CASWELL 6

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

012044

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

SUBJECT: 129100. Monoacid Degradate of Thiazopyr: Review of 4-Week Feeding Study in Rats

PC Code 129100
DP Barcode D224972
Case No. 008262
MRID No. 43946203

Tox. Chem. No. 849AA
Reregistration Case No. N/A
ID No. 000707-ELN

TO: Steve Robbins, Chemical Manager
Risk Characterization
and Analysis Branch
Health Effects Division (7509C)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 8/22/96
Section I, Toxicology Branch I
Health Effects Division (7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner 8/30/96 9/5/96
Section I, Toxicology Branch I
Health Effects Division (7509C)

Background and Request:

Rohm and Haas Company submitted a 4-week feeding study in the rat on the monoacid degradate of thiazopyr that occurs in leaching studies and may be in ground water. The purpose of the study is to prove that the monoacid is relatively non-toxic. The Toxicology Branch (TB-I) has been asked to review the study and comment.

Toxicology Branch Response:

TB-I has reviewed the study and has determined that although it is not a guideline study, it is acceptable for regulatory purposes. The study is classified as acceptable non-guideline. It appears that the major target organs for the monoacid are similar to those of the parent compound, thiazopyr: the liver and thyroid. Hepatocellular hypertrophy was observed in both sexes as well as hepatocellular vacuolization in females. Increases in thyroid weights were also observed in males. With the parent

compound, there was hepatocellular hypertrophy and microfollicular goiter after a 90-day oral exposure in rats, starting at a dose level of 1000 ppm. The thyroid changes were always of minimal or mild severity. Therefore, it is not surprising that microscopic changes in the thyroid were not observed in the study with the monoacid. Microscopic changes with the thyroid would probably be observed with a longer exposure period, although they would probably be minimal as with the parent compound. The NOEL for the study on the monoacid is set at 1000 ppm and the LEL is set at 5000 ppm, based on hepatocellular hypertrophy, increases in liver and thyroid weights and slight increases in alkaline phosphatase levels.

In response to whether or not the monoacid is considered to be relatively non-toxic, it depends upon the exposure. If the monoacid acts in a similar manner as the parent compound, based on information on thiazopyr itself, the data suggest that the chemical affects the liver by inducing an increase in glucuronidation and deiodination of T_4 to T_3, an increase in the rate of clearance of T_4 from the blood and an increase in the excretion of the hormone and its metabolites in the bile. The result would be a reduction in the level of circulating T_4 in the body, thus inducing the thyroid to increase in size in order to meet the requirement for more T_4. The induction of various liver enzymes also results in hypertrophy of the liver. The process is not necessarily a toxic response in itself. However, high doses over a long period of time can induce some non-reversible effects (i.e. thyroid neoplasia).

The following paragraphs summarize the results of the study.

In a 4-week feeding study (MRID 43946203), MON 5794 (97% a.i.) was administered to 5 Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 100, 300, 1000, 5000 or 20000 ppm (0, 7.71, 22.6, 75.8, 385 or 1591 mg/kg/day in males or 0, 8.63, 25.7, 84.5, 444 or 1740 mg/kg/day in females).

At 5000 ppm, hepatocellular hypertrophy (3/5 females and 1/5 males) and hepatocyte vacuolization (1/5 females) were observed. An increase in mean relative liver weight when compared to the control group was also observed in males (115%). At 20000 ppm, hepatocellular hypertrophy (4/5 males (panlobular), 1/5 males centrilobular/midzonal), 4/5 females (centrilobular/midzonal) and hepatocyte vacuolization (2/5 females) were observed. In addition, there were increases in absolute (142% (♂), 136% (♀)) and relative liver weights (137% (♂), 138% (♀)) in both sexes and a statistically significant increase in absolute thyroid weight in males (130% absolute, 125% relative). The liver changes were supported by a slight increase in alkaline phosphatase levels (139% of control value). There were also very slight but statistically significant decreases in hemoglobin and hematocrit levels (91% of controls).

The LOEL is 5000 ppm, based on hepatocellular hypertrophy, increases in liver and thyroid weights and slight increases in alkaline phosphatase levels. The NOEL is 1000 ppm.

