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

JUN 16 1987

005940

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Twenty-Four-Month Combined Chronic Oral Toxicity and Oncogenicity Study in Rats Utilizing Atrazine 98.9% Technical [Accession Nos. 262714-262727]

TOX Chem No. 63

FROM: Henry Spencer, Ph.D. HS 6/11/87
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) ABK 6/15/87
for 6/16/87

The Ciba-Geigy Corporation has submitted the subject study in response to the DCI Notice of the Atrazine Registration Standard. The Data Evaluation Record (DER) is attached and the conclusions are as follows:

- Atrazine was oncogenic in CD-1 Sprague-Dawley rats. An increase in carcinomas of the mammary gland was observed in females fed 70, 500, or 1000 ppm atrazine for 2 years. There was also an increase in the incidence of fibroadenomas/adenomas (1000 ppm) as well as all mammary tumors in females receiving 500 and 1000 ppm when compared to controls. There was a decrease in mean body weights of males and females receiving 500 and 1000 ppm. Survival was decreased in high-dose females but increased in high-dose males. Red cell parameters (hemoglobin, hematocrit, and red cell count) were decreased in high-dose females but not in males. The serum glucose level was decreased in high-dose females at 3, 6, and 12 months and serum

triglyceride levels tended to be decreased in high-dose males throughout the study; however, the toxicologic importance of the clinical chemistry findings is unclear. There were decreases in organ-to-body weight ratios in high-dose animals, which were probably the result of body weight decreases. Hyperplastic changes in high-dose males (mammary gland, bladder, and prostate) and females (myeloid tissue of bone marrow and transitional epithelium of the kidney) were of questionable toxicologic importance. There was an increase in retinal degeneration and in centrilobular necrosis of the liver in high-dose females and an increase in degeneration of the rectus femoris muscle in high-dose males and females when compared to controls. Based on decreased body weight gain, the LOEL for chronic toxicity in males and females is 500 ppm and the NOEL is 70 ppm.

Maximum Tolerated Dose:

An MTD in this study was considered to have been attained based on statistically significant body weight decreases in males and females.

Core Classification:

Core Minimum. The study can be upgraded to Core Guideline if individual animal disposition data can be provided.

The study results and conclusions will be submitted to the Toxicology Branch Peer Review Committee for an oncogenic evaluation and classification pending completion of the entire atrazine data base.

Note: The statistical evaluation presented in the DER was of a cursory nature. The data are being more extensively evaluated, and the final results of that separate evaluation will be submitted as an addendum to this DER.

The more extensive evaluation is not expected to significantly alter the positive oncogenic outcome of the report.

Attachment

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

005940

EPA: 68-02-4225
DYNAMAC No. 2308
March 20, 1987

DATA EVALUATION RECORD

ATRAZINE

Chronic Oral Toxicity/Oncogenicity Study in Rats

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 3-20-87

005940

EPA: 68-02-4225
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DATA EVALUATION RECORD

ATRAZINE

Chronic Oral Toxicity/Oncogenicity Study in Rats

REVIEWED BY:

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EPA Section Head (VII)

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005940

DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Chronic oral toxicity/oncogenicity study in rats.

ACCESSION NUMBER: 262714-262727.

TEST MATERIAL: Atrazine technical.

SYNONYMS: 2-Chloro-4-ethylamino-6-isopropylaminotriazine.

STUDY NUMBER(S): 410-1102.

SPONSOR: CIBA GEIGY Corp., Greensboro, NC.

TESTING FACILITY: American Biogenics Corporation, Decatur, IL.

TITLE OF REPORT: Twenty-four month combined chronic oral toxicity and oncogenicity study in rats utilizing atrazine technical.

AUTHOR(S): Mayhew, D. A., Taylor, G. D., Smith, S. H., and Banas, D. A.

REPORT ISSUED: April 29, 1986.

CONCLUSIONS:

Under the conditions of the study, atrazine was oncogenic in CD-1 Sprague-Dawley rats; an increase in carcinomas of the mammary gland was observed in females fed 70, 500, or 1000 ppm atrazine for 2 years. There was also an increase in the incidence of fibroadenomas/adenomas (1000 ppm) as well as all mammary tumors in females receiving 500 and 1000 ppm when compared to controls. There was a decrease in mean body weights of males and females receiving 500 and 1000 ppm. Survival was decreased in high-dose females but increased in high-dose males. Red cell parameters (hemoglobin, hematocrit, and red cell count) were decreased in high-dose females but not in males. The serum glucose level was decreased in high-dose females at 3, 6, and 12 months and serum triglyceride levels tended to be decreased in high-dose males throughout the study; however, the toxicologic importance of the clinical chemistry findings is unclear. There were decreases in organ-to-body weight ratios in high-dose animals, which were probably the result of body weight decreases. Hyperplastic changes in high-dose males (mammary gland, bladder, and prostate) and females (myeloid tissue of bone marrow and transitional epithelium of the kidney) were of questionable toxicologic importance. There was an increase in retinal degeneration and in centrolobular necrosis of the liver in high-dose females and an increase in degeneration of the rectus femoris muscle in high-dose males and females when compared to controls. Based on decreased body weight gain, the LOEL for chronic toxicity in males and females is 500 ppm and the NOEL is 70 ppm.

