicular epithelial hypertrophy in the thyroid in males and retinal atrophy in females. Treatment-related lesions occurring only at concentrations ≥500 ppm included cytoplasmic change in the liver, hepatocyte centrilobular hypertrophy, thyroid follicular epithelial hypertrophy, skeletal muscle atrophy, and lens degeneration in females; colloid alteration and pigment in the thyroid of males and females; and sciatic nerve degeneration and cholesterol clefts in the pituitary of males;. The following treatment-related lesions occurred only at 1000 ppm: hepatocellular vacuolation in males; and sciatic nerve degeneration, cholesterol clefts in the spinal cord, thyroid follicular epithelial hyperplasia, sinus histiocytosis in the mesenteric lymph nodes, and skeletal muscle degeneration and mononuclear infiltration in females. Increased incidences of liver lesions were also observed at ≥500 ppm in male and female rats sacrificed after 1 year. **The LOAEL**

is 50 ppm (2.5 and 3.3 mg/kg/day for male and female rats, respectively) based on liver toxicity (hepatocellular hypertrophy and cytoplasmic change) Higher doses result in thyroid toxicity (follicular epithelial hypertrophy) in males and oculotoxicity (retinal atrophy) in females. The corresponding NOAEL is 25 ppm (1.2 and 1.6 mg/kg/day for male and female rats, respectively).

This study is **Acceptable/Guideline** and satisfies the guideline requirements for a chronic toxicity/carcinogenicity study in rats [OPPTS 870.4300; OECD 453].

EXECUTIVE SUMMARY: In a 24-month carcinogenicity study (1998, MRID 44927710 and MRID 44927711) YRC 2894 (96.8-97.2% a.i., batch # 290894) was administered to a total of 60 SPF-bred B6C3F_1 mice/sex/dose in the diet at concentrations of 0, 30, 1250, or 2500 (equivalent to 0, 5.7, 234.1, or 546.4 mg/kg bw/day for males and 0, 10.9, 475.3, or 872.5 mg/kg bw/day for females). Ten mice/sex/dose were examined after 12 months of treatment.

There were no treatment-related effects on mortality or clinical signs. The **body weight** gain of males at 2500 ppm was decreased by 14% after 24 months of treatment and the food consumption was increased by 21%. **Food efficiency** was decreased by 22% in high-dose males. Body weight and food consumption were not affected significantly in males in the lower dose groups or in females in any dose group. The **total leucocyte count** was increased in males at weeks 53, 79, and 104 in the 1250 ppm (by 31, 46, and 12%, respectively) and 2500 ppm groups (by 64, 37, and 38%, respectively). In females, the leucocyte count was increased at week 53 in the 1250 ppm group (by 27%) and at week 79 (by 38%), but not at week 104. The absolute and relative (to body) **liver weights** were increased in males by 25% and 32%, respectively, at 2500 ppm after 12 months of treatment, and relative liver weight was increased by 8% at 1250 ppm and 17% ($p<0.01$) at 2500 ppm. At the 24-month sacrifice, only the liver-to-body-weight ratio was increased in males (by 17%). Absolute and relative liver weights were increased in females by 33% and 32%, respectively, after 12 months at 2500 ppm and by 29% and 22% after 24 months compared to the control (all $p<0.01$). The relative liver weight in females was increased by 9% ($p<0.05$) after 24 months at 1250 ppm. Incidences of **hepatocellular hypertrophy, vacuolization, and fatty changes**, graded slight to minimal, were increased in males at 2500 ppm after 12 months and in both sexes after 24 months compared to the controls. Incidences of hepatocellular necrosis were increased in males in the 1250 ppm group (12%, $p<0.05$) and in the 2500 ppm group (62%, $p<0.01$) and in females in the 2500 ppm group (50%, $p<0.05$) after 24 months compared to the control groups (incidences of 10% in males in the control group and 30% in females in the control group). Incidences of **hepatocellular degeneration** were increased in males at 1250 ppm (10%, $p<0.05$) and at 2500 ppm (32%, $p<0.01$) after 24 months compared to the controls (2%). Hepatocellular degeneration was not seen in treated females. Increased incidences and severity of **vacuolation in the mesenteric and mandibular lymph nodes** were seen at 1250 ppm and 2500 ppm in both sexes. Incidences of hemorrhage in the mesenteric lymph nodes were also

increased at 1250 ppm and 2500 ppm in both sexes compared to the controls. Decreased incidences of proximal tubule vacuoles were seen in the **kidneys** of males after 12 months at 2500 ppm (80%) compared to the control (0%, $p<0.01$), but not at 24 months. The incidence and severity of **adrenal X-zone vacuolization** in females was increased after 12 months at 2500 ppm (80%) and after 24 months at 1250 ppm (96%) and 2500 ppm (100%) compared to the controls (12 months, 20%; 24 months, 67%, $p<0.01$). The numbers of females with **increased eosinophilic luteinized cells in the ovaries** were increased at 1250 ppm (10%, NS) and at 2500 ppm (17%, $p<0.01$) compared to the control (6%). **The LOAEL is 1250 ppm for males (234.1 mg/kg/day) and females (475.3 mg/kg/day) based on liver toxicity and microscopic lymph node changes in both sexes and increased X-zone vacuolization of the adrenal glands in female mice. The NOAEL is 30 ppm for males (5.7 mg/kg/day) and females (10.9 mg/kg/day).**

This is **Acceptable/Guideline** and satisfies guideline requirements for a carcinogenicity study [OPPTS 870.4200b; OECD 451] in mice.

