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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Thiazopyr

TO: Joanne Miller, PM Team # 23
Registration Division (7505C)

FROM: Pamela M. Hurley, Ph.D. Pamela M. Hurley 5/5/94
Toxicologist, Section I
Toxicology Branch I
Health Effects Division (7509C)
and
Esther Rinde, Ph.D. E. Rinde
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

THROUGH: Penelope A. Fenner-Crisp 5/24/94
Penelope Fenner-Crisp, Ph.D.
Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on March 9, 1994 to discuss and evaluate the weight-of-the-evidence on thiazopyr with particular reference to its carcinogenic potential. The Peer Review Committee agreed that thiazopyr should be classified as a Group C - possible human carcinogen and recommended that for the purpose of risk characterization a Margin of Exposure (M.O.E.) approach should be used for evaluation of the consequences of human exposure.

The decision to classify thiazopyr as a Group C carcinogen was based on a 2-year dietary feeding study coupled with several special studies conducted with thiazopyr in the rat. In the chronic feeding study, thiazopyr induced a statistically significant increase in thyroid follicular cell tumors in males. In addition, there were numerical increases in renal tubular adenomas (a rare tumor type) in both sexes; however, there was a statistically significant positive trend in females only. The Committee agreed that due to the rarity of the renal tumors in males, the adenomas observed in this sex were also biologically significant, even though the appearance of tumors was not dose-related.

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The M.O.E. approach was selected because the non-neoplastic toxicological evidence (thyroid growth, thyroid follicular cell hypertrophy/hyperplasia, thyroid and pituitary hormonal changes and data on the site of action (hepatocellular hyperplasia/hypertrophy, enhanced liver metabolism and excretion of T_4)) indicated that thiazopyr was inducing a disruption in the thyroid-pituitary hormonal status. In addition, there was no evidence of genotoxicity. Therefore, a threshold consideration was to be applied in estimating risk.

The Committee selected a no observable effect level (NOEL) of 4.4 mg/kg/day and a lowest observable effect level (LOEL) of 44.2 mg/kg/day as the dose levels to be used in the M.O.E. carcinogenicity risk assessment. These dose levels were selected because they represented the majority of the NOEL's and LOEL's for the endpoints examined. Most of the other NOEL's and LOEL's were higher than those selected. The endpoints considered included: thyroid tumors, thyroid hypertrophy/hyperplasia, increases in thyroid weights, TSH, T_3, and reverse T_3 levels, increases in T_4 UDPGT activity and decreases in T_4 levels in rat studies and hepatocellular hypertrophy and increases in liver weights in the rat, mouse and dog studies. The kidney tumors and kidney pathology were considered in the weight of evidence for classification of carcinogenic potential but were not considered for the M.O.E. calculation. The kidney pathology consisted of renal nephropathy and increases in kidney weights in both rats and mice. In addition, renal tubular hyperplasia was observed in 1 high dose female rat. Although the kidney pathology indicated that the kidney was a target organ, it was not necessarily associated with the kidney tumor response.

For comparison purposes, the NOEL selected for calculation of the reference dose (RfD) for thiazopyr is 0.8 mg/kg/day, based on hepatocellular hypertrophy observed in the chronic feeding study in the dog (see HED RfD decision document).

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A. Individuals in Attendance at the Meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Reto Engler

William Burnam

Karl Baetcke

Marcia Van Gemert

Kerry Dearfield

Elizabeth Doyle

Esther Rinde

Richard Hill

Yin Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Pamela Hurley[1]

Roger Gardner

Lucas Brennecke[2]
(PAI/Clement)

Lori L. Brunsman

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

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B. Material Reviewed

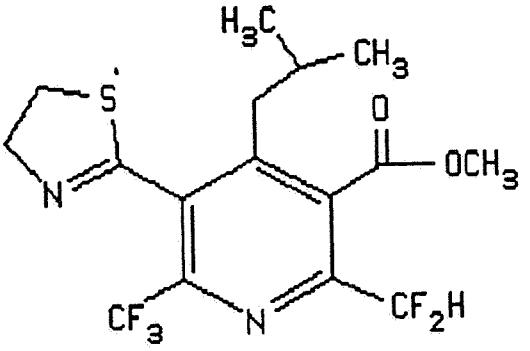
The material available for review consisted of a rat chronic/carcinogenicity feeding study, a mouse carcinogenicity study, a one-year dog feeding study, a subchronic feeding study in the rat, a 4-week and a subchronic feeding study in the dog, a 21-day dermal study in the rat, developmental toxicity studies in the rat and rabbit, a 2-generation reproduction study in the rat, mutagenicity studies and metabolism and special mechanistic studies summarized by Pam Hurley; tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

Thiazopyr is a new pesticide which will be used as an herbicide for selective weed control in several crops including cotton, peanuts, alfalfa, soybeans, tree crops and vines. In addition to these crops, limited testing has indicated potential for its use in sunflowers, sugarcane, transplant vegetables, maize, potatoes, soft fruit, forestry and industrial situations. Its highest unit activity is against grass species. Its best performance is as a soil-applied residual product. It has low water solubility and low movement in the soil. There are three formulations under evaluation at this time: an emulsifiable concentrate containing 2.0 lbs active ingredient/US gallon, a granule containing 5% active ingredient and a water disperable granule containing 50% active ingredient.

The Caswell (or Tox. Chem.) Number for thiazopyr is 849AA
The Chemical Abstracts Registry Number (CAS No.) is 117718-60-2
The PC Code is 129100.

The structure of thiazopyr is:



Thiazopyr

D. Evaluation of Carcinogenicity Evidence

1. 2- Year Chronic/Oncogenicity Study in Rats

Reference: Naylor, M. W., McDonald, M. M. (1992) Chronic Study of MON 13200 Administered in Feed to Albino Rats: Project Number ML-88-247/EHL 88148. Testing Facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, Missouri; Submitted by: Monsanto Agricultural Company, St. Louis, Missouri. MRID No. 426197-24.

a. Experimental Design

Male and female Sprague-Dawley (CD) rats were fed 0, 1, 10, 100, 1000 or 3000 ppm thiazopyr (Technical, 94.8% pure) in the diet for 24 months. This was calculated to be 0, 0.04, 0.4, 4.4, 44.2 or 136.4 mg/kg/day for males and 0, 0.06, 0.6, 5.6, 56.3 or 177.1 mg/kg/day for females. The test material was mixed thoroughly with the basal diet using high speed mixers. Sixty animals/sex were assigned to each dose level. Ten/sex/dose level were sacrificed at 12 months. An additional 6 animals/sex were assigned to satellite groups which were sacrificed at 6 and 12 months for evaluation of hepatocellular proliferation. Periodic determinations of body weight and food consumption were conducted and the animals were examined for treatment-related changes in clinical signs, ophthalmology, hematology, clinical biochemistry, urinalysis and histopathology parameters.

b. Discussion of Tumor Data

Male rats had significant increasing trends in thyroid follicular cell adenomas/cystadenomas, carcinomas, and combined adenomas/cystadenomas and/or carcinomas, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls at $p < 0.05$ and of the 3000 ppm dose group with the controls at $p < 0.01$ for thyroid follicular cell adenomas/cystadenomas, and combined adenomas/cystadenomas and/or carcinomas. These statistical analyses were based upon the Exact trend test since there were small numbers of tumors observed in selected instances. The Fisher's Exact test was used for pair-wise comparisons. The following table reports the male tumor analysis results.

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Thiazopyr - Charles River Sprague-Dawley Rat Study

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

	Dose (ppm)					
	0	1	10	100	1000	3000
Adenomas/ Cystadenomas (%)	1/50 (2)	2/47 (4)	0/49 (0)	2/47 (4)	8/49 (16)	12[a]/48 (25)
p =	0.000**	0.477	0.505[n]	0.477	0.014*	0.001**
Carcinomas (%)	1[b]/50 (2)	1/47 (2)	0/49 (0)	0/47 (0)	1/49 (2)	4/48 (8)
p =	0.008**	0.737	0.505[n]	0.516[n]	0.748	0.169
Combined (%)	2/50 (4)	3/47 (6)	0/49 (0)	2/47 (4)	9/49 (18)	14[c]/48 (29)
p =	0.000**	0.470	0.253[n]	0.668	0.024*	0.001**

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

[n]Negative change from control.

[a]First adenoma/cystadenoma observed at week 69, dose 3000 ppm.

[b]First carcinoma observed at week 81, dose 0 ppm.

[c]Two animals in the 3000 ppm dose group had both carcinomas and adenomas/cystadenomas.

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 $p < 0.01$.

Historical control data for thyroid histopathologic findings were submitted by the registrant. The data are from GLP studies but the compilation of the data were not audited by their Quality Assurance Unit. Data on thyroid lesions were submitted separately for both sexes. It is noted that in males, the percent with thyroid follicular cell adenomas and combined adenomas and carcinomas for both the 1000 and 3000 ppm dose groups were greater than the historical control rates for these particular tumors. In addition, the percent with thyroid follicular cell carcinomas at 3000 ppm in males were also greater than the historical control rate. The historical control data for the follicular adenoma, carcinoma and follicular adenoma and carcinoma combined for males are summarized here.