[MONOACID DEGRADATE OF THIAZOPYR] **4-Week Oral Study (no guideline #)**

EPA Toxicologist: Pamela Hurley Pamela M Hurley, Date 8/22/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Roger Gardner Roger Gardner, Date 8/30/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [4-week feeding]-[rat]

DP BARCODE: D224972 SUBMISSION CODE: S503231
P.C. CODE: 129100 TOX. CHEM. NO.: 849AA

TEST MATERIAL (PURITY): MON 5794

SYNONYMS: Monoacid of thiazopyr

CITATION: Dudek, B. and D. Thake (1994) Four-week range finder feeding study of MON 5794 in CD® Rats (screening study). Monsanto Company, The Agricultural Group Environmental Health Laboratory (EHL) St. Louis, MO. Monsanto Study No. ML-93-376, Lab Project No. EHL 93151, December 15, 1994. MRID 43946203. Unpublished.

SPONSOR: Rohm and Haas Company, Independence Mall West, Philadelphia, PA 19105

EXECUTIVE SUMMARY:

In a 4-week feeding study (MRID 43946203), MON 5794 (97% a.i.) was administered to 5 Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 100, 300, 1000, 5000 or 20000 ppm (0, 7.71, 22.6, 75.8, 385 or 1591 mg/kg/day in males or 0, 8.63, 25.7, 84.5, 444 or 1740 mg/kg/day in females).

At 5000 ppm, hepatocellular hypertrophy (3/5 females and 1/5 males) and hepatocyte vacuolization (1/5 females) were observed. An increase in mean relative liver weight when compared to the control group was also observed in males (115%). At 20000 ppm, hepatocellular hypertrophy (4/5 males (panlobular), 1/5 males centrilobular/midzonal), 4/5 females (centrilobular/midzonal) and hepatocyte vacuolization (2/5 females) were observed. In addition, there were increases in absolute (142% (♂), 136% (♀)) and relative liver weights (137% (♂), 138% (♀)) in both sexes and a statistically significant increase in absolute thyroid weight in males (130% absolute, 125% relative). The liver changes were supported by a slight increase in alkaline phosphatase levels (139% of control value). There were also very slight but statistically significant decreases in hemoglobin and hematocrit levels (91% of controls).

The LOEL is 5000 ppm, based on hepatocellular hypertrophy, increases in liver and thyroid weights and slight increases in alkaline phosphatase levels. The NOEL is 1000 ppm.

This 4-week feeding study is classified as acceptable non-guideline. It does not satisfy any particular guideline requirement but provides the information for which it was intended.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: MON 5794
   Description: white powder
   Lot #: NPD-9308-5448-T
   Purity: 97% ai.
   Stability of compound: stable over a period of approximately 3 months
   CAS #: not readily available

2. Vehicle and/or positive control: Lot/Batch # N/A

3. Test animals: Species: male and female rats
   Strain: Sprague-Dawley (CD)
   Age and weight at study initiation: 7 weeks; 224-267 g (♂); 166-205 g (♀)
   Source: Charles River Laboratory, Raleigh, NC
   Housing: individual stainless steel cages with wire mesh bottoms suspended over paper bedding
   Diet: PMI Feeds, Inc. Certified Rodent Diet #5002 ad libitum
   Water: St. Louis public water supply ad libitum (sodium zeolite conditioned upon entering the laboratory)
   Environmental conditions: Temperature: not stated
   Humidity: not stated
   Air changes: not stated
   Photoperiod: 12 hours dark/12 hours light starting at 6:30 AM
   Acclimation period: 13 days

B. STUDY DESIGN:

1. In life dates - start: 12/20/93 end: 1/21/94

2. Animal assignment

Animals were assigned to the test groups in table 1 using computer randomization by weight.

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)	Male	Female
Control	0	0	5	5
1	100	7.71 (♂) 8.63 (♀)	5	5
2	300	22.6 (♂) 25.7 (♀)	5	5
3	1000	75.8 (♂) 84.5 (♀)	5	5
4	5000	385 (♂) 444 (♀)	5	5
5	20000	1591 (♂) 1740 (♀)	5	5

3. Diet preparation and analysis

The neat test material was mixed thoroughly with the basal diet using high speed mixers. The test diets were prepared once and dispensed weekly. It was not stated how they were stored; however stability and homogeneity analyses were conducted in such a way that it appears that they were stored frozen for a time and then stored at room temperature. Stability analyses of samples from the 100 ppm and 20000 ppm dietary levels were conducted. Samples were either stored in the freezer in closed containers for 25 days and then stored in open containers for 7 days or were kept frozen in a closed container for 32 days. Homogeneity analyses were conducted with samples taken from the top, middle and bottom of the mixer from the 100 ppm and the 20000 ppm diets. Concentration analyses were conducted for all dietary levels at the start of the study using gas chromatography with an electron capture detector.

