US EPA ARCHIVE DOCUMENT

8-2-92

[Fich=]

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

009647

MEMORANDUM:

AUG 0 2 1992

SUBJECT: Review of the 90-Day Subchronic Rat Study - Hydroxyatrazine.

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Tox Chem No 486K
HED Project No. 0-0269
Record No. 255841
MRID 41293501

TO: Kathy Pearce, PM 76
SRB,
SRRD (H7505C)

FROM : Henry Spencer, Ph.D. 1/29/92
Acting Section Head, Review Section 3
Toxicology Branch I
Health Effects Division (H7509C)

THRU: Karl Baetcke, Ph.D. Karl Baetcke 7/29/92
Chief,
Toxicology Branch I
Health Effects Division (H7509C)

REQUESTED ACTION:

Review the 90-Day Oral Toxicity Study In The Rat With Hydroxy Atrazine.

CONCLUSIONS:

The study in rats with hydroxyatrazine (MRID 412935-01) demonstrated that the kidney was the target organ. 300 and 600 ppm of hydroxyatrazine in the diet produced marked effects in both sexes. Effected were the hemopoietic system with reductions in RBCs, hemoglobin values,and hematocrits; Renal system with increased urinary outputs with decreased specific gravity. Renal changes included rough, pitted kidneys with variations in tubular dilitation and basophilic staining of cells. Microscopically, hyperplastic inflammation, cellular casts and crystal depositions were noted in these animals at the two highest doses. A NOEL was

Printed on Recycled Paper

1 of 26

established at 100 ppm and an LEL was determined to be 300 ppm of dietary hydroxyatrazine.

The DER of the study is transmitted to SRRD/SRB for inclusion in their files.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

009647

EPA No.: 68D80056
DYNAMAC No.: 274-A
TASK No.: 2-74A
August 20, 1990

DATA EVALUATION RECORD

HYDROXYATRAZINE

Subchronic Oral Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 8/17/90

3

009647

EPA No.: 68D80056
DYNAMAC No.: 274-A
TASK No.: 2-74A
August 20, 1990

DATA EVALUATION RECORD

HYDROXYATRAZINE

Subchronic Oral Toxicity Study in Rats

REVIEWED BY:

Margaret E. Brower, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: 8/17/90

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 8/17/90

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: 8/17/90

Henry Spencer, Ph.D.
EPA Reviewer, Section II
Toxicology Branch I
(H-7509C)

Signature: Henry Spencer
Date: 9/13/90

Marion Copley, D.V.M., D.A.B.T.
EPA Section Head, Section II
Toxicology Branch I
(H-7509C)

Signature: Marion Copley
Date: 9/13/90

4

DATA EVALUATION RECORD

GUIDELINE §_82-1_

STUDY TYPE: Subchronic oral toxicity study in rats.

MRID NUMBER: 412935-01.

TEST MATERIAL: Hydroxyatrazine.

SYNONYMS: N/A.

STUDY NUMBER: 882146.

SPONSOR: Agricultural Division, CIBA-GEIGY Corporation, Greensboro, NC.

TESTING FACILITY: Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ.

TITLE OF REPORT: Hydroxyatrazine; 90-Day Oral Toxicity Study in Rats.

AUTHORS: Rudzki, M. W., McCormick, G. C., and Arthur, A. T.

REPORT ISSUED: October 25, 1989.

CONCLUSIONS: The 13-week dietary administration of hydroxyatrazine to male and female Sprague-Dawley rats at dose levels of 0, 10, 100, 300, or 600 ppm resulted in minimal to marked compound-related effects at the two highest doses. No deaths occurred. Mean body weight gains of males and females fed 600 ppm and food consumption of males fed this same dose were reduced from study weeks 8 to 13; water consumption of these animals was increased. The target organ was the kidney. Reductions occurred in erythrocyte counts, hemoglobin, and hematocrit concentrations of these high-dose animals; leukocyte and platelet counts, serum blood-urea-nitrogen, and creatinine and electrolyte (sodium, chloride, potassium) levels were increased. Urinary volumes were increased in males fed 300 and 600 ppm and females fed 600 ppm, while urinary specific gravity was decreased in these females. Absolute and relative kidney and spleen weights were increased at the highest dose. Macroscopic renal changes at 300 and 600 ppm included discolored rough or pitted kidneys; minimal to severe tubular dilation and basophilia with minimal to chronic hyperplastic inflammation, cellular casts, and anisotropic crystals were exhibited microscopically in the kidneys of these animals. A high incidence of ultimobronchial bodies was found in the thyroids of males and females fed 600 ppm. Based on the incidence and severity of renal toxicity, the LOEL is 300 ppm and the NOEL is 100 ppm hydroxyatrazine.

Classification: CORE Minimum.