Historical Control Information for Thyroid Histopathologic Findings In Males
(All Deaths; Thiazopyr Study is Study # 10; Data Current as of 1/12/94)

Lesion	Study	Terminal Necropsy Date	Months of Study	# Observed	# Affected	% Affected
Benign Follicular Adenoma[a]	1	Jul-83	24	66	7	11.0
	2	Feb-85	23	60	3	5.0
	3	Oct-85	24	70	1	1.0
	4	Jun-88	24	55	3	5.0
	5	Sep-88	24	60	1	2.0
	6	Jan-89	24	60	0	0.0
	7	Mar-89	24	60	2	3.0
	8	Aug-89	24	60	2	3.0
	9	Jun-90	24	60	5	8.0
	10	Jul-90	24	60	1	2.0
	11	Mar-92	24	59	0	0.0
Malignant Follicular Carcinoma[b]	1	Jul-83	24	66	2	3.0
	2	Feb-85	23	60	1	2.0
	3	Oct-85	24	70	0	0.0
	4	Jun-88	24	55	0	0.0
	5	Sep-88	24	60	1	2.0
	6	Jan-89	24	60	1	2.0
	7	Mar-89	24	60	0	0.0
	8	Aug-89	24	60	0	0.0
	9	Jun-90	24	60	0	0.0
	10	Jul-90	24	60	1	2.0
	11	Mar-92	24	59	0	0.0
Follicular adenoma and carcinoma, combined	1	Jul-83	24	66	9	14.0
	2	Feb-85	23	60	4	7.0
	3	Oct-85	24	70	1	1.0
	4	Jun-88	24	55	3	5.0
	5	Sep-88	24	60	2	3.0
	6	Jan-89	24	60	1	2.0
	7	Mar-89	24	60	2	3.0
	8	Aug-89	24	60	2	3.0
	9	Jun-90	24	60	5	8.0
	10	Jul-90	24	60	2	3.0
	11	Mar-92	24	59	0	0.0

[a]Also referred to as benign follicular adenoma/cystadenoma
[b]Also referred to as malignant follicular adenocarcinoma or malignant follicular cell carcinoma

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There were no statistically significant increases in thyroid tumors in females. The following table summarizes the incidences of these tumors in females.

Incidences of Thyroid Tumors in Female Rats - All Animals

Dose Level (ppm)	0	1	10	100	1000	3000
Organ			Females			
Thyroid	(60)	(60)	(60)	(58)	(60)	(59)
Follicular cell						
Adenoma/cystadenoma	0	1	0	1	4	2
Carcinoma	0	0	1	0	0	0
Combined adenoma/carcinoma	0	1	1	1	4	2

Female rats had a numerical increase in renal tubular adenomas at the high dose which was not statistically significant in a pairwise comparison with controls. However, there was a statistically significant positive trend (brief statistical analyses were conducted on summary data from both sexes). The rate exceeded the historical control range (0/70 - 1/60 in one 23 month study and in eight 24 month studies, ranging in dates from 7/83 to 6/90). Two of the tumors were observed at study termination and the third was observed in an animal which died just one month prior to termination (cause of death was not stated but appeared to be due to an enlarged pituitary pressing on the brain). Two of the tumors were classified as focal and the third one from a terminal sacrifice female was classified as multifocal. The tables below summarize the incidences of these tumors and the available historical control data.

In males, renal tubular tumors were observed at 10, 100 and 1000 ppm, but not at 3000 ppm (see table below). The incidences of these neoplasms in males were not dose-related. Historical control data on renal tubular adenomas for males were not obtained from the registrant.

Incidences of Renal Tubular Adenomas in Rats - All Animals

Dose Level (ppm)	0	1	10	100	1000	3000
Organ			Males			
Kidney (N)	(60)	(60)	(60)	(60)	(61)	(60)
Tubular adenomas	0	0	2	1	2	0
			Females			
Kidney (N)	(60)	(60)	(60)	(60)	(60)	(60)
Tubular adenomas	0	0	0	0	0	3

Historical Control Information for Kidney Histopathologic Findings in Females (All Animals)*

Lesion	Study	Terminal Necropsy Date	Months of Study	# Observed	# Affected	% Affected
Adenoma	1	Jul-83	24	70	0	0.0
	2	Feb-85	23	60	0	0.0
	3	Oct-85	24	70	0	0.0
	4	Jun-88	24	60	0	0.0
	5	Sep-88	24	60	0	0.0
	6	Jan-89	24	60	0	0.0
	7	Mar-89	24	60	0	0.0
	8	Aug-89	24	60	0	0.0
	9	Jun-90	24	60	1	1.7
Carcinoma	1	Jul-83	24	70	0	0.0
	2	Feb-85	23	60	0	0.0
	3	Oct-85	24	70	0	0.0
	4	Jun-88	24	60	0	0.0
	5	Sep-88	24	60	0	0.0
	6	Jan-89	24	60	0	0.0
	7	Mar-89	24	60	1	1.7
	8	Aug-89	24	60	0	0.0
	9	Jun-90	24	60	0	0.0

*Note: These data have not been subject to Monsanto peer review.

> In their discussion of the kidney tumors, the investigators who conducted the chronic rat bioassay provided the following remarks which were considered by the CPRC in their evaluation. In one of the studies from which the historical control data were taken, renal tubular adenomas were observed in 3/60 females from the mid-dose group but not in any females from the control, low or high-dose groups (thus indicating that the historical control rate may be slightly low). The investigators believed that "since chemically-induced renal tubular neoplasia generally occurs more frequently in male rats than in female rats, a treatment-related effect in female rats alone would be unexpected. Thus, the treatment relationship of the tubular adenomas observed in the high-dose females was considered to be equivocal."

Nevertheless, the CPRC considered the presence of these tumors to be biologically significant in both sexes because they are so rare (the Committee agreed that for males, the historical control rate for renal tubular adenomas in this particular strain of rat is less than 1%; the value was verified at the meeting by pathologist, Dr. Lucas Brennecke from available Charles River data, although these data were not from the performing laboratory within the time frame of this study).

c. Non-Neoplastic Effects and Other Considerations

Mortality

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of thiazopyr in male rats. Female rats showed a statistically significant decreasing trend in mortality with increasing doses of thiazopyr.

Clinical Signs

The report stated that "the only clinical sign considered possibly related to treatment was protruding eyes, which occurred more frequently in females at the two highest levels." None of the other clinical signs were considered to be related to treatment.

No treatment-related ophthalmological effects were observed in any dose group when compared to controls.

There appeared to be some evidence of mild anemia in both sexes at various times throughout the study. Slight-to-moderate statistically significant decreases in red blood cells, hemoglobin and hematocrit were observed in both sexes at various times throughout the study at either 3000 ppm alone or at both the 3000 and 1000 ppm dose levels. By the end of the study, effects were present only in high dose females. Mean cell volume values were also significantly decreased in both sexes. In addition, there were increases in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values which followed similar patterns to the above parameters.

Serum glutamate pyruvate transaminase (SGPT) was generally increased over the controls in the high dose group for both sexes. However, the increases were not consistent for all time points. For the other liver indicators, alkaline phosphatase was statistically significantly raised over the control groups in both sexes at the high dose, but only at 6 months, and serum glutamate oxalacetate transaminase (SGOT) was not significantly different from the control groups at any time point. Gamma-glutamyl transferase (GGT), however, was consistently significantly increased over the control groups in both sexes at the highest dose level. At 1000 ppm, GGT was increased over the controls at 18 and 24 months in males; the levels were 5-6 times less than the high dose group and 2-3 times greater than the control values. Cholesterol was significantly increased over controls in the high dose group in both sexes (only 24 months in males) and at 18 and 24 months in the 1000 ppm females. There were some relatively consistent increases in globulin levels in both sexes, but these increases were small. The same holds true for blood urea nitrogen (BUN) levels. None of the other differences between the treated and control groups were biologically significant because of lack of consistency or dose-response.

TSH, T_3 and T_4 values were measured at 6 months only. From the data, it appears that T_4 was significantly decreased in males and T_3 was significantly increased in females when compared to controls. However, the report stated that "since special precautions were not taken to ensure adequate samples (the animals were fasted, blood samples were obtained from the retro-orbital sinus and, in several cases, an insufficient quantity of serum remained), many of the individual values could not be measured and the data were considered unreliable."

The results from the hepatocellular proliferation assays indicated that there were no statistically significant differences in BrdU-labelled nuclei in rats injected with BrdU, when the high dose was compared to controls.

The report stated and the tables indicated that at 24 months, males at the highest dose level had a statistically significant increase in total urine volume. The report also stated that total urine volume was also increased (but not significantly so) at both 18 and 24 months at 1000 and 3000 ppm in males.

Non-neoplastic Effects

At the two highest dose levels in both sexes, mean absolute and relative liver weights were significantly increased at both month 12 and at terminal sacrifice (terminal sacrifice: absolute liver weights 122% and 178% of controls at 1000 and 3000 ppm, relative liver weights 143% and 206% of controls at 1000 and 3000 ppm for males; absolute liver weights 130% and 165% of controls at 1000 and 3000 ppm, relative liver weights 158% and 245% of controls at 1000 and 3000 ppm for females).

For the kidney, the mean absolute weights were increased in the male and female mid- and high dose groups at 12 and/or 24 months. The mean relative weights were increased in a similar fashion (terminal sacrifice: absolute kidney weights 118% and 124% of controls at 1000 and 3000 ppm, relative kidney weights 142% and 145% of controls at 1000 and 3000 ppm for males; absolute kidney weights 134% and 109.8% of controls at 1000 and 3000 ppm, relative kidney weights 164% and 158% of controls at 1000 and 3000 ppm for females).

Absolute thyroid weights were significantly increased in the high dose males at 24 months (297% of controls) and in the 1000 ppm females at 12 months. Relative thyroid weights were significantly increased in the high dose group males and in the 1000 ppm and high dose group females at 12 months, and in both the 1000 ppm and high dose groups of both sexes at 24 months (132% and 326% of controls for males and 155% and 165% of controls for females at the mid- and high doses, respectively). The report stated that these latter increases may have been a result of decreased body weights.