Results - Homogeneity Analysis: The homogeneity of the dietary mixtures were determined to be adequate for the study. At 100 ppm, the means for the top, middle and bottom were 83, 81 and 80, respectively. At 20000 ppm, the means for the top, middle and bottom were 18400, 19500 and 19500, respectively. The coefficient of variation at the 100 ppm level was 8.4% and the coefficient of variation at the 20000 ppm level was 3.3%.
Stability Analysis: Stability appeared to be adequate for the study. For samples that were stored in the freezer in a closed container and then stored in an open container for 7 days at room temperature, the concentrations of the test material were 88 and 96% of the concentrations at day 0 for the 100 ppm sample and for the 20000 ppm sample, respectively. For samples that were stored in the freezer in a closed container for 32 days, the percent of the concentrations at day 0 ranged from 88-111% for the 100 ppm dietary level and from 93-99% for the 20000 ppm dietary level.
Concentration Analysis: Concentrations of the diet were adequate for the study. The following table summarizes the mean analytical concentration for dietary level.

	T1	T2	T3	T4	T5
Target Level (ppm)	100	300	1000	5000	20000
Analytical concentration (ppm)	81	289	785	4690	19100

4. Statistics - Statistical analyses were conducted using a decision-tree logic. The data were tested for normality. If the total sample is ≤ 20, the homogeneity of variances (Bartlett's) test was used. If $p \leq 0.01$ for Skewness or Kurtosis or if the homogeneity of variances test indicated that non-parametric procedures should be used, then the Kruskal-Wallis test or the Jonckheere's test was used. If $p \leq 0.05$ with either of these tests, then the Mann-Whitney test was used. If the results of the homogeneity of variances test indicated that parametric analyses should be used, then the Dunnett's test or Linear Regression was used. Categorical data were analyzed with an Uncorrected Chi-square test. Dunnett's and Mann-Whitney tests to detect group differences were performed two-tailed.

012044

The following paragraphs are a direct quote from the study: "Dunnett's Multiple Comparison Test (two-tailed): Inlife body weights, cumulative body weight changes and food consumption data"

"EHL decision-tree analyses: Hematology data, clinical chemistry data, terminal body weights, absolute organ weights and organ/body weight ratios were evaluated by decision-tree statistical analyses which, depending on the results of tests for normality and homogeneity of variances [Bartlett's Test], utilized either parametric [Dunnett's Test and Linear Regression] or nonparametric [Kruskal-Wallis, Jonckheere's and/or Mann-Whitney Tests] routines to detect differences and analyse for trend."

"Grubb's test was used to detect outliers in the organ weight data."

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity, moribundity and mortality. Detailed observations for clinical signs of toxicity were conducted weekly.

2. Body weight

Animals were weighed prior to randomization and once weekly thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly.

4. Blood was collected at termination from the posterior vena cava of CO_2-anesthetized, fasted rats for hematology and clinical analysis from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Direct bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
x	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

5. Urinalysis - not conducted

6. Sacrifice and Pathology

All animals that were sacrificed on schedule were subjected to gross pathological examination and the thyroid/parathyroid from all males and the liver from all males and females were microscopially examined. Adrenals, kidneys, liver, spleen, thyroids/parathyroids and testes were weighed and saved. The epididymides were saved but were not weighed. The eyes (not mentioned above) were fixed in 5% neutral buffered formalin/0.5 % glutaraldehyde and the remained tissues

were fixed in 10% neutral buffered formalin. Fixed tissues were stained with H & E. Histological stains for bilirubin and lipofuscin were used to identify hepatic pigment.

II. RESULTS

A. Observations :

1. Toxicity - There were no clinical signs that were considered to be related to exposure to the test material. One animal had a scab.

2. Mortality - There were no mortalities.

B. Body weight and weight gain: There were no statistically significant differences in mean body weights in the treated groups when compared to the control group. There also did not appear to be any differences in mean body weight gain in the treated groups when compared to the control groups (mean bodyweight gain data calculated by reviewer - no statistical analysis was conducted). The following table summarizes mean bodyweights at pretest and after 4 weeks of treatment.