A. MATERIALS:

1. Test Compound: Hydroxyatrazine; description: N/A; batch No.: FL870869; purity: 97.1%.

2. Test Animals: Species: Rat; strain: Sprague-Dawley [Crl:CD Br]; age: 6 weeks at study initiation; weight: males--167.5 to 234.4 g, females--76.9 to 188.3 g; source: Charles River Laboratories, Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: Following approximately 3 weeks of acclimation, animals were assigned to the following test groups by computer randomization:

Test Group	Dose in Diet[a] (ppm)	Mean Daily Dose (mg/kg/day) Males	Mean Daily Dose (mg/kg/day) Females	Main Study (13 Weeks) Males	Main Study (13 Weeks) Females
1 Control	0	0	0	15	15
2 Low (LDT)	10	0.64	0.75	15	15
3 Mid 1 (MDT)	100	6.30	7.35	15	15
4 Mid 2 (MDT)	300	18.89	22.73	15	15
5 High (HDT)	600	37.47	45.64	15	15

[a]Dietary level was not adjusted for percent purity.

Animals were housed individually in a room with temperature and humidity controls set at 73 ± 5°F and 50 ± 20%, respectively, with a 12-hour light/dark cycle.

2. Diet Preparation: The test diets were prepared as admixtures of hydroxyatrazine in the basal rodent chow. Frequency of preparation was not indicated; however, the study authors reported that the frequency of preparation of the test diet was based upon available stability data. Homogeneity and stability analyses were performed prior to study initiation; the concentration of the test material in the test diet was analyzed at weeks 1, 5, 9, and 13.

   Results: The test diets were found to be homogeneous; mean concentrations of the test material were 92 ± 1.4, 93.7 ± 3.4, 98.3 ± 3.3, and 95.3 ± 2.4% of nominal for three samples (top, middle, bottom) of the 10-, 100-, 300-, and 600-ppm diets, respectively. The test compound was stable in the diet; after storage at room temperature for 21 days, concentrations of 10- and 5000-ppm diets were both 100% of nominal. The concentrations of the test material in the diets were within 11% of nominal concentrations. The mean concentrations for four intervals of analysis were 0, 9.3 ± 0.29, 94.5 ± 2.2, 293.5 ± 7.9, and 568.8 ± 17.5 ppm hydroxyatrazine for the 0-, 10-, 100-, 300-, and 600-ppm diets, respectively.

3. Food and Water Consumption: Animals received food (Purina Rodent Chow Meal #5001 or #5002) and water ad libitum.

4. Statistics: Body weight, food consumption, clinical observations, water consumption, and organ weights were examined for outliers and tested for homogeneity of variance. Dunnett's test was performed for data with homogeneous variance and without outliers. For data with

7

outliers or heterogeneous variance, the procedures utilized included data transforms, nonparametric Dunnett's test, and multiple comparison procedures with unequal variances. Other parameters that were not normally distributed were evaluated using nonparametric tests.

5. Quality Assurance: A quality assurance statement was signed and dated October 25, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for morbidity and mortality and once daily for clinical signs. Physical and auditory examinations were conducted 2 weeks prior to study initiation and at study termination.

   Results: No deaths occurred during the study. There was no effect of dosing on clinical observations, physical examinations, or auditory examinations.

2. Body Weight: Rats were weighed 7 and 14 days prior to study initiation, at study initiation, and weekly thereafter, as determined by individual and summary data. The study protocol indicated that initial body weights were to be determined 8 and 15 days prior to study initiation.

   Results: Representative data on mean body weights and body weight gains are summarized in Tables 1 and 2. Percent weight gains for the 13 weeks of the study were decreased 15% in both males and females fed 600 ppm as compared to respective control weight gains. The study authors reported this effect to be similar to that found in a previously conducted 4-week feeding study.[1] Cumulative body weight percent gains of males fed 600 ppm were slightly reduced (6 to 11% reduction when compared to concurrent controls) between study weeks 2 and 7 and significantly ($p < 0.01$) reduced between study week 8 and study termination (14 to 16% reduction when compared to concurrent controls). Mean body weights of these animals were slightly (6 to 7% reduction when compared to concurrent controls) but nonsignificantly reduced between study week 8 and study termination. Body weight percent gains of females fed 600 ppm were slightly reduced (6 to 10% reduction when compared to concurrent controls) between study weeks 3 and 10 and significantly ($p < 0.05$)

---

[1]Hazelette, J. R., and Arthur, A. T. 1989. Hydroxyatrazine: Pilot 4-Week Oral Feeding Study in Rats. Toxicology/Pathology Report 88031.