Treatment-related changes were observed in the liver, kidney and thyroid. Other effects, which appeared at a greater incidence in the two highest dose levels, but not considered to be treatment-related were observed in the adrenals, the ovaries and the lungs. Lesions and/or effects which were considered to be age-related were observed in all dose groups. Some changes were statistically significantly increased in one or more dose groups, but there was no indication of a dose response. These are not considered to be biologically significant. Selected changes in liver and thyroid were statistically analyzed by the Science Analysis Branch.

Male rats had significant increasing trends in liver random lipid vacuolization and centrilobular hepatocellular hypertrophy, both at $p < 0.01$. Significant increasing trends also existed in thyroid follicular epithelium hyperplasia and hypertrophy/ hyperplasia, both at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls for liver midzonal lipid vacuolization ($p < 0.05$) and centrilobular hepatocellular hypertrophy ($p < 0.01$). There were significant differences in the pair-wise comparisons of the 3000 ppm dose group with the controls for liver random lipid vacuolization ($p < 0.01$) and centrilobular hepatocellular hypertrophy ($p < 0.01$), and thyroid follicular epithelium hyperplasia ($p < 0.05$) and hypertrophy/hyperplasia ($p < 0.01$).

These statistical analyses were based upon the Exact trend test since there were small numbers of effects observed in selected instances. The Fisher's Exact test was used for pair-wise comparisons. The following tables summarize the statistical analyses of effects observed in the liver and thyroid of male rats.

Male Rat Liver Effect Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)					
	0	1	10	100	1000	3000
Midzonal Lipid Vacuolization (%)	6[a]/60 (10)	2/60 (3)	8/60 (13)	10/59 (17)	15/61 (25)	9/60 (15)
p =	0.112	0.136[n]	0.389	0.200	0.029*	0.291
Random Lipid Vacuolization (%)	20/60 (33)	22/60 (37)	17/60 (28)	16/59 (27)	16/61 (26)	35[b]/60 (58)
p =	0.000**	0.424	0.346[n]	0.295[n]	0.256[n]	0.005**
Centrilobular Hepatocellular Hypertrophy (%)	0/60 (0)	0/60 (0)	0/60 (0)	0/59 (0)	47[c]/61 (77)	52/60 (87)
p =	0.000**	1.000	1.000	1.000	0.000**	0.000**

[+]Number of animals with liver effect/Number of animals examined, excluding those that died or were sacrificed before observation of the first effect.
[n]Negative change from control.
[a]First midzonal lipid vacuolization observed at week 52, dose 0 ppm.
[b]First random lipid vacuolization observed at week 27, dose 3000 ppm.
[c]First centrilobular hepatocellular hypertrophy observed at week 26, dose 1000 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 $p < 0.01$.

Thiazopyr - Charles River Sprague-Dawley Rat Study

Male Rat Thyroid Follicular Epithelial Change Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)					
	0	1	10	100	1000	3000
Hyperplasia (%)	1/60 (2)	1/57 (2)	1/59 (2)	4/57 (7)	5[a]/59 (8)	8/58 (14)
p =	0.001**	0.739	0.748	0.166	0.100	0.014*
Hypertrophy (%)	0/60 (0)	0/59 (0)	0/59 (0)	0/59 (0)	0/60 (0)	1[b]/60 (2)
p =	0.168	1.000	1.000	1.000	1.000	0.500
Hypertrophy/ Hyperplasia (%)	2[c]/60 (3)	0/59 (0)	0/59 (0)	1/59 (2)	5/60 (8)	12/60 (20)
p =	0.000**	0.252[n]	0.252[n]	0.506[n]	0.220	0.004**

[+]Number of animals with thyroid effect/Number of animals examined, excluding those that died or were sacrificed before observation of the first effect.

[n]Negative change from control.

[a]First hyperplasia observed at week 52, dose 1000 ppm.

[b]First hypertrophy observed at week 27, dose 3000 ppm.

[c]First hypertrophy/hyperplasia (not hyperplasia and/or hypertrophy combined; for definition, see page 30 of DER) observed at week 52, dose 0 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 $p < 0.01$.

Female rats had significant increasing trends in liver midzonal lipid vacuolization ($p < 0.01$), peripherolobular lipid vacuolization ($p < 0.05$), centrilobular hepatocellular hypertrophy ($p < 0.01$), and cystic degeneration ($p < 0.05$). Significant increasing trends also existed in thyroid follicular epithelium hyperplasia ($p < 0.05$) and hypertrophy/hyperplasia ($p < 0.01$). There were significant differences in the pair-wise comparisons of the 10 ppm dose group with the controls for liver cystic degeneration at $p < 0.05$. There were significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls for liver centrilobular hepatocellular hypertrophy and thyroid follicular hypertrophy/ hyperplasia, both at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 3000 ppm dose group with the controls for liver midzonal lipid vacuolization ($p < 0.01$), peripherolobular lipid vacuolization ($p < 0.05$), centrilobular hepatocellular hypertrophy ($p < 0.01$), and cystic degeneration ($p < 0.05$), and thyroid follicular epithelium hypertrophy/hyperplasia ($p < 0.01$).

These statistical analyses were based upon Peto's prevalence test since there was a statistically significant negative trend for mortality in female rats with increasing doses of thiazopyr. The following tables summarize the statistical analyses of effects observed in the liver and thyroid of female rats.

Thiazopyr - Charles River Sprague-Dawley Rat Study

Female Rat Liver Effect Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)					
	0	1	10	100	1000	3000
Midzonal Lipid Vacuolization (%)	3/59 (5)	3/58 (5)	2/59 (3)	3[a]/57 (5)	4/59 (7)	10/60 (17)
p =	0.000**	0.524	0.633[n]	0.614	0.344	0.003**
Peripherolobular Lipid Vacuolization (%)	1/47 (2)	0/44 (0)	4[b]/44 (9)	5/45 (11)	4/46 (9)	7/48 (15)
p =	0.011*	0.810[n]	0.086	0.066	0.092	0.024*
Centrilobular Hepatocellular Hypertrophy (%)	1/59 (2)	0/59 (0)	0/59 (0)	0/58 (0)	33[c]/60 (55)	58/60 (97)
p =	0.000**	0.853[n]	0.863[n]	0.890[n]	0.000**	0.000**
Cystic Degeneration (%)	0/45 (0)	1[d]/41 (2)	3/42 (7)	0/44 (0)	1/43 (2)	5/48 (10)
p =	0.037*	0.164	0.028*	-	0.147	0.027*

[+]Number of animals with liver effect/Number of animals examined, excluding those that died or were sacrificed before observation of the first effect.
[n]Negative change from control.
[a]First midzonal lipid vacuolization observed at week 52 at 100 ppm.
[b]First peripherolobular lipid vacuolization observed at week 65, dose 10 ppm.
[c]First centrilobular hepatocellular hypertrophy observed at week 50, dose 1000 ppm.
[d]First cystic degeneration observed at week 71, dose 1 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 $p < 0.01$.

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Thiazopyr - Charles River Sprague-Dawley Rat Study

Female Rat Thyroid Follicular Epithelial Change Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)					
	0	1	10	100	1000	3000
Hyperplasia (%)	1/32 (3)	0/26 (0)	0/30 (0)	2/24 (8)	3[a]/29 (10)	3/37 (8)
p =	0.047*	0.825[n]	0.880[n]	0.402	0.156	0.197
Hypertrophy/ Hyperplasia (%)	1/59 (2)	0/58 (0)	0/59 (0)	4[b]/56 (7)	12/59 (20)	18/59 (31)
p =	0.000**	0.841[n]	0.841[n]	0.066	0.000**	0.000**

[+]Number of animals with thyroid effect/Number of animals examined, excluding those that died or were sacrificed before observation of the first effect.

[n]Negative change from control.

[a]First hyperplasia observed at week 93, dose 1000 ppm.

[b]First hypertrophy/hyperplasia (not hyperplasia and/or hypertrophy combined; for definition, see page 30 of DER) observed at week 52, dose 100 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 authors of the report stated that the mean severity of the centrilobular hepatocellular hypertrophy was generally minimal to slight and was, at most marginally increased with dose. The authors believed that these changes along with the vacuolation and vacuolar change were all related to treatment with the test material. In addition, the authors believed that the midzonal lipid/vacuolization was an effect at 100 ppm, mostly because of data observed at 12 months. This effect did not appear to have a dose-response, and was not very apparent after 12 months.

The thyroid follicular non-neoplastic proliferative changes, hypertrophy/hyperplasia and/or epithelial hyperplasia "were present as early as the month 12 interim sacrifice and mean severity (generally minimal to slight) was similar in the affected groups. Hypertrophy/hyperplasia denoted a diffuse change which collectively included enlargement and/or increased number of follicular epithelial cells, while epithelial hyperplasia denoted discrete focal areas of hypercellularity without increased cell size. The combined diagnosis 'hypertrophy/hyperplasia' indicates that while both of these changes appear to occur in most affected glands, the relative degree to which each is present can be determined only by utilizing quantitative techniques which were not used in this study."

The following tables summarize the most significant non-neoplastic lesions in the kidney.

Incidences of Non-neoplastic Lesions in the Kidney of Male Rats - All Animals

Dose Level (ppm)	0	1	10	100	1000	3000
Kidney (N)	(60)	(60)	(60	(60)	(61)	(60)
Nephropathy	52	48	45	52	59*	59*

*Statistically significant ($p \leq 0.05$) from control using Fisher's exact test.