Core Classification: Core Minimum. The study can be upgraded to Core Guideline if individual animal disposition data can be provided.

A. MATERIALS:

1. Test Compound: Atrazine technical; description: a white powder, batch No. FL0821575; stated purity: 98.9 percent--contaminants were not described.

2. Test Animals: Species: rat; strain: Sprague-Dawley [Crl:COBS CD (SD) BR]; age: 30 days at receipt and 37 to 38 days at initiation; weight (mean): 110-111 g for females and 145-146 g for males; source: Charles River Breeding Laboratories, Portage, MI. Animals were acclimated for 7 to 8 days.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following groups using a computer-randomized program:

	Test Group	Dose in Diet (ppm)	Chronic Toxicity Subgroup		Oncogenicity Subgroup		Interim Sacrifices 12 mos.		Interim Sacrifices 13 mos.[b]	
			M	F	M	F	M	F	M	F
1	Control	0	20	20	70	70	10	10	9	10
2	Low (LDT)	10	20	20	50	50	—	—	—	—
3	Mid low	70	20	20	50	50	—	—	—	—
4	Mid (MDT)	500	20	20	50	50	—	—	—	—
5	High (HDT)	1000	20	20	70	70	10	10	10	10

[a]The chronic toxicity animals were primarily for clinical laboratory analyses.

[b]These rats received control diets for 1 month prior to sacrifice.

2. Diet Preparation: Diets were prepared weekly and presented to animals within 3 days of preparation. Homogeneity and stability were assayed prior to study initiation and diets were analyzed monthly for concentration of test compound. The test material was also assayed monthly to determine its stability.

Results: The test material was stable throughout the study; the mean (±2 SD) purity was 95.9±2.3 (range, 93.3-98.0) percent. Homogeneity was acceptable and the concentration of the test material recovered from the diets after 2 weeks of storage at ambient temperature was greater than 92 percent. Mean concentrations of the test material in diets for 25 intervals of analysis and at 95 percent confidence limits (±2 SD) were 9.90±0.79, 70.2±3.0, 503±19, and 999±35 ppm for nominal levels of 10, 70, 500, and 1000 ppm, respectively.

3. Animals received Purina Certified Rodent Chow No. 5002 (with or without test compound) and water ad libitum. Animals were caged individually in temperature- and humidity-controlled rooms where the air was filtered through HEPA and charcoal.

4. Statistics: Body weight, food consumption, clinical pathology, and organ weight data were analyzed by ANOVA and significant differences examined by Tukey's (equal populations) or Scheffe's (unequal populations) tests for multiple comparisons. Non-parametric data were analyzed by the Kruskal-Wallis test. Survival was examined by the Cox-Tarone test using life tables as well as by the nonparametric-ranked score test of Gehan and Breslow for trend and heterogeneity. Graphical evaluation of survival was determined by Kaplan-Meir product-limit estimates.

   Nonneoplastic incidences were evaluated by the Cochran-Armitage trend test and the Fisher-Irwin exact test with the Bonferroni adjustment for significance level ($p = 0.05/4, 0.0125$).

   Some neoplastic incidences were analyzed by the method of Dinse and Lagakos[1] because of observed intercurrent mortality differences. This method allows delineating the effects of dose x age on prevalence; continuity corrections were applied. Where appropriate, adenomas and carcinomas were combined (and in some cases hyperplasia) for analysis; carcinomas alone were also evaluated. Analysis of pituitary tumors in females combined adenoma and carcinoma, and both prevalence and life-table analyses were performed.

5. A quality assurance statement was signed and dated April 29, 1986.

C. METHODS AND RESULTS:

1. Observations: All animals were observed twice daily for mortality, morbidity, and overt toxic signs; individual animals were examined weekly and palpated for tissue masses.

   Results: There was an increase in the incidence of tissue masses in females receiving 70, 500, or 1000 ppm (Table 1). Irritability was noted at an increased incidence in males receiving 500 ppm (n=22) and 1000 ppm (n=26) when compared to controls (n=13). Other signs of toxicity were those commonly seen in rats and were of a similar incidence in both dosed and control groups.

   Mortality and percent survival at selected intervals are summarized in Table 2. In males, survival was increased in a dose-related manner ($p < 0.003$, using the Cox Tarone test) and was significantly higher in males receiving 1000 ppm when compared to controls ($p = 0.0055$, using pairwise comparison). In contrast,

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[1] Dinse, G. E. and Lagakos, S. W. (1983) Regression analysis of tumor prevalence data, J. R. Stat. Soc. Ser. 32:236-248.