TABLE 1. Representative Results of Mean Body Weights for Rats Fed Hydroxyatrazine for 13 Weeks[a]

Dose Group (ppm)	Mean Body Weight (g ± S.E.[b]) at Weeks: 1	3	7	10	13
			Males		
0	258 ± 3.91	362 ± 7.21	479 ± 11.17	538 ± 12.62	544 ± 12.22
10	259 ± 4.04	361 ± 5.83	480 ± 9.08	534 ± 10.32	545 ± 10.39
100	260 ± 5.34	357 ± 7.19	471 ± 10.08	525 ± 10.89	533 ± 10.14
300	261 ± 4.82	361 ± 8.34	473 ± 13.00	523 ± 14.73	531 ± 15.30
600	259 ± 4.18	361 ± 6.67	461 ± 12.67	498 ± 15.21	507 ± 16.16
			Females		
0	178 ± 3.55	222 ± 5.52	267 ± 7.22	288 ± 7.45	292 ± 7.07
10	175 ± 4.64	219 ± 6.40	273 ± 8.53	294 ± 9.07	297 ± 8.91
100	185 ± 6.13	234 ± 4.65	283 ± 5.95	300 ± 6.86	307 ± 7.07
300	175 ± 5.56	222 ± 6.05	274 ± 8.40	294 ± 8.66	297 ± 8.15
600	180 ± 3.96	220 ± 4.67	261 ± 5.37	280 ± 4.24	277 ± 3.94

[a]Based on 15 rats/sex/dose.

[b]S.E. = Standard Error.

009647

TABLE 2. Representative Results of Mean Body Weight Percent Gains for Rats Fed Hydroxyatrazine for 13 Weeks[a]

Dose Group (ppm)	Mean Body Weight Percent Gain (% ± S.E.) at Weeks: 0-3	0-5	0-7	0-10	0-13
			Males		
0	82 ± 2.53	117 ± 3.82	141 ± 4.86	171 ± 5.82	174 ± 5.96
10	79 ± 2.36	115 ± 3.68	139 ± 4.73	166 ± 5.41	172 ± 5.02
100	78 ± 2.04	112 ± 3.24	135 ± 3.73	162 ± 4.06	166 ± 3.94
300	78 ± 2.22	109 ± 3.18	133 ± 3.89	157 ± 4.88	161 ± 5.35
600	77 ± 2.21	107 ± 3.31	126 ± 4.77	144 ± 6.06**	149 ± 6.61**
			Females		
0	42 ± 1.51	59 ± 2.10	72 ± 2.07	85 ± 2.39	88 ± 2.33
10	43 ± 2.30	63 ± 3.21	77 ± 3.41	90 ± 3.95	92 ± 3.63
100	44 ± 1.55	63 ± 2.23	74 ± 3.17	85 ± 3.59	89 ± 3.53
300	43 ± 2.05	65 ± 2.63	77 ± 2.70	90 ± 2.77	91 ± 3.08
600	39 ± 2.39	56 ± 2.36	66 ± 2.73	77 ± 2.60	76 ± 3.04*

[a]Based on 15 rats/sex/group.

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

009647

reduced between study weeks 11 and 13 (14 to 15% reduction). Mean body weights of these animals were slightly (5 to 6% reduction when compared to controls) but nonsignificantly reduced between study weeks 11 and 13; prior to week 11, body weights of high-dose females were similar to those of concurrent controls. Body weights of females fed 100 ppm were slightly (5%) increased when compared to concurrent controls at study initiation and remained slightly increased throughout the study. Body weights and body weight percent gains of other dosed males and females were similar to concurrent controls. The study authors indicated that reduction in body weight gain in high-dose males was associated with the concomitant reduction in food consumption of these animals.

3. Food Consumption and Compound Intake: Food consumption was determined, and mean daily dietary consumption was calculated at study initiation and weekly thereafter. Water consumption was determined prior to study initiation on 10 animals/sex and at study termination on 10 animals/sex/group.

   Results: Representative results of mean food consumption and mean water consumption are summarized in Tables 3 and 4, respectively. Mean food consumption was slightly depressed in males fed 600 ppm when compared to concurrent controls from study weeks 6 to 13 (significant at $p < 0.05$ at weeks 9 and 11 and $p < 0.01$ at week 10; 8 to 10% reduction when compared to controls). Food consumption of other dosed males and all dosed females was similar to that of concurrent controls. Mean water consumption was significantly ($p < 0.01$) increased at study week 13 in males and females fed 600 ppm; increases were 2.27 and 1.93 times those of concurrent controls for males and females, respectively. These changes correlated with increased urine volumes, kidney weights, and pathological renal changes. Water consumption measured at study initiation was not reported.

4. Ophthalmology: Ophthalmological examinations were performed 3 weeks prior to dosing and at study termination.

   Results: Ophthalmological findings (fundus hyperreflectivity of one male fed 300 ppm and synechia of the iris of one female fed 600 ppm) were considered to be incidental changes common to the rat strain.