Mean Body Weights (Pretest and 4 Weeks) (g)			
Group	Pretest	Week 4	Mean Bodyweight Change[a]
Males			
Control	249.6	432.0	182.4
100 ppm	249.0	454.6	205.6
300 ppm	250.8	444.9	194.1
1000 ppm	248.2	457.9	209.7
5000 ppm	246.0	446.6	200.6
20000 ppm	249.8	455.1	205.3

Mean Body Weights (Pretest and 4 Weeks) (g)			
Group	Pretest	Week 4	Mean Bodyweight Change[a]
Females			
Control	188.6	276.6	88.0
100 ppm	189.3	272.8	83.5
300 ppm	185.2	275.4	90.2
1000 ppm	189.8	271.4	81.6
5000 ppm	186.8	287.2	100.4
20000 ppm	186.6	274.1	87.5

[a]Calculated by reviewer from mean data.

C. Food consumption and compound intake:

1. Food consumption - Food consumption was slightly increased in males at 20000 ppm. It was statistically significantly increased ($p \leq 0.05$) only during the third week when the high dose males ate 32.8 mean grams/day and the controls ate 27.9 mean grams/day. There were no differences in the treated females when compared to the control group.

2. Compound consumption - The overall study averages for consumption of the test material were 0, 7.71, 22.6, 75.8, 385 or 1591 mg/kg/day for males and 0, 8.63, 25.7, 84.5, 444 or 1740 mg/kg/day for females.

D. Blood work:

1. Hematology - The report stated that there were slight decreases in hemoglobin and hematocrit values in high dose males. In addition, reticulocytes were decreased at all treatment levels in females; however, all values were within the historical control range. The reticulocyte data did not appear to be available in the report. The following table summarizes results of interest.

Summary of Selected Hematology Data				
Parameter	Group	Sex	Mean	% Control
Hemoglobin	Control	♂	16.22	-
	1	♂	15.68	97
	2	♂	15.42	95
	3	♂	15.52	96
	4	♂	15.40	95
	5	♂	14.70**	91
Hematocrit	Control	♂	50.64	-
	1	♂	48.90	97
	2	♂	47.16*	93
	3	♂	47.84	94
	4	♂	48.24	95
	5	♂	46.12**	91

*Statistically significant (Dunnett's, $p \leq 0.05$)
**Statistically significant (Dunnett's, $p \leq 0.01$)

2. Clinical Chemistry - In the high dose males, there were mild increases in alkaline phosphatase and creatine phosphokinase levels and a slight decrease in mean glucose levels. The following table summarizes results of interest.

Summary of Selected Clinical Chemistry Data				
Parameter	Group	Sex	Mean	% Control
Alkaline phosphatase	Control	♂	351.8	-
	1	♂	438.2	125
	2	♂	369.2	105
	3	♂	342.8	97
	4	♂	429.8	122
	5	♂	487.8*	139
Creatine phosphokinase	Control	♂	148.6	-
	1	♂	191.8	123
	2	♂	145.8	90
	3	♂	134.6	110
	4	♂	155.6	130
	5	♂	219.8	157
Creatine phosphokinase	Control	♀	109.0	-
	1	♀	134.4	123
	2	♀	98.2	90
	3	♀	119.4	110
	4	♀	142.2	130
	5	♀	171.2*	157

Summary of Selected Clinical Chemistry Data				
Parameter	Group	Sex	Mean	% Control
Glucose	Control	♂	285.6	-
	1	♂	239.8	84
	2	♂	287.2	101
	3	♂	243.6	85
	4	♂	310.0	109
	5	♂	197.0**	69

*Statistically significant (Dunnett's, $p \leq 0.05$)
**Statistically significant (Dunnett's, $p \leq 0.01$)

E. Sacrifice and Pathology:

1. Organ weight - Increases in mean absolute and relative hepatic weights were observed in high dose males and females and in males in the 5000 ppm dose group. Increased thyroid weights were observed in high dose males. The following table summarizes the results.