TABLE 1. Palpable Tissue Mass Observations in Rats Fed Atrazine for 2 Years

Location	Number of Animals with Mass									
	Dose Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Abdomen	19	21	17	19	22	33	36	40	52	58
Axilla	0	1	0	0	0	2	3	6	9	9
Chest	2	0	1	4	2	10	14	18	27	32
Perianal	0	0	0	2	0	4	2	4	20	15
Perineum	5	1	1	2	7	9	14	23	20	35
Back	6	4	5	8	7	2	2	3	2	12
Side	2	3	0	7	8	19	21	31	38	47

TABLE 2. Cumulative Mortality and Percent Survival[a] at Selected Intervals in Rats Fed Atrazine for 2 Years

Dose Group (ppm)	Mortality (Percent Survival) at Week		
	52	78	Termination
		Males	
0	2 (97)	13 (83)	40 (44)
10	3 (96)	11 (84)	37 (47)
70	3 (96)	10 (86)	31 (56)
500	1 (99)	10 (86)	30 (57)
1000	1 (99)	6 (94)	23 (67)
		Females	
0	2 (97)	11 (84)	35 (50)
10	5 (93)	16 (77)	39 (44)
70	0(100)	12 (83)	40 (43)
500	2 (97)	13 (81)	44 (37)
1000	2 (97)	17 (76)	52 (26)

[a]Percent Survival was based on 70 rats/sex/group except for control males (n=71).

survival in females was decreased in a dose-related manner, (p value for a negative trend = 0.0016) and was significantly lower ($p = 0.0042$) in high-dose females when compared to controls.

2. Body Weight: Rats were weighed weekly for 13 weeks and monthly thereafter. Body weights at scheduled sacrifices were determined for fasted animals.

   Results: Representative body weight data are summarized in Table 3. Mean body weights were significantly depressed in both males and females receiving 500 and 1000 ppm with the exception of the mean weights of males in the last 2 months of the study. The 24-month weight gain in the high-dose animals was 76 percent of control for the males and 64.5 percent of control for the females. In the recovery groups, the weight gain for month 13 for males previously receiving 1000 ppm was 63±14.6 g, compared to 20±17.9 g for controls ($p \leq 0.01$), and for females previously receiving 1000 ppm it was 56±31.6 g, compared to 16±11.9 g for controls ($p \leq 0.01$). However, the mean weight at 13 months in these males was still significantly ($p \leq 0.01$) lower than controls.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

   Results: Representative food consumption data are summarized in Table 4. Food consumption values were significantly decreased in mid- and high-dose males for the first 13 months, for the mid-dose females for the first 12 weeks of the study, and for the high-dose females for the first 6 months. No significant differences in food consumption were noted in recovery males or females during month 13, although there were significant increases in weight gain in male and female groups that had previously received 1000 ppm. Food efficiency was similar in dosed and control groups.

4. Ophthalmology: Ophthalmologic examinations were performed on control and high-dose rats prior to study initiation, prior to sacrifice at 12 and 13 months, and prior to terminal sacrifice.

   Results: There were no adverse findings at the interim sacrifices. The changes seen at terminal sacrifice, mild keratitis, neovascularity, or cataracts, were considered due to use of powdered food, to orbital bleeding, or to aging. These finding occurred at a similar frequency in control and high-dose males and females.

5. Blood Parameters: Blood was collected before treatment and at 3-month intervals for hematology and clinical chemistry analysis from all surviving animals in the chronic toxicity subgroups (initially 20/sex/group). It was also collected from 10/sex in the control and high-dose groups scheduled for sacrifice at 12 and 13 months. The CHECKED (X) parameters were examined.

TABLE 3. Mean Body Weights at Selected Intervals in Rats Fed Atrazine for 2 Years

Dose Level (ppm)	Mean Body Weight (g±SD) at Week						
	0	1	13	26	52	78	104
				Males			
0	146±18.2	212±21.3	532±44.2	637±63.8	727±91.4	767±110.7	704±124.7
10	146±18.2	213±19.7	541±47.2	646±71.2	737±98.4	783±139.3	739±140.5
70	146±17.6	209±19.0	516±41.6	617±61.0	713±82.6	779± 97.2	742± 97.7
500	145±18.0	199±19.3**	467±39.9**	548±50.6**	635±70.3**	678± 95.4**	646±119.4
1000	145±17.2	189±19.9**	436±37.6**	508±42.3**	574±52.1**	610± 61.8**	572± 97.0**
				Females			
0	110±11.8	154±14.1	283±27.2	330±36.0	408±59.3	467± 95.2	496±105.7
10	111±12.1	155±13.8	282±25.2	328±39.7	404±67.2	475± 95.1	477±132.6
70	111±11.3	153±13.2	275±26.0	324±38.1	405±67.2	471± 89.4	480±106.8
500	111±12.4	145±13.3**	252±20.3**	289±26.4**	342±42.8**	371± 73.7**	402±106.8*
1000	111±12.2	139±11.2**	239±21.4**	271±27.4**	308±36.1**	341± 59.1**	361±81.1**

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

TABLE 4. Food Consumption Data at Selected Intervals in Rats Fed Atrazine for 2 Years