5. Hematology and Clinical Chemistry: Blood was collected from the right orbital sinus of all animals at study termination for hematology and clinical analyses. In addition, baseline determinations were performed on 10 animals/sex prior to dosing, after which time the animals were sacrificed. The CHECKED (X) parameters were examined:

TABLE 3. Representative Results of Mean Food Consumption for Rats Fed Hydroxyatrazine for 13 Weeks

Dose Group (ppm)	Food Consumption (g/rat/week ± S.E.) at Weeks: 1	3	7	10	13
			Males		
0	169 ± 2.79	195 ± 4.19	181 ± 3.65	188 ± 3.15	165 ± 3.02
10	169 ± 3.86	195 ± 4.09	185 ± 4.10	189 ± 2.95	163 ± 2.34
100	170 ± 4.07	188 ± 3.29	179 ± 3.90	182 ± 3.87	159 ± 3.02
300	168 ± 3.77	190 ± 4.87	183 ± 5.24	178 ± 4.26	157 ± 4.42
600	164 ± 2.44	193 ± 4.32	171 ± 5.16	169 ± 5.47**	159 ± 5.17
			Females		
0	118 ± 2.46	131 ± 3.32	129 ± 3.57	126 ± 2.72	116 ± 2.52
10	123 ± 3.20	134 ± 4.08	131 ± 3.16	130 ± 3.24	113 ± 3.59
100	120 ± 5.46	143 ± 2.72	135 ± 2.97	132 ± 3.12	118 ± 2.62
300	110 ± 6.92	139 ± 4.22	140 ± 4.71	130 ± 3.30	116 ± 3.48
600	121 ± 3.05	130 ± 3 41	131 ± 5.00	129 ± 3.60	112 ± 2.95

**Significantly different from control value ($p < 0.01$).

009647

TABLE 4. Mean Water Consumption for Rats Fed Hydroxyatrazine for 13 Weeks

Dietary Level (ppm)	Water Consumption (mL/rat ± S.E.) at Week 13:	
	Males	Females
0	46.6 ± 2.1	40.2 ± 4.8
10	46.7 ± 3.0	41.8 ± 3.1
100	43.3 ± 2.9	32.4 ± 2.4
300	54.0 ± 4.0	48.2 ± 2.3
600	105.7 ± 5.2**	77.7 ± 3.9**

**Significantly different from control value ($p \leq 0.01$).

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count†
X	Hemoglobin (HGB)†		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†		Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†		Mean corpuscular volume (MCV)
X	Platelet count†	X	Prothrombin time
X	Reticulocyte count (RETIC)[a]		
X	Red cell morphology		
X	Heinz body determination		

Results: Table 5 summarizes mean hematological data of rats fed hydroxyatrazine for 13 weeks. Erythrocyte counts, hemoglobin concentration, and hematocrit values of males fed 600 ppm were significantly ($p < 0.05$ and $p < 0.01$) decreased by 5, 6, and 7%, respectively, when compared to concurrent controls. Leukocyte counts of these animals were nonsignificantly increased by 20%, and neutrophils were significantly ($p < 0.05$) increased (78%) when compared to concurrent controls. Erythrocyte counts and hematocrit values of females fed 600 ppm were significantly ($p < 0.05$ and $p < 0.01$) decreased by 4 and 8%, respectively, when compared to concurrent controls, while the hemoglobin concentration was nonsignificantly decreased by 7%. Leukocyte counts of high-dose females were significantly ($p < 0.01$) increased by 50%, and neutrophil counts were nonsignificantly increased by 30% when compared to concurrent controls. Platelet counts were significantly ($p < 0.01$) increased by 29% in females fed 600 ppm. Baseline laboratory control data were not provided for these indices. Reticulocyte counts of high-dose males were similar to those of concurrent controls; reticulocyte counts of high-dose females were not reported owing to technical difficulties. The study authors considered the changes in erythroid parameters to be reflective of decreased body weight gains and/or food consumption. Even though the changes in erythroid parameters were below 10% and were within the historical range for these parameters,[2] they were consistently found in both sexes and were considered by the reviewers to be related to dosing. Changes in leukocyte parameters were considered incidental or were not addressed by the study authors; these changes were also considered by the reviewers to be related to dosing.

[a]Reticulocyte counts and Heinz body determinations were performed on 10 animals of the control and 600-ppm dose groups.

[2]Hazleton Laboratories, 1984. Representative Historical Control Data.

†Recommended by Subdivision F (November 1984) Guidelines for 90-Day Subchronic Oral Toxicity Studies.

TABLE 5. Representative Hematology Results (± S.E.) for Rats Fed Hydroxyatrazine for 13 Weeks