Summary of Mean Absolute and Relative Organ Weight Data				
Parameter	Group	Sex	Mean	% Control
Absolute liver wt.	Control	♂	13.6714	-
	1	♂	14.0662	103
	2	♂	14.2660	104
	3	♂	14.8642	109
	4	♂	16.2314	119
	5	♂	19.4370**	142
Relative liver wt.	Control	♂	3.3766	-
	1	♂	3.3135	98
	2	♂	3.4270	101
	3	♂	3.5222	104
	4	♂	3.8873*	115
	5	♂	4.6318**	137
Absolute liver wt.	Control	♀	8.4420	-
	1	♀	8.4750	100
	2	♀	8.2798	98
	3	♀	8.1362	96
	4	♀	9.0396	107
	5	♀	11.4826**	136

Summary of Mean Absolute and Relative Organ Weight Data				
Parameter	Group	Sex	Mean	% Control
Relative liver wt.	Control	♀	3.3310	-
	1	♀	3.3651	101
	2	♀	3.2614	98
	3	♀	3.2682	98
	4	♀	3.4512	104
	5	♀	4.5887**	138
Absolute thyroid wt.	Control	♂	0.0234	-
	1	♂	0.0208	89
	2	♂	0.0224	96
	3	♂	0.0256	109
	4	♂	0.0262	112
	5	♂	0.0304*	130
Relative thyroid wt.	Control	♂	0.0058	-
	1	♂	0.0050	85
	2	♂	0.0055	94
	3	♂	0.0061	105
	4	♂	0.0063	109
	5	♂	0.0073	125

*Statistically significant (Dunnett's, $p \leq 0.05$)
**Statistically significant (Dunnett's, $p \leq 0.01$)

2. Gross pathology - No gross pathological changes were observed in any treated group.

3. Microscopic pathology - Hepatocellular hypertrophy was observed (panlobular or centrilobular/midzonal) was observed in the 20000 and the 5000 ppm males and females. Hepatocellular vacuolization was also observed in the 20000 and 5000 ppm females as well. The following table summarizes the findings in the liver and the thyroid.

Summary of Microscopic Findings in the Liver and Thyroid						
Dose (ppm)	0	100	300	1000	5000	20000
Observation						
Males						
Liver (# examined)	5	5	5	5	5	5
Mononuclear cell infiltrate, random	5	4	5	3	4	0
Necrosis with inflammation	1	0	0	0	1	0
Hepatocellular hypertrophy, panlobular	0	0	0	0	0	4*
Hepatocellular hypertrophy, centrilobular/midzonal	0	0	0	0	1	1
Thyroid (# examined)	5	5	5	5	5	5
Vacuolation, follicular epithelium	0	0	0	0	0	1
Ectopic tissue, thymic	0	4*	1	2	0	0
Females						
Liver (# examined)	5	5	5	5	5	5
Mononuclear cell infiltrate, random	2	5	5	3	5	4
Necrosis with inflammation	1	0	0	0	0	0
Hepatocellular hypertrophy centrilobular/midzonal	0	0	0	0	3	4*
Hepatocyte vacuolization, periportal	0	0	0	0	1	2

*Statistically significant ($p \leq 0.05$) Fisher's Exact Test
**Statistically significant ($p \leq 0.01$) Fisher's Exact Test

III. DISCUSSION

It appears that the target organs for this chemical are the liver and thyroid. Increases in mean liver weights were observed in both sexes and increases in mean thyroid weights were observed in males. Hepatocellular hypertrophy (panlobular or centrilobular/midzonal) was observed in both sexes as well as hepatocellular vacuolization in females. These findings were supported by increases in alkaline phosphatase levels in males. The only finding in the thyroid was vacuolation of the follicular epithelium in one male. This was not considered to be related to treatment. This study is not a guideline study, however, the data are useful in that it appears that the target organs for this monoacid degradate of thiazopyr are similar to those of the parent

compound, thiazopyr. It is also of interest that si changes in hematological parameters were observed i 90-day rat study conducted with the parent compound although more parameters were affected. With the p compound, there were microscopic changes in the th after a 90-day oral exposure in rats (hypertrophy/hyperplasia, diffuse, starting at a level of 1000 ppm). These changes were always of or mild severity. Therefore, it is not surprising th microscopic changes were not observed in this study. They probably would have been observed with a longer exposure period. The NOEL for this study is set at 10 ppm and the LEL is set at 5000 ppm, based on hepatocellular hypertrophy, increases in liver and thyroid weights and slight increases in alkaline phosphatase levels.

012044

13544

033437

Chemical:	**Thiazopyr**
PC Code:	**129100**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/11/96**
File ID:	**TX012044**
Accession Number:	**412-02-0280**

HED Records Reference Center
04/09/2002