5. Mode of Action Studies

Data from studies attempting to define a mode of action as it relates to induction of thyroid tumors in rats are described in Appendix 1. The CARC concluded that there are insufficient data at this time to accept the proposed mechanistic theory for induction of thyroid tumors based on increased destruction by the liver of thyroid hormones with subsequent activation of the pituitary to induce thyroid stimulating hormone to stimulate compensatory increases in circulating thyroid hormones. Although there were data demonstrating that the liver is a target for thiacloprid toxicity and there were large increases in hepatic hypertrophy, cytoplasmic change and enzyme activity as well as evidence of thyroid hypertrophy and other non-neoplastic changes, the CARC determined that there was a deficiency of critical data such as studies with labelled iodine to determine if the uptake and excretion of thyroid hormones was affected. There were also problems with the assessment of circulating thyroid hormones being in the opposite direction than expected and otherwise inconsistent with regard to dose and time.

There were also several studies that attempted to relate the increase in hepatic aromatase (the enzyme responsible for converting androgens to estrogens) with the increase in uterine tumors in rats and ovary tumors in mice that were reviewed by the Mechanisms SARC of HED. The overall conclusion was that there is insufficient data at this time to relate an apparent increase in hepatic aromatase with these tumors. A major problem was the lack of specificity in the assay method used for determining the *apparent* increase in aromatase. In particular, the method included starting with a tritium labeled androgen and following the production of tritiated water as an index of enzyme activity. This method may be subject to artifact especially when other hepatic enzymes are also increased. There was also a problem in that there were no consistent increases in circulating estrogens to support the hypothesis. The MTARC report is filed separately (Memo, J. Doherty, "Mechanism of Toxicity SARC Report: Thiacloprid (PC

Code 014019)", 02/19/03, TXR No. 0051476). The CARC also concurred with the conclusions of the MTARC that there was insufficient support for the theory that uterine tumors in rats and ovary tumors in mice are induced by imbalances in circulating estrogens due to increased hepatic aromatase.

The CARC concluded that there was insufficient support to regulate thiacloprid based on a threshold mechanism for tumor induction.

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

1. Carcinogenicity

The CARC concluded that thiacloprid showed evidence of carcinogenicity based on the following:

The rat and mouse studies demonstrated that thiacloprid was associated with thyroid tumors in both sexes in rats, uterine tumors in rats, and ovarian tumors in mice.

Thyroid Tumors

- Male Wistar rats had a significant increasing trend, and a significant difference in the pair-wise comparison of the 1000 ppm dose group with the controls, for thyroid follicular cell adenomas, both at $p<0.01$. There was also a significant difference in the pair-wise comparison of the 500 ppm dose group with the controls for thyroid follicular cell adenomas at $p<0.05$. The incidence of thyroid follicular cell adenomas in males was 0/48, 0/49, 1/50, 5/49, and 8/48 for the 0, 25, 50, 500, and 1000 ppm dose groups, respectively. The incidence of follicular cell adenomas for males in the 500 ppm (10%) and 1000 ppm (17%) dose groups exceeded the historical control range (range 0-5.1%). The single incident (2%) in the 50 ppm dose group was within the historical control range. However, since there were no follicular cell adenomas in either the control or 25 ppm dose groups in this study, the single incidence at 50 ppm was considered to be treatment-related. Thus, the CARC considered the increase in thyroid follicular cell adenomas to be treatment-related in males. No thyroid follicular cell carcinomas were observed at any dose.

- In female Wistar rats, thyroid follicular cell adenomas occurred in 0/50, 1/50, 1/50, 1/50 and 2/48 rats in the control group, 25, 50, 500 and 1000 ppm dose groups, respectively. The historical control incidence of thyroid follicular cell adenomas was 6 adenomas out of 707 rats for 14 studies. Six of the 14 studies had one incident each of thyroid follicular cell adenomas (range 0-2%); 8 of the 14 studies had no adenomas. The two follicular cell adenomas in the 1000 ppm dose group exceeded the historical control data for this tumor type. Although these data did not attain statistical significance for either a positive trend or pair-wise comparisons, the CARC considered the tumors to be possibly related to treatment. This possibility is supported by the fact that this same type of tumor was found to be statistically significant in males as well as in excess of the historical control range.