Dose Level (ppm)	Daily Mean Food Consumption (g/rat) at Week					
	1	13	26	52	78	104
			Males			
0	22.2	27.2	27.1	26.7	24.5	22.2
10	22.1	26.6	26.9	27.2	25.9	23.7
70	21.4	26.1	26.2	26.6	24.1	22.9
500	19.7**	24.1**	24.6**	25.3	23.0	22.1
1000	17.7**	22.7**	23.4**	23.8**	23.2	21.0
			Females			
0	17.5	18.1	19.7	20.7	18.8	18.1
10	17.8	18.3	19.3	20.5	20.2	17.4
70	17.9	18.0	19.6	20.0	19.8	17.3
500	16.1**	17.0	19.1	20.0	17.9	18.1
1000	14.4**	16.7**	18.4*	19.8	17.8	16.5

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

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

a. Hematology

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Coagulation time
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Heinz bodies

Results: In females receiving 1000 ppm, significantly lower mean RBC, HGB, and HCT were noted at 6, 12, and 18 months when compared to controls (Table 5). The values were somewhat depressed in high-dose females at 24 months, however, only four females were used for clinical studies. RBC, HGB, and HCT were also decreased in the 10 females receiving 1000 ppm and scheduled for sacrifice at 12 months. The values approached control levels at 13 months in the 1000-ppm recovery group. All values for parameters in dosed males were similar to those in the control groups with the exception of an increased mean platelet count at 6 months in rats receiving 1000 ppm. Increased platelet counts were also seen at 6 and 12 months in females receiving 1000 ppm.

b. Clinical Chemistry

Electrolytes
X Calcium†
X Chloride†
Magnesium†
X Phosphorus†
X Potassium†
X Sodium†

Enzymes
X Alkaline phosphatase
Cholinesterase (RBC and serum)
Creatinine phosphokinase†
Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)†
X Serum aspartate aminotransferase (also SGOT)†
X Gamma-glutamyl transpeptidase (GGT)
X Creatine phosphokinase

Other
X Albumin†
Blood creatinine†
X Blood urea nitrogen† (BUN)
X Cholesterol†
X Globulins and A/G ratio
X Glucose†
X Total bilirubin†
X Total protein†
X Triglycerides

Results: The level of serum triglycerides in high-dose males was, in general, lower than control values throughout the study (Table 6); however, the decrease was significant only at 6 months. In groups scheduled for the 12-month sacrifice, the level in high-dose males (103.10±47.81 mg/dL) was significantly lower ($p \leq 0.01$) than in control males (228.30±95.44 mg/dL). At the

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 5. Selected Hematology Data on Female Rats Fed Atrazine for 2 Years

Parameter/ Monthly Interval	Dietary Level (ppm) 0	500	1000
Erythrocytes (10^6/mm^3)			
3	7.74±0.30 (20)[a]	7.66±0.41 (20)	7.40±0.27 (20)
6	7.43±0.39 (19)	7.17±0.33 (20)	6.84±0.41** (20)
12	6.74±0.39 (19)	6.14±0.81 (18)	5.95±1.13* (18)
18	7.72±1.00 (17)	7.04±0.63 (15)	6.29±1.09** (12)
24	7.20±1.21 (8)	6.14±1.43 (6)	6.36±0.90 (4)
Hemoglobin (g/dL)			
3	16.33±0.59 (20)	16.23±0.66 (20)	15.76±0.59 (20)
6	15.70±0.77 (20)	15.29±0.65 (20)	14.66±0.84** (20)
12	13.55±0.80 (19)	12.63±0.98 (18)	12.15±2.19* (18)
18	14.18±1.64 (17)	13.19±1.22 (15)	12.13±1.86* (12)
24	13.60±1.62 (8)	12.12±2.01 (6)	12.30±1.21 (4)
Hematocrit (%)			
3	41.86±1.44 (20)	41.52±0.84 (20)	40.26±1.58 (20)
6	39.46±1.89 (20)	38.50±1.90 (20)	36.91±2.18** (20)
12	42.04±3.03 (19)	39.08±3.73 (18)	37.53±6.73* (18)
18	42.98±4.87 (17)	39.79±3.40 (15)	36.46±5.41* (12)
24	42.06±5.11 (8)	37.68±6.54 (6)	38.08±3.35 (4)

*Significantly different from control value ($p \leq 0.05$).
**Significantly different from control value ($p \leq 0.01$).

[a]The numbers of animals included in calculation of the mean ±SD are given in parentheses.

TABLE 6. Serum Triglyceride Levels (±SD) in Male Rats Fed Atrazine for 2 Years

Dose Level (ppm)	Serum Triglyceride Level (mg/dL) at Month				
	3	6	12	18	24
0	98.37± 64.16	139.26± 88.35	221.05±145.16	210.12±192.78	141.82± 72.35
10	105.79± 62.29	147.74± 98.68	241.16±103.89	223.75±124.14	120.88± 54.49
50	89.55± 55.64	123.85± 84.87	251.32±149.51	232.13±143.48	211.31±116.93
500	62.21± 14.42	87.21± 66.94	171.89± 58.32	171.89± 69.51	141.73± 64.90
1000	53.00± 14.36	56.55± 18.12*	110.45± 43.08	133.00± 59.88	107.85± 62.45

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

13-month sacrifice, the triglyceride level was similar in control males and those that had previously received 1000 ppm atrazine. In females, glucose levels were decreased ($p < 0.01$) in the high-dose group at 3, 6, and 12 months when compared to controls (Table 7). Other changes in clinical chemistry parameters occurred sporadically in high-dose animals and were not considered compound related since there were no patterns consistent with dose or time.