Incidences of Non-neoplastic Lesions in the Kidney of Female Rats - All Animals

Dose Level (ppm)	0	1	10	100	1000	3000
Kidney (N)	(60)	(60)	(60	(60)	(60)	(60)
Nephropathy	39	39	44	42	53**	59**
Renal tubular hyperplasia	0	0	0	0	0	1

**Statistically significant ($p \leq 0.01$) from control using Fisher's exact test.

One high dose female had "an area of renal tubular hyperplasia (distinct from regenerative, hyperplastic tubules which are a feature of nephropathy)". The incidence of this finding was within

the historical control range provided in the report (0/70 - 2/70 in one 23 month and eight 24 month studies ranging in dates from 7/83 to 6/90).

The mean severity of the renal nephropathy ranged from slight (mild) to moderate. The authors considered the increased incidences and/or severity to be "a test material-related exacerbation of a normal aging process in the rat." The following table, taken directly from the report summarizes incidences and severity of the nephropathy.

Incidences and Severity of Nephropathy in Males and Females

Dose Level (ppm)	Males		Females	
	Incidence	Severity[a]	Incidence	Severity
0	52/60	2.4	39/60	1.9
1	48/60	2.4	39/60	2.0
10	45/60	2.5	44/60	2.1
100	52/60	2.5	42/60	2.3
1000	59/61*	3.1	53/60**	2.5
3000	59/60*	2.9	59/60**	2.8

[a]Severity grades: 0 = none; 1 = minimal; 2 = slight (mild); 3 = moderate; 4 = moderate; 5 = very severe; mean severity = sum of severity grades of all animals in group/number of lesion-bearing animals in group.
*Statistically significant, $p \leq 0.05$, Fisher's Exact Test
**Statistically significant, $p \leq 0.01$, Fisher's Exact Test

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of thiazopyr, based on increases in absolute and relative liver, thyroid and kidney weights; hepatocellular vacuolation and hypertrophy; hyperplasia and hypertrophy of the follicular epithelium of the thyroid and an increase in the severity of nephropathy. In addition, there was some supporting clinical chemistry evidence for liver as a target organ (increases in SGPT, cholesterol and GGT).

2. 18 - Month Carcinogenicity Feeding Study in Mice

Reference: Naylor, M. W.; Raju, N. R. (1992) Chronic Study of MON 13200 Administered in Feed to Albino Mice. Project Number ML-88-248/EHL 88147. Testing Facility: Monsanto Company, The Agricultural Group, Environmental Health Laboratory, St. Louis, Missouri; Submitted by: Monsanto Agricultural Company, St. Louis, Missouri. MRID No. 426197-23.

a. Experimental Design

Male and female CD-1 albino mice were fed 0, 1, 10, 100, 400 or 800 ppm thiazopyr (Technical, 94.8% pure) in the diet for 18 months. This was calculated to be 0, 0.17, 1.6, 16.9, 66.3 or 128.4 mg/kg/day in males and 0, 0.24, 2.6, 26.8, 108.1 or 215.9 mg/kg/day in females. The neat test material was mixed thoroughly with the basal diet using high speed mixers. For the main study, 70 animals/sex were assigned to each dose level. For each of the interim sacrifices (5 days, 1 month, 5 months and 12 months), 7 satellite animals/sex/group were added to the original 70 animals/sex/dose for use in evaluating hepatocellular proliferation. At months 5 and 12, 10 animals/sex/group from the main study were sacrificed for the interim studies. Periodic determinations of body weight and food consumption were conducted and the animals were examined for treatment-related changes in clinical signs, hematology (limited), clinical biochemistry (limited) and histopathology parameters.

b. Discussion of Tumor Data

There were no statistically significant increases in any neoplastic lesions in either sex. The following table summarizes selected neoplastic lesions for all animals examined, including those from scheduled sacrifices and those which died during the study or were sacrificed *in extremis*.

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Summary of Selected Neoplastic Lesions in Male and Female Mice

Group (ppm)	0	1	10	100	400	800
Observation			Males			
Kidney						
Tubular adenoma	2/71	0/4[a]	-	0/51	1/50	1/70
Liver						
Hepatocell. adenoma	8/71	11/74	8/70	5/68	3/68	10/69
carcinoma	1/71	2/74	2/70	2/68	3/68	3/69
Testis						
Interstitial cell tumor	0/71	0/4	-	1/51	2/49	0/67
			Females			
Liver						
Hepatocell. adenoma	1/70	1/69	2/68	0/69	3/71	3/71
carcinoma	0/70	0/69	0/68	0/69	0/71	1/71
Mammary gland						
Adenocarcin-oma	0/40	-	-	1/37	0/18	0/31
Uterus						
Stromal sarcoma	1/69	-	-	3/50	4/51	0/70

[a]The denominator is the number of selected organ examined.

c. Non-neoplastic Effects and Other Considerations

Mortality

The report stated that there were no statistically significant differences in mortality in any of the treated groups when compared to the controls. Survival ranged from 54% to 82%.

Clinical Signs

The report stated that the only clinical sign which was possibly related to treatment was a distended abdomen, which occurred slightly more frequently in a few high dose males. No other treatment-related clinical signs were observed.

In general, there were no effects on body weight in either males or females throughout the study. Body weight gains followed a similar

pattern as the body weights. After the first year of the study, body weight gains began to drop in the treated males when compared to the controls. However, the decrease in cumulative body weight gains did not become statistically significantly less than controls until the last measurement (weeks 1-541), at which time the body weight gains were significantly less than controls for all treated groups (71, 75, 69, 60 and 66% of the control value for the five dose levels in consecutive order). Overall, it is judged that there were no significant effects on either body weights or body weight gains for either sex at any dose level.

There were no consistent statistically significant differences in food consumption when compared to controls for any of the treated groups in either sex.

No consistent treatment-related differences were observed in any hematological parameters.

Statistically significant increases in SGPT, SGOT and alkaline phosphatase were observed at various intervals in both sexes. ALP was elevated in the two highest dose groups for males, but only significantly so at 12 months (both doses) and at 18 months (high dose). ALP was slightly elevated in the two highest dose female groups throughout the study, but was only statistically significant at 18 months at the highest dose level. SGOT was slightly elevated in both sexes at the two highest dose levels at various times throughout the study, but only significantly so at 12 and at 18 months in the high dose males. SGPT values were statistically significantly elevated over controls in the high dose males at 5, 12 and 18 months, approximately twice the control values. These values were significantly elevated in high dose females at 5 and 12 months and in the second highest dose females at 5 months. These values were generally less than twice the control values. The results indicate that there may be some hepatoxic activity in the liver, however, none of the values were greatly elevated over the control values.

Non-neoplastic Effects

Absolute and relative liver weights were statistically significantly increased over controls in both sexes at the highest dose level at many sacrifice times (ranging from 132 - 158% of control in males and 124 - 193% of controls in females) and at the second highest dose level at some sacrifice times (95 - 114% of controls in males and 114 - 125% of controls in females). At terminal sacrifice, decreases in absolute and relative spleen weights were observed in high dose females (75 and 79% of controls, respectively) and increased absolute and relative kidney weights were also observed in high dose females (111% and 114%, respectively). No other treatment-related differences were observed.

Treatment-related changes were observed in the liver, particularly in the two highest dose levels of both sexes. In addition, increased incidences of nephropathy (high dose males) and lymphocytic hyperplasia of the mesenteric lymph node (high dose females) were observed, but only when analyzed for all deaths combined. The report also stated that amyloid deposition was seen in many tissues, particularly in males at the two highest dose levels and some at 100 ppm. Statistically significant increases in other effects were also observed, but were not considered to be treatment-related due to low frequency and/or lack of a dose-response. The following tables summarize selected non-neoplastic microscopic effects for all animals examined, including those from scheduled sacrifices and those which died during the study or were sacrificed *in extremis*.

Summary Incidence of Selected Non-neoplastic Effects in Male Mice (All Animals)

Group	MN	M1	M2	M3	M4	M5
Observation						
Liver						
Hepatocellular hypertrophy	2/71	3/74	2/70	8/68[a]	39/68[b]	61/69[b]
Hepatocellular vacuolation						
Random	5/71	0/74	2/70	2/68	26/68[b]	43/69[b]
Periportal	0/71	0/74	0/70	1/68	0/68	5/69[a]
Hepatocytes, increased eosinophilia	0/71	0/74	0/70	0/68	0/68	39/69[b]
Amyloid deposition						
heart	4/71	0/4	-	10/52[a]	12/50[b]	19/70[b]
adrenal	7/70	0/4	-	12/50[a]	12/48[a]	17/69[a]
ileum	9/64	0/2	-	14/44[a]	15/42[b]	24/66[b]
jejunum	1/58	0/1	-	12/43[b]	10/41[b]	15/64[b]
cecum	0/61	0/1	-	0/43	0/43	5/60[a]
duodenum	2/63	0/1	-	7/43[a]	7/43[a]	10/67[a]
colon	0/66	0/3	-	0/43	1/45	2/63
parathyroid	3/67	0/4	-	6/45	7/39[a]	12/54[b]
stomach	0/71	0/3	-	2/49	6/46[b]	11/68[b]
thyroid	6/70	0/4	-	10/51	10/46[a]	17/69[b]
salivary gland	0/71	0/4	-	0/48	0/48	3/68
Kidney						
Nephropathy	8/71	0/4	-	4/51	2/50	18/70[a]
Thyroid						
Follicular cyst(s)	2/70	0/4	-	0/51	2/46	5/69
Cyst(s), follicular	0/70	0/4	-	1/51	0/46	2/69

[a]Statistically significantly increased over controls ($p \leq 0.05$) using Fisher's Exact test.
[b]Statistically significantly increased over controls ($p \leq 0.01$) using Fisher's Exact test.