Uterine Tumors

- Wistar rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls, for uterine adenocarcinomas and combined adenomas, adenocarcinomas, and/or adenosquamous carcinomas, all at $p<0.01$. There were also significant differences in the pair-wise comparisons of the 500 ppm dose group with the controls for uterine adenocarcinomas ($p<0.05$) and combined adenomas, adenocarcinomas, and/or adenosquamous carcinomas ($p<0.01$). The incidence of combined adenomas, adenocarcinomas, and/or adenosquamous carcinomas was 6/47, 3/50, 4/47, 16/46, and 21/49 for the 0, 25, 50, 500, 1000 ppm dose levels, respectively. For adenocarcinomas, the incidence in the concurrent control group (13%) was slightly higher than the historical control database range (0.0 to 12.0%). However, the 500 ppm (30%) and 1000 ppm (37%) incidence of adenocarcinomas greatly exceeded the historical control database range. The CARC agreed that, due to the rarity of uterine adenomas and the absence of adenomas in the control or 25 ppm dose groups, the incidence of adenomas in the 50 ppm (2%), 500 ppm (2%), and 1000 ppm (4%) dose groups were considered to be treatment-related even though these data did not attain statistical significance. The incidence of uterine adenomas in the 50 and 500 ppm dose groups (2%) was within the historical control range (0-2%) but above the historical control range for the 1000 ppm dose group (4%). Thus, the CARC considered the increase in adenomas, adenocarcinomas, and adenosquamous carcinomas of the uterus to be treatment-related.

- In rats, dosing at the highest dose (1000 ppm) was considered by the CARC to be adequate, but not excessive, based on an overall decrease in body weight gain of 20% in females and 12% in males at 1000 ppm, no treatment-related effects on mortality, increased absolute/relative liver weight (19/31%) in males at 1000 ppm, increases in several hepatic enzymes at ≥ 50 ppm, hepatic hypertrophy and cytoplasmic change at ≥ 50 ppm, and eventual vacuolation at the highest test dose. In addition, there was thyroid hypertrophy, cellular alteration and pigment formation and indications of acceleration of ocular degeneration in females. None of these effects were considered to be severely adverse.

Ovarian Tumors

- Female B6C3F mice had a significant increasing trend in ovarian luteomas at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 1250 and 2500 ppm dose groups with the controls for ovarian luteomas, both at $p < 0.05$. The incidence of ovarian luteomas was 0/49, 1/47, 5/49, and 6/49 for the 0, 30, 1250, 2500 ppm dose groups. The 1250 ppm (10%) and 2500 ppm (12%) dose groups exceeded the historical control range (0-2%) for this type of tumor in this strain of mouse. The single incident at 30 ppm was considered to be treatment-related since this is a rare

tumor type and the concurrent control group did not have this type of tumor.

- There was no treatment-related increase in any tumors in male B6C3F mice.

- In mice, dosing at the highest dose (2500 ppm) was considered by the CARC to be adequate, but not excessive, for carcinogenicity assessment. This was based on no treatment-related effects on survival, a 14% decrease in body weight gain for males (body weight was not affected in females), increases in relative liver weight and minimal hypertrophy, as well as enzymes (possibly including aromatase) and eventual hepatic fatty change and minimal necrosis in both sexes. Male mice had centrilobular hepatocellular degeneration at 1250 and 2500 ppm (severity was slight to minimal). There were also increases in vacuolation and atrophy of the X-zone of the adrenal in females and there was an increase in "eosinophilic luteinized cells" in the ovary to indicate possible disruption of the endocrine systems. None of these effects were considered to be severely adverse.

2. Mutagenicity

- The CARC determined that the mutagenicity data base is complete and satisfies the 1991 guideline criteria. All of the studies were negative and, thus, there is no mutagenicity concern.

3. Structure Activity Relationship

- Thiazopyr is a structural analogue to thiacloprid. Thiazopyr is classified as a "Group C-Possible human Carcinogen"Thiazopyr was classified as a "Group C - Possible Human Carcinogen" with a Margin of Exposure approach recommended. Thiazopyr has been demonstrated to cause thyroid tumors (male rats), but not uterine or ovarian tumors. There was no evidence of genotoxicity.

4. Mode of Action

- The CARC concluded that there are insufficient data at this time to accept the proposed mechanistic theory for induction of thyroid tumors based on increased destruction by the liver of thyroid hormones with subsequent activation of the pituitary to induce thyroid stimulating hormone to stimulate compensatory increases in circulating thyroid hormones. The CARC also concurred with the conclusions of the MTARC that there was insufficient support for the theory that uterine tumors in rats and ovary tumors in mice are induced by imbalances in circulating estrogens due to increased hepatic aromatase.
  Thus, there is no established mode of action at this time for the increases in thyroid and uterine tumors in rats and ovary tumors in mice.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the CARC classified thiacloprid into the category **"Likely to be Carcinogenic to Humans"**, by the oral route based on the following weight-of-the-evidence considerations:

(i) the presence of relatively rare tumors (follicular adenomas) in the thyroid of rats in both sexes

(ii) presence of treatment-related adenomas, and malignant adenocarcinomas and adenosquamous carcinomas in the uterus of rats

(iii) presence of benign ovarian luteomas in mice

(iv) limited SAR support for thyroid tumors

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended that a linear low-dose extrapolation approach for the quantification of human cancer risk be applied to the experimental animal tumor data and that quantifications of risk be estimated for male rat thyroid, rat uterine, and mouse ovarian tumors for thiacloprid. The data did not support a mode of action.