Dose Group (ppm)	Erythrocyte Count (10^6/mm^3)	Hemoglobin (g/dL)	Hematocrit (%)	Neutrophils (%)	Leukocytes (10^3/mm)	Platelets (10^3/mm^3)
			Males			
0	7.4 ± 0.06	15.7 ± 0.12	45.8 ± 0.34	9.3 ± 1.76	13.4 ± 0.91	1015.9 ± 27
10	7.2 ± 0.22	15.2 ± 0.39	44.5 ± 1.09	12.5 ± 1.66	14.5 ± 1.45	1023.1 ± 46
100	7.5 ± 0.09	15.8 ± 0.18	46.1 ± 0.55	11.5 ± 2.11	13.4 ± 1.18	1001.6 ± 37
300	7.4 ± 0.07	15.7 ± 0.15	45.6 ± 0.43	13.8 ± 3.05	12.1 ± 0.88	1047.0 ± 25
600	7.0 ± 0.06*	14.7 ± 0.13**	42.5 ± 0.31**	16.6 ± 1.52*	16.1 ± 0.69	1135.3 ± 35
			Females			
0	6.8 ± 0.08	15.1 ± 0.17	43.3 ± 0.44	12.3 ± 1.89	11.2 ± 1.11	968.9 ± 30
10	6.9 ± 0.07	15.2 ± 0.09	43.8 ± 0.33	14.7 ± 2.42	10.7 ± 0.70	1015.6 ± 23
100	6.7 ± 0.08	15.1 ± 0.16	43.4 ± 0.50	11.9 ± 1.41	10.2 ± 0.57	1050.4 ± 32
300	6.9 ± 0.08	15.2 ± 0.08	43.7 ± 0.29	12.7 ± 1.68	11.0 ± 0.75	1024.1 ± 28
600	6.5 ± 0.10*	14.0 ± 0.23	40.0 ± 0.67**	16.0 ± 1.43	16.7 ± 1.06**	1252.9 ± 38**

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

0096647

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol
X	Sodium†		Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein†
	Creatine phosphokinase	X	Triglycerides
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
X	Gamma glutamyltransferase (GGT)		

Results: Table 6 summarizes mean clinical chemistry data. Mean blood-urea-nitrogen (BUN) and creatinine levels of males and females fed 600 ppm were significantly ($p < 0.01$) increased when compared to concurrent controls at study week 13. BUN levels were increased by 76 and 80% for males and females, respectively, and creatinine by 38% for both sexes. Serum electrolyte (sodium, chloride) levels of males and females fed 600 ppm were slightly but significantly ($p < 0.01$) increased when compared to concurrent controls. In addition, serum chloride levels were significantly ($p < 0.01$) increased in females fed 300 ppm, and potassium levels were significantly ($p < 0.05$) increased in females fed 600 ppm. These changes were reported to be associated with the compound-related renal effects found in these animals. Similar biochemical changes were reported by the study authors in the previously conducted 4-week feeding study. Other changes in clinical chemistry levels (cholesterol, total bilirubin, glucose, albumin, albumin/globulin ratios, and phosphorous) were within ranges recorded for historical controls[3] and were considered to be incidental by the reviewers. The study authors considered these changes to be incidental because of a lack of dose-response and the "proximity" of the individual values to concurrent control values.

†Recommended by Subdivision F (November 1984) Guidelines for 90-Day Subchronic Oral Toxicity Studies.

[3]Harlan Sprague Dawley Inc., 1989. Mid-Atlantic Regional Laboratory-Serum Chemistry data.

TABLE 6. Representative Clinical Chemistry Results for Rats Fed Hydroxyatrazine for 13 Weeks

Dietary Level (ppm)	Selected Clinical Data (mean ± S.E.) at Week 13: BUN (mg/dL)	Creatinine (mg/dL)	Sodium (meq/L)	Chloride (meq/L)	Potassium (meq/L)
			Males		
0	21.4 ± 0.46	0.60 ± 0.01	141 ± 0.16	102 ± 0.34	4.9 ± 0.08
10	21.5 ± 0.66	0.61 ± 0.02	141 ± 0.25	102 ± 0.39	4.7 ± 0.07
100	21.9 ± 0.61	0.61 ± 0.01	141 ± 0.22	102 ± 0.40	4.7 ± 0.07
300	22.5 ± 0.69	0.60 ± 0.01	141 ± 0.21	103 ± 0.30	4.7 ± 0.07
600	37.7 ± 1.61**	0.83 ± 0.03**	143 ± 0.44**	104 ± 0.30**	4.7 ± 0.10
			Females		
0	21.6 ± 0.73	0.61 ± 0.02	141 ± 0.24	102 ± 0.51	4.0 ± 0.08
10	22.3 ± 0.91	0.60 ± 0.02	141 ± 0.19	103 ± 0.26	4.1 ± 0.08
100	21.2 ± 0.67	0.60 ± 0.02	140 ± 0.34	103 ± 0.58	4.2 ± 0.10
300	22.9 ± 1.01	0.65 ± 0.02	141 ± 0.29	104 ± 0.38**	4.1 ± 0.07
600	38.8 ± 2.57**	0.84 ± 0.04**	142 ± 0.35**	105 ± 0.44**	4.4 ± 0.10*

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

009647

6. Urinalysis: Urine was collected from 10 animals/sex/group prior to study initiation and during week 13. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen

Results: Representative urinalysis data are presented in Table 7. The urine volume of males fed 300 ppm ($p < 0.05$) and males and females fed 600 ppm ($p < 0.01$) was significantly increased during study week 13. The mean volume changes were 38 (300 ppm) and 228% (600 ppm) in males and 147% (600 ppm) in females when compared with concurrent controls. At this same time, a slight but significant ($p < 0.05$) decrease in specific gravity was exhibited in females fed 600 ppm. These changes correlated with the increased water consumption and other renal effects found in these animals. No other urinary changes were noted. Urinalysis determinations conducted prior to study initiation were not reported.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

†Recommended by Subdivision F (November 1984) Guidelines for 90-Day Subchronic Oral Toxicity Studies.