6. Urinalyses: Urine was collected from fasted animals (chronic toxicity groups) at 3, 6, 12, and 18 months and prior to termination. It was also collected from animals of the control and high-dose groups prior to sacrifice at 12 and 13 months. The CHECKED (X) parameters were examined.

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

Results: Urinalysis data in dosed groups were comparable to control data and within the normal ranges.

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

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta†	XX	Brain† (3 levels)
X	Salivary glands†	X	Heart†	X	Peripheral nerves (sciate)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	X	Pituitary†
	(3 areas)	X	Spleen†	X	Eyes†
X	Duodenum†	X	Thymus†		Glandular
X	Jejunum†		Urogenital	XX	Adrenals†
X	Ileum†	XX	Kidneys†		Lacrimal gland
X	Cecum†	X	Urinary bladder†	X	Mammary gland†
X	Colon†	XX	Testes†	X	Parathyroids†
X	Rectum†	XX	Epididymides	XX	Thyroids†
XX	Liver†	XX	Prostate		Other
	(2 sections)	X	Seminal vesicle	X	Bone (femur)
	Gall bladder†	XX	Ovaries	X	and bone marrow†
X	Pancreas†	X	Uterus/cervix	X	Skeletal muscle†
	Respiratory			X	Skin
X	Trachea†			X	Harderian glands
X	Lung/bronchi†			X	All gross lesions and masses

†Recommended by Subdivision F (October 1982) guidelines for chronic studies.

TABLE 7. Serum Glucose Levels (mg/dL) in Female Rats Fed Atrazine for 2 Years

Interval (Month)	Dose Level (ppm)	
	0	1000
3	135.95± 14.91[a]	117.25± 12.16*
6	148.79± 15.31	129.79± 20.40*
12	127.16± 20.52	103.50± 22.74*
12[b]	121.80± 14.82	99.70± 19.32*
18	112.18± 29.75	104.00± 22.28
24	112.06±118.40	88.25± 20.01

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

[a]Mean ± standard deviation.

[b]Rats (10) from the oncogenicity group scheduled for interim sacrifice.

Results:

a. Organ Weights: The absolute weight of liver and kidney in high-dose males sacrificed at 12 months was significantly ($p \leq 0.05$) lower than controls. The mean weight of the liver was 14.71±2.81 g for the high-dose group and 19.70±3.46 g for the controls; the mean weight of the kidneys was 3.67±0.40 g for high-dose males, compared to 4.39±0.58 g for controls.

At 24 months, the mean absolute weights of liver and kidney in high-dose males were lower than those of controls, but the decrease was not statistically significant. There were no other changes in absolute organ weights of males and females.

There were several increases in organ-to-body weight ratios in high-dose animals that were significant ($p \leq 0.05$) when compared to controls but they were not accompanied by changes in absolute organ weights. These changes were the result of decreased body weights. At 12 months, the organ-to-body weight ratios for brain and kidney were increased in high-dose males; at 13 months, the ratios for brain, kidney, and testes were increased and at 24 months the ratios for brain and testes were increased in high-dose males. In high-dose females, the organ-to-body weight ratios for adrenal, brain, kidney, and liver were increased at 12 and 24 months. There was an increase in the ovary-to-body weight ratio at 13 months in the recovery group that had previously received 1000 ppm.

b. Gross Pathology: For most organs there were very few gross abnormalities noted at necropsy. There were no notable abnormalities at the 12- and 13-month interim sacrifices when high-dose rats were compared to controls. In the main study, there was an increased incidence of abnormal pelvic contents in the kidneys of high-dose males (10/65) when compared to controls (4/67). In females, there were some increases in the incidence of masses in the abdominal, thoracic, and axillary regions in rats receiving 70, 500, and 1000 ppm when compared to controls. Correlation of gross and microscopic findings indicated that most of the masses were mammary tumors. Masses found on weekly palpations were correlated with gross finding for all individual males and all high-dose females. An excellent correlation was found (see Discussion).

c. Microscopic Pathology:

1. Nonneoplastic: A summary of selected nonneoplastic lesions is presented in Table 8. Several proliferative lesions occurred with increased frequency in atrazine-dosed rats. Acinar hyperplasia of the mammary gland and epithelial hyperplasia of the prostate were increased in males receiving 1000 ppm when compared to controls. In females receiving 500 or 1000 ppm there was an increased