VIII. BIBLIOGRAPHY (in MRID order) - for key studies including studies referenced in Appendix I. The mutagenicity studies are referenced in the Summary Table in *Section IV, 2 Mutagenicity.*

MRID No.	CITATION
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44927711	Wirnitzer, U. (1998) YRC 2894: Oncogenicity study in B6C3F_1 mice (administration in feed over 2 years). Supplemental submission to AC No.108358. Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-233, D-42096 Wuppertal, Germany. Bayer Report No. 27247 A, Study No. T9059195, August 18, 1998.
44927712	Bomhard, E., A. Popp, C. Ruhl-Fehlert (1998) YRC 2894: combined chronic toxicity carcinogenicity study in Wistar rats dietary administration over 2 years. Institute of Toxicology, Bayer AG, Friedrich-Ebert-Strasse 217- 333, D-42096 Wuppertal, Germany. Laboratory Report No. 27480, May 13, 1998.
44927713	Christenson, W.R. (1998) Further examination of the increased occurrence of dystocia and stillbirths observed in a reproductive bioassay with an experimental cyanamide (YRC 2894). Bayer Corporation, Stilwell, KS. Laboratory Project Study Id.: 96-972-KF, August 31, 1998.
44927714	Krotlinger, F. and V. Geiss (1997) Investigations of Subchronic Toxicity in Wistar Rats (Feeding Study Over 12 Weeks With a Subsequent Recovery Period Over 5 Weeks. Bayer AG, Department of Toxicology, Freidrich- Ebert-Strasse 217-233, D-42096 Wuppertal, Germany. Bayer AG Report No. PH-26239 and T 9055540, March 21, 1997.
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44927720	Andrews, P., W. Bomann, F. Krotlinger, et. al. (1998) YRC 2894: Mechanistic studies on aromatase induction and toxicokinetics in rats (4-week feeding studies). Bayer AG Dept. of Toxicology, Wuppertal, Germany. Laboratory Report/Study Nos.: PH-27717/T 0061940 and T3062311, July 27, 1998.

44927734 Krotlinger, F. (1996) YRC 2894: Study for subacute oral toxicity in rats (feeding study over 2 weeks). Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Report/Study Nos.: 25720/T 6058111, November 29, 1996.

44927633 Wirnitzer, U, Ruhl-Fehlert, C. (1995) YRC 2894 SUB-CHRONIC range-finding study for a two-year study in B6C3F$_1$ mice (administration in feed over about 14 weeks). Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Bayer Report No. 23834, Study No. T8055585, Jan. 30, 1995.

44927634 Wirnitzer, U, Ruhl-Fehlert, C. (1998) Supplemental submission to AC No.106868, YRC 2894 SUB-CHRONIC range-finding study for a two-year study in B6C3F$_1$ mice (administration in feed over about 14 weeks). Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Bayer Report No. 23834 AA, Study No. T8055585, Aug. 18, 1998.

44927636 Wirnitzer, U. (1994) YRC 2894 Pilot study on subacute toxicity in B6C3F$_1$ mice (administration in feed over 3 weeks). Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Bayer Report No. 23450, Study No. T0055541, July 17, 1994. MRID 44927736. Unpublished.

45307403 Andrews, P. (2000) YRC 2894 (c.n.: Thiacloprid) Special study for subacute oral toxicity in rats (feeding study for 3 weeks). Bayer AG Dept. of Toxicology, Wuppertal, Germany. Laboratory Project Id.: Bayer report/study No. PH 29674/T 5069235, March 10, 2000.

---------- Memo, L. Brunsman, "Thiacloprid Qualitative Risk Assessment Based on Wistar Hsd Cpd:WU Rat and B6C3F1 Mouse Dietary Studies", January 8, 2003, TXR No. 0051446.

---------- Memo, L. Brunsman, "Thiacloprid Quantitative Risk Assessment (Q1*) Based on Wistar Hsd Cpd:WU Rat and B6C3F1 Mouse Dietary Studies with ¾'s Interspecies Scaling Factor", February 20, 2003, TXR No. 0051572.

---------- Memo, J. Doherty, "Mechanism of Toxicity SARC Report: Thiacloprid (PC Code 014019)", 02/19/03, TXR No. 005147.

---------- Memo, P. Hurley and E. Rinde, "Carcinogenicity Peer Review of Thiazopyr", May 25, 1994, TXR No. 0050215.