TABLE 7. Representative Urinalysis Data for Rats Fed Hydroxyatrazine for 13 Weeks

Dietary Level (ppm)	Mean Urinalysis Data (Mean ± S.E.) at Week 13: Males		Females	
	Specific Gravity	Volume (mL)	Specific Gravity	Volume (mL)
0	1.027 ± 0.005	24.6 ± 2.27	1.032 ± 0.004	22.2 ± 3.83
10	1.023 ± 0.005	26.2 ± 1.70	1.027 ± 0.004	20.3 ± 2.31
100	1.021 ± 0.004	25.0 ± 2.24	1.032 ± 0.004	17.0 ± 2.13
300	1.027 ± 0.002	34.0 ± 2.33*	1.023 ± 0.002	28.0 ± 2.13
600	1.015 ± 0.000	80.6 ± 3.95**	1.014 ± 0.000*	54.8 ± 3.95**

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain
XX	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†a
X	Duodenum†	XX	Spleen	X	Eyes (optic nerve)
X	Jejunum†	XX	Thymus		
X	Ileum†				
X	Cecum†				
X	Colon†				
X	Rectum		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals
	Gallbladder†	X	Urinary bladder†	X	Lacrimal gland
X	Pancreas†	XX	Testes†	X	Mammary gland†
		XX	Epididymides	XX	Thyroids with parathyroids†a
		XX	Prostate	X	Harderian glands
		X	Seminal vesicle		
	Respiratory	XX	Ovaries		
X	Trachea†	XX	Uterus		
XX	Lung†	X	Vagina		
					Other
				X	Bone (sternum, femur)†
				X	Skeletal muscle†
				X	Skin
				X	All gross lesions and masses†

Results:

a. Organ Weights: Table 8 presents data for kidney and spleen weights. Kidney weights were increased 34% in high-dose males and females and kidney-to-body weight and kidney-to-brain weight ratios were increased 44% and 30% in high-dose males, respectively, and 45% and 34% in high-dose females, respectively. These changes were significant at $p < 0.01$. The changes in kidney weights reflect the pathological renal effects found in these animals. Spleen weights, spleen-to-body weight, and spleen-to-brain weight ratios were also increased in these animals; increases were significant ($p < 0.05$ and $p < 0.01$) in high-dose females (increases of 16, 27, and 18% in spleen weights, spleen-to-body

[a]Weights were taken after fixation for pituitary and thyroid with parathyroid glands; all other weights were taken before fixation.

†Recommended by Subdivision F (November 1984) Guidelines for 90-Day Subchronic Oral Toxicity Studies.

TABLE 8. Mean Organ Weights, Organ-to-Body Weight, and Organ-to-Brain Weight Ratios in Rats Fed Hydroxyatrazine for 13 Weeks

Dietary Level (ppm)	Kidney			Spleen		
	Absolute (g ± S.E.)	Relative (% of Body Weight ± S.E.)	Relative (% of Brain Weight ± S.E.)	Absolute (g ± S.E.)	Relative (% of Body Weight ± S.E.)	Relative (% of Brain Weight ± S.E.)
		Males			**Males**	
0	3.65 ± 0.092	0.707 ± 0.021	161 ± 4.91	0.84 ± 0.03	0.16 ± 0.003	37.1 ± 1.23
10	3.64 ± 0.089	0.705 ± 0.013	163 ± 4.14	0.91 ± 0.04	0.18 ± 0.007	40.8 ± 1.79
100	3.73 ± 0.137	0.730 ± 0.022	164 ± 5.21	0.88 ± 0.04	0.17 ± 0.007	38.5 ± 1.52
300	3.64 ± 0.085	0.716 ± 0.015	166 ± 4.08	0.80 ± 0.03	0.16 ± 0.006	36.5 ± 1.31
600	4.89 ± 0.153**	1.02 ± 0.032**	210 ± 6.16**	0.95 ± 0.04	0.20 ± 0.004**	40.7 ± 1.33
		Females			**Females**	
0	2.11 ± 0.073	0.774 ± 0.018	105 ± 3.33	0.60 ± 0.03	0.22 ± 0.008	29.6 ± 1.31
10	2.05 ± 0.070	0.742 ± 0.018	103 ± 3.30	0.57 ± 0.02	0.21 ± 0.010	28.5 ± 0.78
100	2.08 ± 0.055	0.728 ± 0.022	103 ± 2.50	0.57 ± 0.02	0.20 ± 0.008	28.2 ± 1.17
300	2.04 ± 0.047	0.737 ± 0.017	100 ± 1.76	0.63 ± 0.03	0.23 ± 0.009	30.9 ± 1.22
600	2.82 ± 0.078**	1.123 ± 0.043**	141 ± 4.23**	0.70 ± 0.03*	0.28 ± 0.012**	34.8 ± 1.32*