TABLE 8.- Nonneoplastic Lesions in Rats Fed Atrazine for 2 Years[a]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Mammary gland	(58)[b]	(59)	(61)	(64)	(65)	(66)	(64)	(68)	(65)	(64)
Acinar hyperplasia	7	1	5	7	21**T	0	0	0	0	0
Bone marrow—femur	(65)	(63)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(64)
Myeloid hyperplasia	23	30	21	21	27	25	23	24	38**	52**T
Bone marrow—sternum	(65)	(65)	(67)	(67)	(66)	(68)	(65)	(69)	(65)	(64)
Myeloid hyperplasia	23	28	21	27	26	21	21	20	33*	46**T
Spleen	(65)	(65)	(67)	(67)	(67)	(67)	(65)	(69)	(65)	(65)
Extramedullary hematopoiesis	6	10	14	9	9	12	14	18	22*	28**T
Kidney	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(68)	(65)	(65)
Pelvic calculi	15	16	11	17	31**T	57	52	57	55	60
Micro calculi	3	5	3	7	8	3	0	0	3	2
Hyperplasia, transitional epithelium	9	8	12	13	13	17	10	5**	19	31**
Urinary bladder	(65)	(65)	(67)	(67)	(67)	(67)	(65)	(69)	(65)	(64)
Hyperplasia, transitional epithelium	4	2	5	3	5	4	0	1	3	10*
Prostate	(65)	(63)	(66)	(67)	(66)					
Epithelial hyperplasia	12	16	11	17	29**T					
Muscle—rectus femoris	(64)	(65)	(67)	(66)	(67)	(67)	(65)	(69)	(64)	(64)
Degeneration	6	7	7	10	28**T	5	4	9	8	13*T
Eye	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Retinal degeneration	2	2	5	5	7	12	9	13	16	22*T
Liver	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Centrolobular necrosis	6	3	1	2	2	3	3	1	4	12**

[a]Includes animals at terminal sacrifice and those that died or were sacrificed moribund from month 13 to study termination.

[b]The number of tissues examined is given in parentheses.

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

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

[T]Positive dose-related trend ($p \leq 0.01$).

incidence of myeloid hyperplasia in the bone marrow of both the femur and sternum. It was reported that the bone marrow changes, as well as an increase in extramedullary hematopoiesis in the spleen, were sequellae related to mammary fibroadenomas and adenocarcinomas. The myeloid hyperplasia was characterized by a decrease in the number of fat cells in the marrow and an increase in hematopoietic tissue, particularly cells of the granulocytic series. In females receiving 1000 ppm, there was also an increased incidence of hyperplasia of the transitional epithelium of both the kidney ($p < 0.01$) and urinary bladder ($p > 0.05$). The incidence of calculi in the renal pelvis in high-dose males was increased compared to controls. Muscle degeneration (femoral muscle) was found in both high-dose males and females. Retinal degeneration was increased in both dosed males and females; the incidence being significantly ($p \leq 0.05$) higher in the high-dose females than in controls. In high-dose females there was an increase in coagulative centrolobular necrosis in the liver. There was a slight increase in chronic pododermatitis in females receiving 500 and 1000 ppm (16-17%) when compared to controls (1%) but the incidence was much lower than in all groups of males including controls (30-39%).

2. Neoplastic: Table 9 summarizes the neoplastic lesions found in rats that died from month 13-24 or were sacrificed at termination. There was an increased incidence of mammary adenocarcinoma in females receiving 70, 500, or 1000 ppm atrazine and an increase in fibroadenoma in high-dose females. Several females had multiple mammary tumors. There was also an increase in adenocarcinoma in high-dose females at the 12- and 13-month sacrifices (Table 10). Statistical analysis by the report authors included all animals on study and used life-table analysis and pairwise comparison with the Cox-Tarone test and Gehan-Breslow test. These results are included in Table 10. There were statistically significant increases ($p < 0.05$) in carcinomas for females receiving 70, 500, and 1000 ppm atrazine, in adenomas and fibroadenomas for females receiving 1000 ppm, and in total mammary tumors in females receiving 500 and 1000 ppm. There were significant ($p < 0.00005$) positive dose trends for all three categories (sarcomas, fibroadenomas plus adenomas, and all mammary tumors).

D. STUDY AUTHORS' CONCLUSIONS:

Atrazine was oncogenic in female CD Sprague-Dawley rats. There were increased incidences of mammary carcinomas at 70, 500, and 1000 ppm, all mammary tumors at 500 and 1000 ppm, and of mammary adenoma and fibroadenoma at 1000 ppm. Survival was significantly higher in males

TABLE 9. Neoplastic Lesions in Rats Fed Atrazine for 2 Years[a]