Appendix 1: Consideration of the Use of the Threshold Model for Thyroid Tumors

Introduction

The rat chronic feeding/carcinogenicity study demonstrated there were 0, 0, 1, 5 and 8 incidents of thyroid follicular cell adenomas in males and 0, 1, 1, 1 and 2 incidents in females for Wistar strain rats (50/group) dosed with 0, 25, 50, 500 or 1000 ppm of thiacloprid. The males at 500 (10%, $p < 0.05$) and 1000 (16%, $p < 0.01$) ppm were well in excess of the historical control from the testing laboratory. Thyroid adenoma in female Wistar rats is very rare being only one incident out of 706 rats in 15 studies meaning that the occurrence of this tumor in dosed animals only may suggest an association with treatment. The following presents data that may allow a conclusion that the thyroid tumors are associated with increased hepatic thyroid hormone metabolism with subsequent increased thyroid synthesis of thyroid hormones and subsequent induction of thyroid hyperplasia and neoplasia (adenomas).

FACTOR I. Consideration of whether the thyroid tumors associated with administration of thiacloprid can be attributed to disruption of the thyroid-pituitary hormonal balance. (In addressing this factor, the Policy states, 6 indicators (a, b, c, d, e and f) should be considered.)

a. **Goitrogenic activity *in vivo*:**

Table A illustrates the goitrogenic activity of thiacloprid as evidenced by thyroid *pathological* changes from several studies.

Table A shows that thyroid follicular epithelial cell hypertrophy was seen as early as 14 days after treatment (at 2000 ppm, study R4) and apparent after only 22 days at 100 and 400 ppm (although statistical significance was not noted, R3). After one year, males displayed colloid changes and nearly all were affected with the hypertrophy at 500 ppm. After two years, hypertrophy was noted at 50 ppm in males. Females were less sensitive but the same non-neoplastic lesions were noted except that females had a few incidents of *hyperplasia* but the males did not.

The males in the special 14 day study were also reported to have "mitotic index" affected and this was at the 500 and 2000 ppm dose levels. This condition was not reported as in other studies.

Table A. Histopathological changes in the thyroid in rats, mice and dogs.

Lesions	Response									
Rat Studies										
Chronic Feeding/Onco	Males					Females				
	Control	25	50	500	1000	Control	25	50	500	1000
Interim - 1 year (n=10)										
Follicular epi. Hypertrophy	1	1	1	7	**10**	0	0	0	0	**4**
"Colloid clumping"	3	5	3	**10**		1	1	1	1	
					10					**8**
Terminal (n=50)										
Follicular epi. Hypertrophy	12	10	**22***	**27****	**34****	6	2	6	**16****	**23****
Follicular epi. Hyperplasia "colloid alteration"	Not reported					0	0	1	1	**3***
	17	16	21	**37****	**41****	6	2	5	**17****	**28****
Pigment	16	11	23	**30****	**32****	0	0	**2**	**4***	**4***
Follicular cell adenoma	0	0	1	**5***	**8***	0	1	1	1	2
Subchronic 90 day (R2)	Control	25	100	400	1600	Control	25	100	400	1600
	No thyroid pathology reported.					No thyroid pathology reported.				
Special 22 day study (R3)	Control	25	100	400	1600	Control	25	100	400	1600
Follicular cell hypertrophy (N=10)	1	1	**3**	**5**	**8****	0	0	1	**2**	**5***
Special 14 day study (R4)	Control	25	100	500	2000	Control	25	100	500	2000
Mitotic index	1	1	1	**4**	**5**	[Not reported]				
Follicular cell hypertrophy	0	0	0	0	**5**	0	0	0	0	1

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Lesions	Response
Mouse Studies	
There were no treatment-related non-neoplastic or neoplastic conditions in the thyroid in either the mouse subchronic or oncogenicity studies.	
Dog Studies	
There were no treatment-related non-neoplastic or neoplastic conditions in the thyroid in either the dog subchronic (D2) or chronic (D1) studies.	

b. Clinical chemistry changes (evidence for reduced circulating thyroid hormones and increased TSH serum concentrations):

Table B summarizes the data generated for circulating thyroid hormones (T3 and T4). Thyroid stimulating hormone (TSH) and thyroxine binding capacity (TBC) in rats and dogs.

Table B. Circulating Thyroid Hormones

Parameter	Response									
Rat Studies										
Chronic Feeding/Onco	Males and Females Control	25	50	500	1000					
T3 T4 TSH TBC	Assessed at weeks 26, 52, 78 and 105. **Data considered inconsistent with large standard deviations and poor dose response and temporal relationships.** T3 and T4 rarely reached statistical differences and did not indicate any dose response. No consistent pattern of statistically significant increases (but occasional increase up to up to 235%) and decreases in ♀. Males at week 26 have large apparent increase (up to 246%) but not statistically significant. In ♂ only a 12% increase at 1000 ppm at week 105. In ♀ only a 15% increase at 500 and 1000 ppm at week 78.									
Subchronic 90 day (with recovery phase)	Males Control	25	100	400	1600	Females Control	25	100	400	1600