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

009647

weight and spleen-to-brain weight ratios, respectively, when compared to concurrent controls). The study authors did not consider these splenic changes to be compound-related. Significant increases in relative [to body (11%, p <0.01) heart weights and nonsignificant increases in absolute (3%) and relative [to brain (4.5%)] heart weights of high-dose females were considered by the reviewers to be a reflection of decreased terminal body weights and were not considered to be compound related. Significant increases in relative [to body (17%, p <0.01) and to brain (10%, p <0.05)] lung weights and nonsignificant increases in absolute lung weights (9%) of high-dose females were the result of inflammatory changes due to infection and were not considered to be compound related. Other changes in organ weights were considered to be incidental based on the lack of corresponding pathological change, decreased terminal body weights, absence of dose-response relationships, or lower than expected organ weights in control animals (pituitary and thyroid/parathyroid weights in females).

b. Gross Pathology: Table 9 summarizes the incidence of frequently occurring gross findings in rats fed hydroxyatrazine for 13 weeks. Rough or pitted kidneys were exhibited in 15 males (100%) and 14 females (93%) fed 600 ppm and 3 males (20%) and 2 females (13%) fed 300 ppm hydroxyatrazine. Renal pallor and tan discoloration were found in 9 males (60%) and 5 females (33%) fed 600 ppm, and green renal discoloration was found in 1 male (7%) fed 300 ppm. Similar gross renal changes were reported by the study authors to have occurred in a previously conducted 4-week feeding study at dietary concentrations ≥500 ppm.[4] Other findings were reported to be of similar incidence and severity in controls and dosed groups and were not considered related to dosing.

c. Microscopic Pathology:

1) Nonneoplastic: Table 10 presents representative nonneoplastic findings. A significantly (p <0.001) increased incidence of severe toxic nephrosis, characterized by marked tubular dilation, tubular basophilia, extensive chronic hyperplastic inflammation in the interstices, and cellular casts, was exhibited in all males and females fed 600 ppm. In addition, anisotropic crystals were exhibited in the papillary renal tubules of 11 males (73%) and 13 females (87%) fed this same dose. Minimal toxic nephrosis, characterized by minimal tubular dilation and tubular basophilia with minimal interstitial inflammation, was found

[4]Hazlette and Arthur, 1989.

TABLE 9. Representative Gross Findings in Rats Fed Hydroxyatrazine for 13 Weeks[a]

Organ/Finding	Dietary Level (ppm): Males 0	10	100	300	600	Females 0	10	100	300	600
Kidney	(15)[b]	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Distended	1	0	0	0	1	1	1	0	0	1
Discoloration, pallid	0	1	0	1	9	0	1	0	0	5
Lesion	0	0	0	1	0	0	0	0	0	2
Pitted	0	0	0	2	0	0	0	0	2	2
Rough texture	0	0	0	1	15	1	0	0	0	12
Cyst	0	0	0	0	0	0	0	0	1	0
Spleen	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Cyst	0	0	0	1	0	0	0	0	0	0

[a]Gross findings compiled from individual data by the reviewers.

[b]Numbers in parentheses denote number of animals examined.

TABLE 10. Representative Nonneoplastic Findings in Rats Fed Hydroxyatrazine for 13 Weeks

Organ/Finding	Dietary Level (ppm): Males 0	10	100	300	600	Females 0	10	100	300	600
Kidney[a]	(15)[b]	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Hydronephrosis, minimal	1	0	0	0	0	1	1	0	0	1
Toxic nephrosis, minimal	0	0	0	7**	0	0	0	0	11***	0
Toxic nephrosis, severe	0	0	0	0	15***	0	0	0	0	15***
Tubule crystals, minimal	0	0	0	0	10***	0	0	0	0	11***
Tubule crystals, moderate	0	0	0	0	1	0	0	0	0	2
Cyst	0	0	0	0	0	1	0	0	1	0
Lung	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Lymphoid hyperplasia, subacute	5	10	10	5	4	8	9	7	8	6
Subacute lymphocytic inflammation	1	6*	1	2	3	3	3	1	3	3
Lymph node	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Congestion	0	0	0	1	1	0	0	0	0	0
Lymphangiectasis	0	2	0	0	0	0	0	0	0	0
Lymphoid hyperplasia	0	2	2	1	0	0	0	0	1	0
Thyroid	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
Subacute lymphocytic inflammation	0	0	1	0	0	0	1	0	0	0
Ultimobronchial body	3	3	1	2	7	1	5	0	2	7*

[a]Nonneoplastic kidney data compiled from individual data by the reviewers.