Summary Incidence of Selected Non-neoplastic Effects in Female Mice (All Animals)

Group Observation	FN	F1	F2	F3	F4	F5
Liver						
Hepatocellular hypertrophy	2/70	1/69	1/68	3/69	17/71[b]	51/71[b]
Hepatocellular vacuolation						
Random	1/70	0/69	0/68	4/69	14/71[b]	17/71[b]
Periportal	0/70	1/69	1/68	0/69	6/71[a]	8/71[b]
Hepatocytes, increased eosinophilia	0/70	0/69	0/68	0/69	2/71	44/71[b]
Amyloid deposition						
heart	7/70	-	-	4/48	7/51	13/70
adrenal	8/69	-	-	10/50	7/51	12/70
ileum	11/68	-	-	17/45	11/46[b]	19/65
jejunum	7/64	-	-	10/41	5/41	13/65
colon	0/69	-	-	0/42	0/47	5/66[a]
parathyroid	6/63	-	-	5/47	7/47	12/62
stomach	0/69	-	-	0/42	0/47	5/66[a]
thyroid	8/70	-	-	7/50	9/51	13/69
salivary gland	0/69	-	-	1/49	3/51	9/71[b]
Thyroid						
Follicular cyst(s)	2/70	-	-	1/50	1/51	0/69
Cyst(s), follicular	2/70	-	-	0/50	0/51	0/69
Follicular dilatation	0/70	-	-	1/50	0/51	1/69
Lymph node, mesenteric						
Hyperplasia, lymphocytic	3/67	-	-	0/48	5/44	9/62[a]

[a]Statistically significantly increased over controls ($p \leq 0.05$) using Fisher's Exact test.
[b]Statistically significantly increased over controls ($p \leq 0.01$) using Fisher's Exact test.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of thiazopyr, based on increases in absolute and relative liver weights in both sexes, hepatocellular hypertrophy and vacuolation, increased amyloid deposition and nephropathy (males) and lymphocytic hyperplasia in females. In addition, there was supporting clinical chemistry in the liver.

E. Additional Toxicology Information

1. Mutagenicity

a. Gene Mutation

1) Mutagenicity of Thiazopyr in *Salmonella typhimurium*

Reference: Bakke, J. P. (1989) Ames/Salmonella Mutagenicity Assay with MON 13200: Study No. ML-88-191/EHL No. 88124. Testing Facility: Monsanto's Environmental Health Lab (EHL), St. Louis, MO; Submitted by Monsanto, St. Louis, MO. MRID No. 42275535.

Thiazopyr was tested for potential to induce reverse mutations in the Salmonella typhimurium strains TA 98, TA 100, TA 1535 or TA 1537; both with and without metabolic activation (S9 mix) at dose levels up to 10,000 µg/plate. The results were negative. The study was considered to be acceptable for regulatory purposes.

2) Mutagenicity of Thiazopyr in Chinese Hamster Ovary Cells (CHO) at the HGPRT Locus Both With and Without Metabolic Activation

Reference: Li, A. P., Myers, C. A. (1989) CHO/HGPRST Gene Mutation Assay with MON 13200: Study No. ML-88-382/EHL No. 88071. Testing Facility: Monsanto's Environmental Health Lab (EHL), St. Louis, MO; Submitted by Monsanto, St. Louis, MO. MRID No. 42275536.

Thiazopyr was tested for potential to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells, either in the presence of or in the absence of metabolic activation at dose levels up to 1000 µg/ml. The following positive controls were run concurrently: ethylmethanesulfonate for the non-activated series and benzo(a)pyrene for the activated series. The results were negative. The study was originally considered to be acceptable for regulatory purposes, however, the CPRC considers this study to be unacceptable because the chemical was not tested at a high enough concentration (no signs of cytotoxicity or solubility problems).

b. Structural Chromosomal Aberration

Induction of Micronuclei In Vivo in Bone Marrow Cells of Mice Treated With Thiazopyr

Reference: L. J. Flowers (1990) Micronucleus Assay with MON 13200: ML-88-390/EHL Study No. 88230. Testing Facility: Monsanto's Environmental Health Lab (EHL), St. Louis, MO; Submitted by Monsanto, St. Louis, MO. MRID No. 42275537.

Thiazopyr was tested for potential to induce chromosomal aberrations in a mouse bone marrow assay. The mice were administered single i.p. doses of the test material at 80, 400 or 800 mg/kg (7%, 35%, 70% of the LD_{50}). They were then sacrificed 24, 48 and 72 hours later and the femoral bone marrow was prepared by conventional smear technique on microscope slides, air-dried overnight, and stained using the Wright-Giemsa Stain Pak. The slides were first coded, then scored for micronuclei in 1000 polychromatic erythrocytes (PCE) per experimental point. The positive control was cyclophosphamide. The results were negative under the conditions of the study. The study was considered to be acceptable for regulatory purposes.

c. Other Genotoxic Effects

Unscheduled DNA Synthesis in Rat Hepatocytes That Have Been Treated In Vitro With Thiazopyr

Reference: J. P. Bakke (1989) Evaluation of MON 13200 to Induce Unscheduled DNA Synthesis in the In Vitro Hepatocyte DNA Repair Assay in the Male F-344 Rat: Study No. SR-88-204/SR1 No. LSC 6327. Testing Facility: SRI International, Menlo Park, CA; Submitted by Monsanto, St. Louis, MO. MRID No. 42275538.

Thiazopyr was tested for induction of unscheduled DNA systhesis in rat hepatocytes treated in vitro up to the solubility limit dose of 3000 µg/ml. Primary hepatocyte cultures from male F-344 rats were simultaneously exposed for 19 hours for both tritiated thymidine and either solvent (1% acetone) or graded concentrations of the test material (5 - 3000 µg/ml). The slides were prepared from the cells, dipped in photographic emulsion and stored in light-tight boxes at -20°C for 7 days. They were then developed and stained with 1% methyl green-pyronin. 2-Acetylaminofluorene was used as a positive control. MON 13200 induced neither cytotoxicity nor increased nuclear grain counts over the control values. The positive control induced a strong positive response. The study was considered to be acceptable for regulatory purposes.

2. Metabolism

A metabolism study was conducted in rats in which thiazopyr was administered as: 1) a single low oral dose (1 mg/kg), 2) a single high dose (oral) (100 mg/kg), 3) a single low i.v. dose (1 mg/kg) or 4) multiple low dose (oral) (14 daily unlabeled 1 mg/kg doses, followed by 1 radiolabeled (oral) dose at 1 mg/kg). MON 13200 with ^{14}C at the 4 position of the pyridine ring and ^{14}C at the 4' and 5' positions of the thiazole ring was tested. The apparent rate of absorption was less for the thiazoline labeled MON and the apparent plasma half-life of the thiazoline labeled material was 5-fold that of the pyridine labeled molecule. The absorption of an orally administered dose was ≥ 90% for both test materials. The overall radiolabel recovery for all study groups was 88.9 ± 0.6%. No significant sex-related differences were observed in the total percent recovery. However, the distribution of recovery was sex-related. There was little radiolabel detected in the tissues at study termination. Preferential sites for localization of the radiolabel included liver, adipose tissue, muscle and bone. Ten metabolites of MON 13200 were found in the excreta, each constituting ≥ 5% of the dose for either sex. The metabolic pathway is essentially an oxidative pathway. Vulnerable sites of the molecule are the thiazoline ring, the isobutyric side chain and the pyridine rings. The test material appears to be rapidly and extensively eliminated with low amounts of residues remaining in the tissues and carcass. However, the percentage of radiolabel remaining in the carcasses following thiazoline labeled MON 13200 was between 6.9 - 10.8%. The study is classified as supplementary until the Registrant provides a satisfactory explanation of the high levels of residual redioactivity in the tissues following the thiazoline labeling portion of the study.

3. Special Thyroid Function Studies

a. Effect of Dietary Exposure of MON 13200 on Serum Levels of T_3, T_4, and TSH, Hepatic Drug Metabolizing Activity and Thyroid Pathology in Male Sprague Dawley Rats

Thiazopyr was tested in a special thyroid function study to investigate the subchronic effects on hormone levels and other biochemical endpoints. The chemical was administered through the diet at either 0 or 3000 ppm and the animals were sacrificed at 7, 14, 28, 56 or 90 days. Statistically significant decreases in body weight gain were observed at 90 days (89% of controls). Starting fairly early on, increases in TSH (ranging from 133% to 200% of controls at scheduled sacrifice times, $p < 0.05$) and decreases in T_4 (ranging from 43% to 76% of controls at scheduled sacrifice times, $p < 0.05$) were observed. In addition, there were increases in liver (137% - 186% of control for absolute and 134% - 197% of control for relative at scheduled sacrifice times, $p < 0.05$) and thyroid weights (124% - 154% of control for absolute and 123% -

160% of control for relative at scheduled sacrifice times, $p < 0.05$) and increases in thyroid follicular cell hypertrophy/hyperplasia. Reverse T_3 was increased at 28 days (only timepoint measured, 147% of control value, $p < 0.05$). T_3 was either not affected or increased.