Thiacloprid - Thyroid analysis

Parameter	Response	
T3 Week 3	–	No difference
Week 12	14%** 18%** 22%** 42%**	– No difference
TBC Week 3	– ~ ~ ~ 30%**	– ~ ~ 5%* 6%*
Week 12		
Recovery-Week 16	– ~ ~ ~ 6%*	– No difference
	– No difference	
Special 22 day study Days 2, 7, 14 and 21	Males Control 25 100 400 1600	Females Control 25 100 400 1600
T3 T4	[- 16% ↓at day 2 only at 1600 ppm] [-24,18 and 24% ↓at day 2, 7 and 21 at 1600 ppm]	@Day 22 ↑9%** ↑17%** ↑19%** @Day 2 ↓32%
TBC	-7%↑at day 14 only at 1600 ppm.	@Day 14 and 22 ↑4 and 5%** at 1000 ppm
TSH -Day 2	– ~ ~ ~	No differences
-Day 7	↓64%**	~ ~ ~ ~
-Day 14	No differences	↑132%**
-Day 21	– ~ ↑87%* ↑71%* ↑202%*	No differences
	No differences	~ ~ ~ ~ ↑164%**
Special 14 day study	Control 25 100 500 2000	Control 25 100 500 2000
T3 and T4	Only slightly ↓(7-13%) at week 1 only at	2000 ppm.
TSH Week 1	– ~ ~ ~ ↑23%	– ↑19% ↑12% ↑21% ↑34%
Week 2	– ~ ~ ↑14% ↑33%	– ~ ~ ~ ↑35%
Special reprod. study	Control 800	Control 800
	T3 and T4, TSH, prolactin, and follicle stimulating hormone remained unchanged. Aromatase apparently increased	
Mouse Studies - No thyroid hormone data.		
Dog Studies		
Subchronic feeding	Control 250 1000 2000	Control 250 1000 2000

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Thiacloprid - Thyroid analysis

Parameter	Response							
T4	–	~	↓15-16%	↓26-47%	–	↓14-29%	↓18-38%	↓18-38%
TBC	–	↑5-9%	↑6-11%	↑9-14%	–	↑4-10%	↑5-11%	↑11-13%

It would be expected that the circulating thyroid hormones (T3 and T4) would be consistently decreased and the thyroid stimulating hormone from the pituitary increased since the thyroid hormones should be metabolized at a greater rate to cause the signal for the thyroid to produce more circulating hormones. However, this ideal pattern was not shown although there were some cases where TSH was apparently increased.

c. Specific evidence of reduced hormone synthesis (inhibited iodine uptake) or increased thyroid hormone clearance (enhanced biliary excretion):

No studies were presented that attempted to *directly* demonstrate reduced or enhanced thyroid hormone synthesis using iodine uptake or assessment of thyroid hormone clearance.

Although the above specific parameters were not investigated, there are studies which demonstrate that hepatic enzymes are induced that may influence the metabolism of thyroid hormones. Tables C and D illustrated the induction of these enzymes in males and females. Similar patterns of enzyme induction were found in both males and females but males were generally found to be more sensitive to induction than females as indicated by induction of enzymes to a greater extent at lower doses.

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Table C. Male Rat Hepatic Enzyme Induction data from four studies.

Dose Study	Liver Path. Hy CC MEF V	ECOD	ALD-E	GS-T	UDP-Glu	EH	N-Dem	O-Dem
25 R1 R2 R5 R6	0 0 4% 28% 0 0 -- -- 0 -- -- --	139%** ? ~ 3%	137%** ? ~ ~	~ ~ ~	~ ~ 24%ns 3%	~ ~ ~	~	~
50 R1	** ** ** **24% 16% 10%** 16%	148%**	159%** *	~	~	~		
100 R2 R5 R6	0 0 -- --	~ 14%	~ ~	~ 15%	~ 98%** 21%	~ ~	~	~
400 R2 R5	**90% 90%** -- --	67%ns	37%	35%	38% 98%**	44%	72%** *	79%** *
500 R1 R6	**95% 82% 30%** **24%** **100%** -- --	257%** 188%	137%** * 76%	133% ** 97%	163%** 78%	16%ns 131%		
1000 R1 R5	** ** ** ** **95% 94% 44%** **48%**	414%**	199%** *	149% **	189%**	197%** *		
1600 R2 R5	**100%100%** -- --	615%**	293%** *	148% **	428% 252%**	353%	102% **	464% **

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Thiacloprid - Thyroid analysis

Dose Study	Liver Path. Hy CC MEF V	ECOD	ALD-E	GS-T	UDP-Glu	EH	N-Dem	O-Dem
2000 R6	100% – -- --	567%	108%	205%	265%	392%		

R1- Chronic feeding/onco study; R2 -Guideline subchronic study, R3- Special aromatase study, R4 - Special one generation reproduction study, R5 - Special Study and R6 - two week feeding study.
ECOD = 7-ethoxycourmarin deethylase, ALD-E = aldrin epoxidase, GS-T = Glutathione S-transferase, UDP-Glu = UDP-glucuronyl transferase, EH = Epoxide hydrolase, –Dem = N-demethylase, O-dem = O-demethylase.
Dose level is in ppm and can be compared with the female data on the following page. See Table 3 for the dose in mg/kg/day for males and the table on aromatase data in the introduction for mg/kg/day in females.

Table D. Female Rat Hepatic Enzyme Induction data from five studies.