Organ/Neoplasm	Dietary Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Brain	(65)[b]	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Chromophobe carcinoma	3	1	1	0	1	2	1	3	8	7
Astrocytoma	1	0	0	0	2	0	0	0	0	0
Adrenal	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Pheochromocytoma	11	9	4	5	5	1	2	2	3	4
Cortical adenoma	1	3	0	0	0	5	5	6	4	3
Pituitary	(59)	(64)	(67)	(66)	(62)	(68)	(63)	(68)	(65)	(63)
Chromophobe adenoma	22	22	29	24	17	47	41	49	47	35
Chromophobe carcinoma	5	7	6	7	4	9	6	9	14	13
Thyroid	(63)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
C-cell adenoma	12	9	8	21	9	8	10	6	5	5
C-cell carcinoma	2	4	2	3	3	0	0	1	1	0
Follicular cell adenoma	5	4	1	2	3	1	1	1	0	0
Kidney	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Liposarcoma	0	0	5	0	2	0	0	0	0	0
Liver	(65)	(65)	(67)	(67)	(67)	(68)	(65)	(69)	(65)	(65)
Hepatocellular carcinoma	4	5	0	1	2	0	1	1	0	0
Hepatocellular adenoma	0	0	1	2	3	1	1	2	1	0
Pancreas	(64)	(65)	(67)	(67)	(67)	(67)	(65)	(69)	(65)	(65)
Islet cell adenoma	4	5	4	2	3	3	0	3	1	0
Islet cell carcinoma	1	1	3	2	0	5	1	2	0	0
Testis	(65)	(65)	(67)	(67)	(67)					
Interstitial cell tumor	1	3	2	2	7					
Uterus						(67)	(65)	(69)	(62)	(65)
Endometrial stromal polyp						4	6	3	0	3

(Continued)

[a]This tabulation includes animals sacrificed at termination or found dead or sacrificed moribund between 13 months and study termination. Statistical notations are not included in this table since the authors' analysis included animals sacrificed at 12 and 13 months.

[b]The number of tissues examined is in parentheses.

TABLE 9. Neoplastic Lesions in Rats Fed Atrazine for 2 Years[a] (Continued)

Organ/Neoplasm	Dietary Level (ppm)									
	Males					Females				
	0	10	70	500	1000	0	10	70	500	1000
Mammary gland	(58)	(59)	(61)	(64)	(65)	(66)	(64)	(68)	(65)	(64)
Adenocarcinoma	0	1	0	0	1	15	15	26	27	35
Fibroadenoma	1	1	1	1	0	29	29	35	38	42
Adenoma	0	0	0	1	1	1	0	1	1	2
Carcinosarcoma	0	0	0	0	0	0	0	0	0	2
Skin	(4)	(6)	(3)	(7)	(6)					
Keratoacanthoma	1	3	0	2	2	0	0	2	0	1
Lipoma	2	0	0	0	0	1	0	0	0	0
Fibroma	0	0	0	0	3	0	0	0	0	0
Trichoepithelioma	0	0	0	2	0	0	0	0	0	0
Head										
Zymbal gland carcinoma	4	2	0	3	1	0	0	1	0	0

(Concluded)

[a] This tabulation includes animals sacrificed at termination or found dead or sacrificed moribund between 13 months and study termination. Statistical notations are not included in this table since the authors' analysis included animals sacrificed at 12 and 13 months.

[b] The number of tissues examined is in parentheses.

TABLE 10. Mammary Tumors in Female Rats Fed Atrazine for 2 Years

	Dose Level (ppm)				
	0	10	70	500	1000
12-month sacrifice/deaths and moribund sacrifices at 0-13 months					
No. tissues examined	12	5	1	5	15
Adenocarcinoma	0	1	1	0	3
Fibroadenoma	0	0	1	1	1
13-month sacrifice					
No. tissues examined	10	--	--	--	10
Adenocarcinoma	0	--	--	--	5
Fibroadenoma	0	--	--	--	2
Terminal sacrifice/deaths and moribund sacrifices at 13-24 months					
No. tissues examined	66	64	68	65	65[a]
No. rats--adenocarcinoma and carcinosarcoma	15	15	26	27	37
No. of adenocarcinomas	17	22	42	48	64
No. rats--fibroadenoma	29	29	35	38	42
No. of fibroadenomas	37	46	48	81	69
Mammary tumor-bearing rats	35	39	47	47	56[a]
All animals on study					
No. of tissues	88	69	69	70	89
Carcinomas	15	16	27	27	45
Adenomas and fibroadenomas	29	29	36	39	46
All tumors	35	40	48	48	65
p values[b]					
Carcinomas--Cox-Tarone			0.0454	0.0071	<0.00005
--Gehan Breslow			0.0290	0.0016	<0.00005
Adenomas and fibroadenomas--Cox-Tarone				0.0685	0.0004
All tumors--Cox-Tarone				0.0071	<0.00005
--Gehan-Breslow				0.0050	<0.00005

[a]Values differ from those in final report by 1.