There were indications of increases in hepatic UDPGT activity (129% - 279% of control for nmol/mg protein/min, $p < 0.05$ at 7, 56 and 90 days; or 102% - 269% of control for μmol/liver, $p < 0.05$ at 56 and 90 days) and significant increases in T_4 UDPGT activity (161% - 264% of control for pmol/mg protein/min, $p < 0.05$ at all times except 90 days; or 285% - 420% of control for nmol/liver, $p < 0.05$ at all time points). Hepatic 5'-monodeiodinase activity was either not affected or decreased. The effects observed in this study are supportive of the theory that MON 13200 may induce thyroid tumors through a disruption in the thyroid-pituitary hormonal feedback mechanisms.

b. <u>A Dose Response and Reversibility Study of Effects of MON 13200 on Biochemical Mechanisms of Thyroid Toxicity in Sprague Dawley Rats</u>

In a second special thyroid function study, groups of twenty 12-week-old male Sprague Dawley Crl:CD SD(BR) strain rats were given diets containing 0 (3 groups), 10, 30, 100, 300, 1000 or 3000 (3 groups) ppm thiazopyr for 56 days. The dose levels are equivalent to 0, 0.5, 1.5, 5, 15, 50 or 150 mg/kg/day, respectively (1 ppm = 0.05 mg/kg/day). Two experiments were conducted with these animals. The first was designed to investigate dose response effects of the test substance on body, liver and thyroid weight, T_4 uridine diphosphate glucuronyl transferase (UDPGT) activity in liver tissue, serum concentrations of thyroxine (T_4), triiodothyroxines (T_3), reverse triiodothyroxine (rT_3) and thyroid stimulating hormone (TSH), and thyroid histology. In the second experiment, two groups receiving the 3000 ppm diet (150 mg/kg/day) were maintained on a control diet following the 56 day treatment period for 56 or 112 days before they were sacrificed along with a group of control animals and observed for reversibility of effects on the parameters observed in the first experiment.

In the dose-response study, liver weights were significantly increased at 300, 1000 and 3000 ppm (14, 34 and 82% above controls, respectively). Thyroid weights were increased by 25 and 46% over control values for the 1000 and 3000 ppm dose groups, respectively ($p < 0.05$). There were no significant effects of the test substance on body weight or body weight gain during the study. The T_4 UDPGT levels were increased by 117 and 376% above controls at the 1000 and 3000 ppm dose levels. The highest dose level (3000 ppm) was associated with increased T_3, TSH, and rT_3 serum concentrations (30, 57 and 51%, respectively, $p < 0.05$), an increased incidence of follicular cell hypertrophy/hyperplasia (7/20 at 3000 ppm vs. 1/20 in the control group), and significantly

decreased T_4 (28% less than controls, $p < 0.05$). Based on the liver weight increases, the NOEL and LOEL in this study are 100 and 300 ppm respectively. Thyroid weight was the only one of the above mentioned parameters that did not return to values comparable to controls. After 56 days on the 3000 ppm diet, thyroid weights were 242% of control values. At the end of the 56 and 112 day recovery periods the thyroid weights were 120 and 123% of control values.

c. Effect of Dietary Exposure of MON 13200 on Biochemical Mechanisms Involved in the Disposition of T_4

In a third special thyroid function study, groups of 5-10 twelve-week-old male Sprague Dawley Crl:CD SD(BR) strain rats were given diets containing 0 (4 groups) or 3000 (4 groups) ppm thiazopyr for 56 days. The dose level is equivalent to 150 mg/kg/day (1 ppm = 0.05 mg/kg/day). A series of four experiments was conducted to evaluate the test substance's effect on blood concentration halflife of T_4, biliary excretion rate of T_4, thyroid gland iodine uptake, and hepatic enzyme activity including T_4 UDPGT, deiodinase, aryl hydrocarbon dehydrase, ethoxy O-dehydrase, and cytochrome P-450.

The 3000 ppm dose level of thiazopyr increased the clearance rate from the blood (approximately twice as fast over 72 hours, $p < 0.05$) and rate of accumulation in the bile (at 4 hours, adjusted % of dose/hour, approximately 1.4 times as fast, $p < 0.05$). The test substance was also associated with increases in T_4 UDPGT activity ($p < 0.05$) and total deiodinase activity in the liver ($p < 0.05$ for μg T_3/mg protein/30 minutes). A two-fold increase in mixed function oxidase enzyme activity was also associated with administration of 3000 ppm thiazopyr in the diet of rats for 56 days ($p \leq 0.01$ for all parameters). These results, along with those of the previously summarized studies suggest that increased glucuronidation, deiodination of T_4 to T_3, and increased rate of clearance of T_4 from the blood and excretion of the hormone and its metabolites in the bile could significantly reduce the level of circulating T_4 in the male rat.

4. Subchronic and Chronic Toxicity

In a 21-day dermal study in rats, thiazopyr was tested at the following dose levels: 0, 100, 500 or 1000 mg/kg/day. The NOEL was 100 mg/kg/day and the LEL was 500 mg/kg/day based on the following: 500 mg/kg/day and above: increases in mean absolute kidney weights (♀) and minimal multifocal or periportal hepatocyte vacuolation (♀); 1000 mg/kg/day: increases in mean absolute and relative liver weights (♂+♀) and in mean relative kidney weights (♀). The study was graded Core Guideline.

In a subchronic feeding study in rats, thiazopyr was tested at the following dose levels: 0, 1, 10, 100, 1000 or 3000 ppm; 0, 0.07, 0.67, 6.6, 68 or 201 mg/kg/day (M); 0, 0.08, 0.79, 8, 79 or 227

mg/kg/day (F). The NOEL was 100 ppm (6.6 mg/kg/day in males) and the LEL was 1000 ppm (68 mg/kg/day in males) based on an increase in liver, thyroid and kidney weights, changes in clinical chemistry and hematological parameters, hepatocellular hypertrophy and microfollicular goiter (diffuse thyroid follicular cell hypertrophy/hyperplasia) at 1000 and 3000 ppm. The study was graded Core Guideline.

In a subchronic feeding study in dogs, thiazopyr was tested at the following dose levels: 0, 10, 100, 1000 or 5000 ppm (0, 3, 6, 35 or 175 mg/kg/day - ♂; 0, 2, 3, 35 or 160 mg/kg/day - ♀). The NOEL was 10 ppm and the LEL was 100 ppm based on decreased body weight gain and increased SGPT levels at 100 ppm and above; decreased total protein and albumin concentration and albumin/globulin ratio, increased AP, hepatocytic hypertrophy, oval cell proliferation and increased hepatocytic fatty content at 1000 ppm and above; and decreased calcium concentration which is thought to be related to the hypoalbuminemia, decreased cholesterol and triglyceride concentrations, slightly increased GGT and ALAT, follicular hyperplasia of thyroid, increased colloid content in follicles and increased relative thyroid weight at 5000 ppm. The study was graded Core Guideline.

In a 1-year feeding study in dogs, thiazopyr was tested at the following dose levels: 0, 20, 200 or 2000 ppm (0, 0.8, 7.8 or 86 mg/kg/day for males and 0, 0.8, 8.8 or 78 mg/kg/day for females). The NOEL was 20 ppm and the LOEL was 200 ppm based upon hepatocellular hypertrophy/hyperplasia, which was observed at both 200 and 2000 ppm. In addition, an increase of approximately 10% in prothrombin time was observed at 2000 ppm with both sexes, as well as increased SGOT, SGPT, GGT and ALK and decreases in cholesterol, albumin, total protein and calcium levels. An increase in absolute and relative liver weights were also observed at 2000 ppm. Enlargement and/or discoloration in some of the high dose animals provided additional evidence of hepatotoxicity. The study was graded Core Guideline.

Thiazopyr was tested in a reproduction study in rats at the following dose levels: 0, 10, 100 or 1000 ppm (0, 0.5, 5 or 50 mg/kg/day). The parental/systemic NOEL was 10 ppm and the LEL was 100 ppm based on the following effects: at 100 ppm and above: centrilobular hepatocellular hypertrophy (♂); and at 1000 ppm: centrilobular hepatocellular hypertrophy (♀), centrilobular hepatocellular vacuolation (♂), periportal hepatocellular vacuolation (♀), hepatocellular degeneration/necrosis (♀); increased absolute and relative liver weights (♂+♀) and discoloration and enlarged livers (♂). The thyroid was not examined. The study was graded Core Guideline.

5. Structure-Activity Relationships

No chemicals from a closely related chemical class which had been tested for potential carcinogenicity could be located for structure-activity comparison purposes. In addition, thiazopyr does not appear to have a structure which would have much electrophilic activity.

F. Weight of Evidence Considerations

The following facts regarding the toxicology data on thiazopyr may be of importance in a weight-of-the-evidence determination of carcinogenic potential.

1. Thiazopyr, when administered in the diet to Sprague-Dawley CD male rats at 0, 1, 10, 100 1000 or 3000 ppm (0, 0.04, 0.4, 4.4, 44.2 or 136.4 mg/kg/day) in the diet for 24 months was associated with a statistically significant increased incidence of thyroid follicular cell adenomas/cystadenomas and combined adenoma/carcinomas by pair-wise comparisons of controls with both the mid- and high dose levels. There was also a statistically significant positive trend for thyroid adenomas/cystadenomas and combined adenomas/carcinomas in males. The carcinomas alone did not show a significantly increased incidence by pair-wise comparison with controls, however, there was a statistically significant positive trend for carcinomas alone.

2. In the chronic/carcinogenicity feeding study in rats, a numerical increase in renal tubular adenomas was observed in females at the high dose which although not statistically significant in a pairwise comparison with the control group, exceeded the historical control rate. There was a statistically significant positive trend. In addition, renal tubular adenomas were observed in males in the 10, 100 and 1000 ppm dose groups, but not at the high dose. Although the incidences of these tumors were not dose-related in males, since they are considered rare (historical rate < 1%), they were considered to be biologically significant.