Dose Study (a)	Liver Path. Hy MEF	CC	Aro m-atase	EC OD	AL D	GS-T	UDP-Glu	EH	Cyto-p450	N-Dem	O-Dem
25											
R1	0 4%	0		~ ~	~ ~	~ ~	~ 18% 32%	~ 38%		~	~
R2	0 –	0		~							
R5	– –	0									
R6											
50							~	~			
R1	2% 25	2%		21 %	~	~					
R6	0 –	--									
100											
R2	0 –	0		~	~	~	17%	21% 32%		↓	~
R5	-- --	0	↓4 %	32 %	33 %	12 %		24%			
R6											
R3											
200 R3			69 %*								
400											
R2	20% –	20%		74 %	83 %	23 %	91%* 64%*	134%	23%	24%*	~
R5											
500											
R1	60%* 12%*	60%*		106 %*	109 %*	34 %*	91%* 50%	191% *			
R6	-- --	60%	97 %*	145 %*	125 %	61 %		120%			
R3											

Thiacloprid - Thyroid analysis

Dose Study (a)	Liver Path. Hy CC MEF	Aro m-atase	EC OD	AL D	GS-T	UDP-Glu	EH	Cyto-p450	N-Dem	O-Dem
800 R4 R3		96 %*						See R4-110% to 330% and 520%	116% to 308% *	150 to 200% *
1000 R1 R5	72%* 68%* 20%*	137 %*	241 %*	149 %*	57 %*	155%*	235% *			
1600 R2 R5	100% 100% –		402 %*	317 %*	125 %* *	359%* *	577% ** 321% **	102%	100% **	122% **
2000 R6	100% – –		719 %*	401 %*	194 %*	215%* *	361% **			

R1- Chronic feeding/onco study, R2 -Guideline subchronic study, R3 - special aromatase study, R4 - Special one generation reproduction study, R5 - Special Study and R6 - two week feeding study. ECOD = 7-ethoxycoumarin deethylase, ALD = aldrin epoxidase, GS-T = Glutathione S-transferase, UDP-Glu = UDP-glucuronyl transferase, EH = Epoxide hydrolase, –Dem = N-demethylase, O-dem = O-demethylase.
~ essentially the same as the control. * statistically significant ($p < 0.05$ or less).
(a) dose level is in ppm and can be compared with the previous table in males also in ppm.

d. Evidence of progression (hypertrophy/hyperplasia, nodular hyperplasia - neoplasia):

Table A above shows the temporal relationship of increased thyroid hypertrophy. Colloid change and pigment to indicate that over time there are more animals affected and the lower doses become affected. Males did not demonstrate the development of thyroid *hyperplasia* but there was *hyperplasia* in females.

e. **Reversibility of effects after exposure is terminated:**

The only study that attempted to assess for reversibility of the effects of thiacloprid on the thyroid and liver is the rat subchronic toxicity study (R2) which included a five week reversibility phase. In this study, male body weight was 10% (not significant) at week 12 and

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after the recovery phase was only 3% less than the control. Female body weight was still 17% ($p < 0.01$) below the control group after recovery and was 22% less than the control at the end of dosing indicating poor body weight recovery.

Thyroid weigh data were vague, but relative thyroid weight in males was apparently increased at the end of dosing (probably about 50%) but was essentially the same as the control after recovery. Female thyroid weight was essentially the same for each group at dosing termination and after recovery. There were no thyroid hypertrophy or hyperplasia reported in this study.

In males, T3 levels were noted to be increased 14%, 18%, 22% and 42% (all $p < 0.01$) for the 25, 100, 400 and 1600 ppm dose group at week 3 but only the 1600 ppm dose group (30%., $p< 0.01$) was increased at week 12. After recovery, there was no difference. Female T3 levels were apparently higher than the control for all doses but there was no dose response.

In males only, thyroxine binding capacity (TBC), was higher ($p <0.05$) by only 6% for the 1600 ppm dose group at week 3 only. In females, TBC was higher ($p < 0.05$) by 5 to 6% for the 400 and 1600 ppm dose groups only at week 3.

Liver weight returned to the control level in males but in females liver weight remained 18% higher after recovery. Several hepatic enzymes that were increased all returned to control levels after recovery. Hepatocellular hypertrophy and cytoplasmic change were reported in 9 males and only 2 females at 400 ppm and all males and females at 1600 ppm with none in the control or lower doses. After recovery there were still 3 males and one female with the hypertrophy in the high dose group (cytoplasmic change was not reported).

f. **SAR to other thyroid tumors:**

Thiacloprid contains two rings that may be considered common to thiazopyr another chemical that was demonstrated to induce thyroid tumors in rats. These are the pyridylmethyl and the thiazolinidine structures.

Summary-Factor I. Based on the overall judgment of the 6 indicators in Factor I, it was concluded that there is *insufficient data* to determine whether or not there is evidence that the thyroid tumors in the male rat associated with administration of thiacloprid may be due to a disruption in the thyroid-pituitary status.