[b]Life-table analysis, pairwise comparison.

receiving 1000 ppm and significantly lower in females receiving 1000 ppm than in controls. There was a significant decrease in body weight throughout the study for males and females in the 500- and 1000-ppm groups. Food consumption was decreased in males receiving 500 and 1000 ppm for the first year of the study, for females receiving 1000 ppm for the first 6 months, and for females receiving 500 ppm for the first 3 months. Red cell parameters were decreased in females receiving 1000 ppm but not in males. Glucose was decreased in 1000 ppm females at 3, 6, and 12 months and triglycerides were decreased in 1000-ppm males at 3 and 6 months. Nonneoplastic findings were limited to animals receiving 1000 ppm. An increase in mammary acinar cell hyperplasia, kidney calculi, and epithelial hyperplasia of the prostate in high-dose males may be associated with increased survival in this group. Retinal degeneration and centrolobular necrosis of the liver were increased in high-dose females; there was an increase in degeneration of the rectus femoris muscle in high-dose males and females. Increased occurrence of transitional cell hyperplasia in the kidney and bladder of high-dose females was of questionable significance. The NOEL for chronic toxicity was considered to be 70 ppm atrazine in the diet.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

In agreement with the report authors, we assess that atrazine was carcinogenic in female CD rats, causing an increase in mammary adenocarcinomas at dietary levels of 70, 500, and 1000 ppm. The report authors found a high level of statistical significance and a positive dose-related trend ($p < 0.00005$) with both the Cox-Tarone test and the Gehan-Breslow test using life-table analysis. The concurrent control incidence of mammary adenocarcinoma (17.05%) was somewhat higher than the mean value for four other studies (9.5 percent, range 3.8-18.9 percent) performed by the testing laboratory. The biological importance of the significant increase in mammary adenoma plus fibroadenoma in high-dose females ($p = 0.004$) is not as clear as that of the adenocarcinomas. The concurrent control incidence of fibroadenoma (29/88, 33 percent) is slightly lower than the historical incidence for the laboratory (mean 41.4 percent, range of four studies 36.3-47.8 percent).

We assess that the study authors correctly interpreted that the increase of interstitial cell tumors of the testes in high-dose males (7/63) when compared to controls (1/65) was probably related to increased survival in the males, which resulted in more late-appearing tumors. The incidence of the finding in final sacrifice males was 6/47 in the high-dose group and 1/31 in controls; in those that died between 13-24 months, the incidence was 1/20 in the high-dose group and 0/34 in controls. Historical control incidence for interstitial cell tumors in terminal sacrifice animals was 8 percent (range, 0-19 percent). The incidence in high-dose males was 12 percent in the current study.

Time to mammary tumor could not be calculated since the individual animal disposition tabulations were not provided. However, we noted that 8/25 high-dose females (compared to 0/22 controls) had mammary adenocarcinomas by 13 months (Table 10).

Weekly palpation data were available for all animals. These data were checked against the gross and histologic findings to determine if all in-life masses were followed through with a gross finding and a histologic diagnosis. The data for all males were checked as well as the data for high-dose females. Masses that disappeared were checked for the first and last day of observation.

In the high-dose females, more than 90 percent of all masses observed in-life persisted until sacrifice or death. There were none that disappeared in the last 2 months of the study. The followup at gross and histologic examination was excellent. For female 8364 there was no section for a mass and for female 8429 the masses were lost; female 8183 had a small mass that was not a tumor and female 8330 had an abdominal mass but no tumor at gross or histologic examination. All other masses had a histologic diagnosis.

In males, there were several masses that were transient. Cell masses that persisted were confirmed by gross or histologic examination. The following lists in-life masses that disappeared in males:

Dose (ppm)	No. animals	No. animals with masses	No. masses
0	90	20	32
10	70	19	36
70	70	17	43
500	70	25	42
1000	90	27	56

In four control males and in two high-dose males masses not seen in-life were found on gross examination and confirmed histologically. None of the males that died by month 13 and none that were sacrificed at 12 months had mammary tumors. Mammary tumors in males were as follows: control, fibroadenoma (8051); 10 ppm, adenocarcinoma (7805), fibroadenoma (7906); 70 ppm, fibroadenoma (7951); 500 ppm, adenoma (7701), fibroadenoma (8017); 1000 ppm, adenocarcinoma (8063), fibroma (7896). Other masses were diagnosed as abcesses, galactocoeles, lipomas, fibromas, papillomas, histiocytic sarcomas, zymbal gland carcinomas, etc. In two males receiving 1000 ppm (8030 and 3818), late appearing masses were not found on gross examination. It is our assessment that the gross and histologic followup of in-life masses was excellent.

There was an increase in several hyperplastic lesions in rats receiving 1000 ppm atrazine. The increase in hyperplasia of the transitional epithelium in kidney and urinary bladder in high-dose females is probably compound related. The increase in acinar hyperplasia of the mammary glands and in epithelial hyperplasia of the prostate in high-dose males could be compound related and/or the result of increased survival in this group. Other lesions noted in high-dose animals such as muscle degeneration (males and females), retinal degeneration (females), and pelvic calculi in the kidney (males) are normally occurring lesions of aging.

The decrease in red cell parameters in high-dose females and increase in myeloid hyperplasia and increased extramedullary hematopoiesis in females receiving 500 or 1000 ppm atrazine may be a consequence of the development of mammary tumors.

The increases in organ-to-body weight ratios noted in the study are primarily due to decreased body weights. The effects of triglycerides and glucose may also be related to the weight loss. When all chronic toxicity parameters are considered it is reasonable to set a LOEL at 500 ppm and a NOEL at 70 ppm.

The study was well conducted and adequately reported. Once individual animal disposition data are provided, the study can be classified Core Guideline.