3. There were no apparent tumor increases in either male or female CD-1 albino mice when thiazopyr was fed in the diet for 18 months for up to 800 ppm (128.4 mg/kg/day for males or 215.9 mg/kg/day for females).

4. In the chronic/carcinogenicity feeding study in rats, statistically significant increases in the incidences of hyperplasia of the follicular epithelium of the thyroid in high dose males and thyroid follicular hypertrophy/hyperplasia in high dose males and in mid- and high dose females were observed. There were also statistically significant positive trends for both hyperplasia and for hypertrophy/hyperplasia of the thyroid follicular epithelium in both sexes. Thyroid follicular cell hyperplasia was not observed in the carcinogenicity study in the mouse. It was observed in both sexes of the high dose group in the subchronic dog study (5000 ppm, 125 mg/kg/day) but not in the chronic dog study (tested up to 2000 ppm, 50 mg/kg/day).

5. In the chronic/carcinogenicity feeding study in rats, statistically significant increases in hepatocellular hypertrophy were observed in both sexes at the two highest dose levels: 1000 or 3000 ppm (44.2 or 136.4 mg/kg/day for males and 56.3 or 177.1 mg/kg/day for females). Hepatocellular hypertrophy was also observed with the three highest doses in male mice and with the top two doses in female mice. In addition, hepatocellular hypertrophy was observed at dose levels of 200 ppm (7.8 mg/kg/day) and above in male and female dogs (chronic study), at dose levels of 1000 (25 mg/kg/day) and 5000 ppm (125 mg/kg/day) in male and female dogs (subchronic study) and at dose levels of 100 ppm (5 mg/kg/day) and above in males and 1000 ppm (50 mg/kg/day) in females in the rat reproduction study.

6. In the chronic/carcinogenicity feeding study in rats, there were increases in kidney weights for both sexes, increases in the incidences and/or severity of renal nephropathy at the mid- and high doses in both sexes and a renal tubular hyperplasia in 1 female at the high dose. In both the subchronic feeding study in rats and the 21-day dermal study in rats, there were also increases in kidney weights in either one or both sexes. In the carcinogenicity study in mice, increases in kidney weights were observed in high dose females. In addition, there were increased incidences of nephropathy in high dose male mice ($p \leq 0.05$).

7. In three special thyroid function studies conducted in the rat, thiazopyr was administered to male rats at dose levels up to 3000 ppm. The rats were sacrificed over a time range from 7 to 112 days. One of the studies examined possible reversibility of effects after a recovery period. In these studies, the following effects were observed: increases in thyroid weight and the incidences of thyroid follicular cell hypertrophy/hyperplasia; increases in serum TSH, T_3 and reverse T_3 levels; decreases in serum T_4 levels; increases in liver weights and hepatic T_4 UDPGT activity; increased clearance rate of T_4 from the blood and rate of accumulation in the bile; increase in total hepatic 5'-monodeiodinase activity and a two-fold increase in microsomal mixed function oxidase enzymatic activity (aryl hydrocarbon dehydrase, ethoxy o-dehydrase, and cytochrome P-450). The increases in liver weights, in hepatic T_4 UDPGT activity, in serum T_3, rT_3, and TSH concentrations and in the thyroid follicular cell hypertrophy/hyperplasia and the decreases in serum T_4 were all shown to be reversible or nearly reversible upon removal of thiazopyr in the diet.

8. The genotoxicity studies conducted with thiazopyr did not indicate genotoxic activity. Thiazopyr was tested in the following studies: a reverse mutation study in *Salmonella typhimurium*, a forward mutation study in chinese hamster ovary cells (CHO) at the HGPRT locus, an in vivo micronucleus test in bone marrow cells of mice and an in vitro unscheduled DNA synthesis study in rat

hepatocytes. The CHO study was not considered to have been tested at a sufficiently high dose level for an adequate negative study.

9. Thiazopyr does not appear to have a structure which would have much electrophilic activity. There was no indication of any structure activity relationships as it relates to carcinogenic activity.

10. **Consideration of the Use of the Threshold Model for Thiazopyr**

When evaluating thiazopyr, the Committee considered the possibility of using the threshold model for thyroid neoplasms. The following discussion has been taken from the Amitrole Peer Review Document and revised for thiazopyr.

The following guidance is given in the Agency's DRAFT Policy Document (Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988):

"Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (eg., iodine deficiency) have demonstrated the significance of long-term thyroid-pituitary hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia. Hyperplasia and sometimes neoplasia of the pituitary may also be seen.. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations ..... the Agency concludes that:

a. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels:

b. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and

c. models that assume thresholds may be used to assess the risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance."

Two basic questions must be addressed before this policy is applied.

"The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second

question concerns the procedures to be employed in estimating the risks of these agents."

"The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assignation is based upon knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response."

Determination of whether neoplasms are due to thyroid-pituitary imbalance

The document goes on to describe the 3 factors which should be considered in making this determination (answering the first question, or "qualitative issue"). These are addressed as they apply to thiazopyr as follows:

FACTOR I. Consideration of whether the thyroid tumors associated with administration of thiazopyr can be attributed to disruption of the thyroid-pituitary hormonal balance. (In addressing this factor, the Policy states, 6 indicators should be considered.)

a. Goitrogenic activity in vivo:

Thyroid follicular cell hyperplasia and/or hypertrophy were observed in high dose males and in mid- and high dose females in the chronic rat study. These effects were also observed in the 90-day feeding studies in both rats and dogs and in the special thyroid function studies in rats (56 and 90 days).

b. Clinical chemistry changes (eg., reduced thyroid hormone and increased TSH serum concentrations):

In the special thyroid function studies in rats, thiazopyr induced significant increases in TSH and decreases in T_4. Reverse T_3 was increased at 28 days. In one study, T_3 was either not affected or increased after 90 days but in the other study, T_3 was increased at 56 days.

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

The following effects were observed in the thyroid function studies: increases in hepatic T_4 UDPGT activity, total hepatic 5'-monodeiodinase activity, microsomal mixed function oxidase enzymatic activity (aryl hydrocarbon dehydrase, ethoxy o-dehydrase, and cytochrome P-450), in the clearance rate of T_4 from the blood and in the rate of accumulation of T_4 in the bile.

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

There was evidence of progression (hypertrophy/hyperplasia to neoplasia) in rats. In the short term studies, thyroid hypertrophy/hyperplasia was evident as early as 56 days. In the two-year rat study, the first thyroid hypertrophy was observed in males at week 27 and the first hyperplasia was observed at week 52. Thyroid follicular cell adenomas were first observed in males at week 69. The first carcinoma was observed in the control group at week 81. Significant increases in thyroid follicular cell tumors were evident in males by the end of the study.

e. Reversibility of effects after exposure is terminated:

In the second special thyroid function study, with the exception of thyroid weight, all of the following effects were shown to be reversible or nearly reversible upon removal of thiazopyr in the diet: increased liver and thyroid weights, hepatic T_4 UDPGT activity, serum T_3 and TSH concentrations and thyroid follicular cell hypertrophy/hyperplasia. In addition, the decrease in serum T_4 levels were reversed.

f. SAR to other thyroid tumorigens: No evidence has been found.

Based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of thiazopyr may be due to a disruption in the thyroid-pituitary status.

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

The genotoxicity data are negative. There is no indication that genotoxicity plays a role in the tumorigenic activity for this chemical. Although the gene mutation study in mammalian cells is inadequate, a new study is not required because the regulatory requirements are satisfied by the other adequate studies.

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

There were no statistically significant increases in any other tumor types in either rats or mice for this chemical. However, there was a numerical increase in renal tubular adenomas in high dose females in the chronic rat feeding study. This increase exceeded the historical control range, and there is a statistically significant positive trend. In addition, these tumors also appeared in three of the treated groups in males, although not at the high dose. These tumors are considered to be rare. These data were supported by nonneoplastic evidence that the kidney is a

target organ: increases in kidney weights for one or both sexes in the chronic and subchronic rat feeding studies, the 21-day dermal study in rats and in the carcinogenicity study in mice; increases in the incidences and/or severity of renal nephropathy at the mid- and high doses in both sexes of rats and in high dose male mice in the oncogenicity studies and renal tubular hyperplasia in 1 female at the high dose in the chronic rat feeding study.

Conclusions: As indicated above, based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of thiazopyr may be due to a disruption in the thyroid-pituitary status. Adding in Factors II and III, this conclusion still stands. All of the criteria for a threshold effect have been met except one: the presence of a second tumor type. The kidney tumors were not statistically significantly increased in a pairwise comparison with the control group. However, since these tumors are rare, they are considered to be biologically significant in both sexes for this chemical. They are therefore considered as a confirmation of the carcinogenic potential and a Group C classification for this chemical.

11. Factors to be Considered in Determining Method to be Used in Estimating the Risks of Thiazopyr

Again, this guidance was taken from the Amitrole Peer Review Document and revised for thiazopyr. The Committee was requested to consider these points when determining which method is to be used for estimating the carcinogenic risk for thiazopyr.