[b]Numbers in parentheses denote number of animals examined.

*Significant incidence at $p < 0.05$ as determined by Fisher's Exact test.

**Significant incidence at $p < 0.01$ as determined by Fisher's Exact test.

***Significant incidence at $p < 0.001$ as determined by Fisher's Exact test.

in 7 males (47%) and 11 females (73%) fed 300 ppm; the study authors reported that this finding often occurred unilaterally in these animals. The rough textured kidneys found macroscopically in one control female were reported by the study authors to be associated with calculi in the urinary bladder and infarction of the kidneys of this animal. The increased incidence of inflammatory changes of the lungs and ultimobronchial bodies of the thyroids of dosed males and females were not considered by the study authors to be related to dosing. The reviewers consider the inflammatory changes in the lungs to be the result of infection. Even though the incidence of ultimobronchial bodies of the thyroid was not dose related, the incidence of this finding (47%) in high-dose males and females was significantly increased when compared to the incidence of cysts of the thyroid of historical controls (1.7%, 9/199 males; 1.4%, 8/199 females)[5]. The reviewers consider these findings possibly to be related to dosing with hydroxyatrazine. Other histologic findings were sporadic and similar in dosed and control males and females.

009647

2) Neoplastic: There were no neoplastic findings.

D. STUDY AUTHORS' CONCLUSIONS:

The 13-week dietary administration of hydroxyatrazine to male and female Sprague-Dawley rats at dose levels of 0, 10, 100, 300, or 600 ppm resulted in minimal to marked compound-related effects at the two highest doses. These effects included the following: (1) reductions in the body weight gain in males and females fed 600 ppm; (2) decreased food consumption in males fed 600 ppm; (3) increased water consumption in males and females fed 600 ppm; (4) reductions in mean erythrocyte counts and hemoglobin and hematocrit concentrations in both sexes fed 600 ppm and increased platelet counts in females fed 600 ppm; (5) increased serum BUN, creatinine, and electrolyte [sodium, chloride (≥300 ppm) and potassium] concentrations in males and females fed 600 ppm; (6) increased mean urine volume in males fed ≥300 ppm and females fed 600 ppm, and decreased urinary specific gravity in females fed 600 ppm; (7) increased kidney weights and kidney-to-body and kidney-to-brain weight ratios in males and females fed 600 ppm; (8) discolored, rough, or pitted kidneys in males and females fed ≥300 ppm; and (9) minimal to marked renal tubular dilation and basophilia with chronic interstitial inflammation in males and females fed ≥300 ppm, and cellular casts and anisotropic crystals in the papillary tubules of both sexes fed 600 ppm. Based on the incidence and severity of the nephrotoxic responses, the NOEL was 100 ppm and the MTD was between 300 and 600 ppm.

[5]Mobay Corporation, Nonneoplastic Histopathology Incidence Summary Report.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete, and the conduct of the study was acceptable. However, the study authors indicated that hematology and clinical chemistry parameters were measured in a subgroup of 10 animals/sex prior to study initiation for baseline data; results were not reported. In addition, water consumption was indicated to have been measured prior to study initiation; results were not reported. These data would be of use in comparing results of dosed animals at study termination and should be provided by the study laboratory. Water consumption should have been measured at regular intervals throughout the study. Dunnett's nonparametric test was referenced in the study report entitled "Summary of Statistical Analyses"; Dunn's test, reported to have been used to analyze nonparametric data on page 18 of the study report, is considered to be an error. Gross pathology data were not summarized by the study authors. There was some technical difficulty in measuring reticulocytes and Heinz bodies of control and dosed animals at study week 13. The reviewers consider the increase in leukocytes (20% increase in males, 50% increase in females), neutrophils (78% increase in males, 30% increase in females) and platelets (29% increase in females) to be compound related. Even though the changes (decreased erythrocyte counts, hemoglobin, and hematocrit) in erythroid parameters were below 10% and were within the historical range for these parameters (Hazleton Laboratories, 1984), they were consistently found in both sexes and were considered by the reviewers to be related to dosing.

The incidence of ultimobronchial bodies of the thyroid was high (47%) in males and females fed 600 ppm when compared to the incidence of cysts of the thyroid of historical controls of another laboratory (1.7% males, 1.4% females); however, the incidence of this finding also appears higher than historical incidence in the concurrent controls or in animals receiving lower doses of hydroxyatrazine, but there was no dose-response relationship. It is possible that this finding is related to dosing with hydroxyatrazine. The test laboratory should provide the historical incidence of Sprague-Dawley rats with this finding in their laboratory. An inventory of histopathological tissues was not provided for individual animals.

We agree with the study authors that based on the incidence and severity of the nephrotoxic response, the LOEL is 300 ppm and the NOEL is 100 ppm hydroxyatrazine.