The data on the actual measurement of thyroid hormones are considered poor and there are no data actually showing increased synthesis of new thyroid hormones (i.e. increased iodine uptake) or increased excretion of degraded thyroid hormones.

Although the parallel association between increased hepatic pathology, enzymes levels and the thyroid hypertrophy and other thyroid effects is recognized, this alone is not considered sufficient to determine that there is a threshold for induction of thyroid tumors.

There are thyroid tumors in the females dosed with thiacloprid even at the low dose of 25 ppm or below levels where there is no induction of hepatic enzymes and there is at least one tumor in the males at 50 ppm where there is the liver starts to show more definite signs of enzyme induction.

FACTOR II. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

The genotoxicity data for thiacloprid and its metabolites (WAK 6999 and KKO 2254) are negative and the regulatory requirements for testing are satisfied for thiacloprid. There is no indication that genotoxicity plays a role in the tumorigenic activity of thiacloprid. Please see the CARC presentation document for a summary table of the genotoxicity data base.

FACTOR III. Evaluation of neoplasms other than thyroid follicular cell tumors (and relevant pituitary tumors).

Thiacloprid was demonstrated to be associated with increased incidence of uterine tumors in rats with there being 6 (12%), 3 (6%), 3 (6%), 16 (35%), and 22 (45%) rats affected (46-50 rats assessed for each dose) for the control, 25, 50, 500 and 1000 ppm dose groups, respectively. These tumors were a combination of adenocarcinomas and adenosquamous carcinomas and adenomas. There were only 1, 1, and 2 incidents of adenomas in the 50, 500 and 1000 ppm dose groups respectively meaning that most were malignant tumors.

Thiacloprid was also demonstrated to be associated with increased incidence of ovary luteomas in mice with there being 0/47, 1/48 (2%), 5/49 (10.2%) and 6/ 47 (12.8%) for the control, 30, 1250 and 2500 ppm dose groups respectively. Except for one incident of a malignant tumor in the high dose group, all other incidents were described as benign.

The possibility that the both the rat uterine tumors and the mouse ovary tumors are related to the hormonal imbalance associated with thiacloprid's potential to increase aromatase and upset hormonal balance (especially estrogens) was considered by HED's Mechanisms SARC (report in preparation). However, the HED Mechanisms SARC determined that there is an insufficient data base to conclude that an increase in hepatic aromatase leads to alterations in circulating estrogens to justify a causative association with the uterine and ovary tumors at this time.

The pituitary gland of male, but not female rats demonstrated an apparent increase in "cholesterol clefts" with there being 0, 1, 2, 4 and 4 of 50 rats examined for the control, 25, 50, 500 and 1000 ppm dose groups, respectively in the chronic feeding study (R1). The 500 and 1000 ppm dose groups were statistically significant ($p < 0.05$). There were 7, 12, 11, 7 and 14 or 14%, 24%, 22%, 14% and 28% ($p = 0.07$, or not significant) tumors of the *pars distalis* in the males for the control 25, 50, 500 and 1000 ppm dose groups in this study.

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Overall Conclusions:

Some of the criteria in Factors I and II for a threshold effect for induction of thyroid tumors in male rats can be considered as having been met. In particular, as indicated above for Factor I, this is based mainly on the dose and temporal relationship for the induction of the non-neoplastic thyroid conditions of hypertrophy, colloid differences and pigment as well as the presence of hepatic hypertrophy and cytoplasmic change and enzyme induction. Adding in the implication in Factors II that thiacloprid is not genotoxic reenforces this association. There are also possible structural characteristics.

However, detracting from a more definite conclusion for determining that there is a threshold effect for thiacloprid on the thyroid/liver/pituitary axis to induce thyroid tumors are the following items:

-the data on measurement of circulating hormones (T3, T4, TSH) as well as data showing alterations in thyroid hormone synthesis by increased iodine uptake by the thyroid or data on increased excretion of thyroid hormones were either inconsistent (i.e. circulating hormone data) or non-existent (metabolism data).

-there were no data showing that the pituitary was affected including consistent increases in TSH (the cholesterol clefts in males are of questionable significance).

-there were 0, 1, 1, 1, and 2 females in the 25, 50, 500 and 1000 ppm dose groups that had a thyroid follicular cell tumor. Thyroid tumors are rare in females of this Wistar strain rat having a frequency of only 6 in 707 rats with only one incident in 50 rats per study in the historical control data base for the rats used at the laboratory from the same supplier.

-there is also one male with the thyroid tumor at 50 ppm a dose where the thyroid starts to show hypertrophy. Since there are no thyroid tumors in the control or 25 ppm dose group, this tumor may also be related to treatment.

Other Factors:

Thiacloprid did not inhibit thyroid peroxidase *in vitro* using hog thyroids as the source up to concentrations of 435 μM.

The CARC concluded that there are insufficient data at this time to accept the proposed mechanistic theory for induction of thyroid tumors based on increased destruction by the liver of thyroid hormones with subsequent activation of the pituitary to induce thyroid stimulating hormone to stimulate compensatory increases in circulating thyroid hormones.