Guidance given in the EPA DRAFT policy for proceeding with the quantitation of risk is as follows:

a. "Threshold considerations should be applied in dose-response assessments for those chemical substances where (1) only thyroid tumors (and relevant pituitary tumors) have been produced; (2) the tumors can be attributed to a disruption in thyroid-pituitary hormonal homeostasis; and (3) potential mechanisms other than thyroid-pituitary imbalance (eg., genotoxicity) can be disregarded.

b. Special attention should be given to chemicals (1) that have induced thyroid tumors (and relevant pituitary tumors) that may be due to thyroid-pituitary imbalance, and (2) where there is also evidence of either a genotoxic potential or the induction of neoplasms at sites other than the thyroid (or pituitary). Generally, those cases will be approached using various principles laid out in the EPA Guidelines for Carcinogen Risk Assessment. A strong rationale must be articulated for handling these agents otherwise.

c. For those chemicals producing thyroid tumors that do not seem to be acting via thyroid-pituitary hormonal inhibition, dose-response assessments will be performed in accordance with the EPA Guidelines for Carcinogen Risk Assessment."[3]

[3]It is noted that a new policy document is in process which currently states these phrases differently: 1. "Threshold considerations will be incorporated into thyroid (and relevant pituitary) cancer dose-response assessments for chemicals that (a) cause disruption of thyroid-pituitary homeostasis and (b) are judged not to have genotoxic activity relevant to carcinogenicity. Dose-response relationships for neoplasms other than the thyroid (or pituitary) should be evaluated using mechanistic information bearing on their induction and various principles laid out in the Agency's cancer risk assessment guidelines. 2. Threshold considerations may be applied in thyroid cancer dose-response assessments on a case-by-case basis for chemicals that (a) produce thyroid-pituitary imbalance and (b) are judged to have genotoxic activity related to carcinogenicity. The implications of the genotoxic events to the thyroid carcinogenic responses need to be carefully evaluated. In some cases thyroid cancer dose-response relationships may be characterized in more than one way. 3. Threshold considerations will not be applied in thyroid cancer dose-response assessments for substances operating through mechanisms not involving thyroid-pituitary imbalance. However, case-by-case exceptions may arise, based on mode of action data."

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Carcinogenicity in animals -- Thiazopyr

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to thiazopyr resulted in an increased incidence of thyroid follicular cell tumors (adenomas and combined adenoma/carcinomas) in male rats and renal tubular adenomas in both male and female rats. The relevance of the tumor data to an evaluation of thiazopyr's potential for human carcinogenicity is discussed elsewhere in this report.

G. Classification of Carcinogenic Potential

The CPRC considered the criteria contained in the EPA's "Guidelines for Carcinogenic Risk Assessment" (FR51: 33992-34003, 1986) for classifying the weight of evidence for the carcinogenicity of thiazopyr.

The consensus of the Committee was that there was limited evidence for carcinogenicity and that thiazopyr may be classified as a Group C - possible human carcinogen, and that for the purpose of risk characterization the M.O.E. approach should be used for quantification of human risk.

The decision to classify thiazopyr as a Group C carcinogen was supported by statistically significant increases in thyroid adenomas and combined adenoma/carcinomas in male rats at 1000 ppm ($p<0.05$) and 3000 ppm ($p<0.01$), with statistically significant increasing trends for adenomas, carcinomas and combined adenoma/carcinomas (all at $p<0.01$). There were also numerical increases in kidney adenomas in both sexes. Although these were not statistically significant by pairwise comparison, the CPRC considered them to be biologically significant, since they are rare tumors in both sexes. However, due to the low response in both males and females and the nature of the response in males, the CPRC did not believe the appearance of the kidney tumors constituted a level of high concern although they are supportive of an overall concern for carcinogenicity.

There were no apparent tumor increases in either sex in a mouse study at dose levels up to 800 ppm.

The CPRC also considered the possibility of using the threshold model for thyroid neoplasms based on the Agency's DRAFT Policy Document, "Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988."

Based on the evidence of thyroid growth (increases in thyroid weight and size and follicular hypertrophy/hyperplasia), hormonal changes and the evidence of the site of action provided in the special studies in the rat, **the CPRC concluded that there appeared to be sufficient evidence for relating the thyroid tumors in the male rat to a disruption of the thyroid-pituitary status** (a full discussion of this analysis is found in the body of the document - Section F, number 12). **However, the presence of kidney tumors in both sexes of the rat, *in addition to* the thyroid tumors was also considered.**

Therefore, based on the evidence that thiazopyr appears to induce thyroid tumors through a disruption in the thyroid-pituitary status, and thus may have a threshold for tumor development, **the Committee recommended that a Margin of Exposure (M.O.E.) approach**

be used for quantitating carcinogenic risk. This decision was supported by the weight of the evidence, considering the neoplastic, related nonneoplastic and/or hormonal effects in the male rat thyroid and liver.

The selection of a NOEL for the M.O.E. approach utilizes only those biological endpoints which are related to tumor development. Therefore, when selecting the most appropriate NOEL and LOEL to use for the carcinogenic risk assessment of thiazopyr, the following endpoints were considered by the CPRC:

Two-year rat study:

- Thyroid tumors
- Thyroid hypertrophy/hyperplasia
- Increase in thyroid weights
- Hepatocellular hypertrophy
- Increase in liver weights

Thyroid special studies in the rat:

- Increase in thyroid weights
- Increase in liver weights
- Decrease in T_4
- Increase in TSH
- Increase in T_3
- Thyroid hypertrophy/hyperplasia
- Increase in reverse T_3
- Increase in T_4 UDPGT activity

All of the endpoints above that were observed either in the thyroid or liver were considered to be directly related to the thyroid neoplastic response in rats. Therefore, in light of the definition given above, all of these endpoints were considered to be appropriate for use in the selection of a NOEL and a LOEL for the carcinogenicity risk assessment on thiazopyr utilizing a M.O.E. approach.

In addition, the same endpoints were examined in other species (with the exception of the tumors which were only observed in the rat). The following endpoints were observed in the chronic feeding study in the dog and in the 18-month carcinogenicity study in the mouse:

1-year dog study:

- Hepatocellular hypertrophy (endpoint upon which RfD is based)
- Increase in liver weights

18-month carcinogenicity study in the mouse:

- o Increase in liver weights
- o Hepatocellular hypertrophy

The following table summarizes the studies, endpoints, NOEL's and LOEL's considered for CPRC's decision. **For the M.O.E. calculation, the Committee selected the NOEL and LOEL which represented the majority of the observations. These were 4.4 mg/kg/day for the NOEL and 44.2 mg/kg/day for the LOEL.** Only two endpoints had NOEL's lower than 4.4 mg/kg/day. These were hepatocellular hypertrophy in the mouse and dog. The hepatocellular hypertrophy in the dog is the endpoint on which the RfD is based.

Kidney tumors were observed in males at 0.4 and 4.4 mg/kg/day. These were not included in the consideration for the M.O.E. calculation for the following reasons: the Committee stated that with regard to the kidney tumors in the male rats at 0.4 and 4.4 mg/kg/day, they did not know how much importance to place on them because it was difficult to judge how critical the evidence was for this effect. The incidences of the tumors were not statistically significantly increased over controls (although there was a statistically significant positive trend in female rats but not in males) and there were no tumors at the high dose level. There were no indications that thiazopyr was tested at an exceedingly high level at the high dose. In addition, the nonneoplastic kidney pathology consisted of renal nephropathy and increases in kidney weights in both rats and mice. Renal tubular hyperplasia was also observed in 1 high dose female rat. Although the kidney pathology indicated that the kidney was a target organ, it was not necessarily associated with the kidney tumor response. **Therefore, the findings of renal tubular adenomas in male rats at dose levels of 0.4 and 4.4 mg/kg/day were considered in the weight of evidence for classification of carcinogenic potential but were not considered for the M.O.E. calculation.**

For comparison purposes, the NOEL selected for calculation of the RfD for thiazopyr is 0.8 mg/kg/day, based on hepatocellular hypertrophy observed in the chronic feeding study in the dog (see HED RfD decision document). The selection of a NOEL for the RfD is based on all toxicological endpoints except for neoplastic endpoints. The RfD is calculated from the NOEL selected from the most sensitive toxicological endpoint from the most susceptible species.

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Factors Considered for Determining NOEL for Margin of Exposure for Thiazopyr			
Study	Endpoint	NOEL (mg/kg/day)	LOEL (mg/kg/day)
2-Year Rat	thyroid tumors thyroid hypertrophy/hyperplasia ↑ thyroid weights hepatocellular hypertrophy ↑ liver weights	4.4 (♂), 177.1 (♀) 44.2 (♂), 5.6 (♀) 4.4 (♂), 5.6 (♀) 4.4 (♂), 5.6 (♀) 4.4 (♂), 5.6 (♀)	44.2 (♂) 136.4 (♂), 56.3 (♀) 44.2 (♂), 56.3 (♀) 44.2 (♂), 56.3 (♀) 44.2 (♂), 56.3 (♀)
18-Mo. Mouse	↑ liver weights hepatocellular hypertrophy	16.9 (♂), 26.8 (♀) 1.6 (♂), 26.8 (♀)	66.3 (♂), 108.1 (♀) 16.9 (♂), 108.1 (♀)
1-Year Dog	liver hypertrophy/hyperplasia ↑ liver weights	0.8 (♂+♀) 7.8 (♂), 8.8 (♀)	7.8 (♂), 8.8 (♀) 86 (♂), 78 (♀)
Thyroid Special Studies	↑ thyroid weights ↑ liver weights ↓ T_4 ↑ TSH ↑ T_3 thyroid hypertrophy/hyperplasia ↑rT_3 ↑ T_4 UDPGT activity	15 (♂) 5 (♂) 50 (♂) 50 (♂) 50 (♂) 50 (♂) 50 (♂) 15 (♂)	50 (♂) 15 (♂) 150 (♂) 150 (♂) 150 (♂) 150 (♂) 150 (♂) 50 (♂)