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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: TRANSMITTAL OF THE REVIEW OF HORMONE LEVELS IN FISCHER -344 RATS TREATED WITH ATRAZINE TECHNICAL (MRID 42146101)

BARCODE D 172940
CASE 838836
S 409411
Tox # 063
PC 080803

TO: KATHLEEN PEARCE, PM # 63
SPECIAL REVIEW BRANCH
SRRD (H7508W)

FROM: HENRY SPENCER, PH.D. 4/15/93.
REVIEW SECTION 3
TOXICOLOGY BRANCH 1
HEALTH EFFECTS DIVISION (H7509C)

THRU: KAREN HAMERNIK, PH.D. 7/21/93 KB 7/23/93
ACTING SECTION HEAD
REVIEW SECTION 3
TOXICOLOGY BRANCH 1
HEALTH EFFECTS DIVISION (H7509C)

ACTION:

Review the study on Hormone levels in the Fischer 344 rats fed Atrazine technical.

CONCLUSIONS:

1. The female rat was used in the study and only a limited number of tissues were examined to include the pituitary gland, uterus, ovaries and mammary glands. No analysis for hormone levels were reported in this submission as the name of the study would imply. Additionally, hematology results were not reported in the submission.

2. There were 70 animals assigned to each dose group and 10 animals per dosage group were sacrificed at each time period. By carrying out multiple sacrifices of these

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small groups, the study was not conducted to meet the requirements of the GL 83-1, or 83-2.

3. Atrazine did not appear to have any effect on the development of tumors in the organ systems examined. Neither the tumor type nor frequency in these organs increased with increasing dosage at any time period evaluated.

4. A NOEL for chronic toxicity based on decreases in body weight & body weight gain, could be established at 70 ppm with an LOEL set at 200 ppm for these effects.

5. The study is classified as core: Supplementary.

DOC930024

FINAL

DATA EVALUATION REPORT
ATRAZINE
DETERMINATION OF HORMONE LEVELS IN FISCHER-344 RATS TREATED WITH ATRAZINE TECHNICAL

Prepared for:

Health Effects Division
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Contract Number: 68D10075
Work Assignment Number: 1-88
Project Officer: Mr. Jim Scott
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Secondary Review by: Karen Hamernik, Ph.D.
Toxicology Branch I/HED

Signature Henry Spencer
Date 12/23/92
Signature Karen Hamernik
Date 7/21/93

DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity/Oncogenicity - Rats

TEST MATERIAL: Atrazine Technical TOX. CHEM. NO.: 063 PC 080803

SYNONYMS: 2-chloro-4-ethylamino-6-isopropylamino-s-triazine

MRID NO.: 421461-01

STUDY NUMBER:

SPONSOR: Ciba-Geigy Corporation
Agricultural Division
P. O. Box 18300
Greensboro, North Carolina 27419

TESTING FACILITY: Hazleton Washington, Inc.
9200 Leesburg Turnpike
Vienna, Virginia 22182

TITLE OF REPORT Determination of Hormone Levels in Fischer-344 Rats Treated with Atrazine Technical

AUTHORS: Ajit K. Thakur, Ph.D.

REPORT ISSUED: November 8, 1991

CONCLUSIONS:

1. This study was conducted to determine the effect of atrazine technical on tumor development in the pituitary gland, uterus, ovaries, and mammary glands.

2. Atrazine technical was administered in the diet to groups of 70 female CDF®(F-344)/ CrlBR rats at concentrations of 0, 10, 70, 200, and 400 ppm. Interim sacrifices were conducted on ten animals/dose group at approximately 1, 3, 9, 13, 15, and 18 months, with a terminal sacrifice at 24 months.

3. At terminal sacrifice, only 5 to 9 animals per dose group remained for examination.

4. This study was not conducted to meet the requirements of any Guidelines (§83-1, Chronic Toxicity, §83-2 Oncogenicity) and was evaluated as supplementary information.

5. Atrazine did not appear to have any effect on the development of tumors in the organ systems evaluated. Neither the tumor type nor frequency in these organs increased with increasing dose at any time period evaluated.

6. A NOEL for chronic toxicity, based on decrease in body weight & Body gains 4/15/93 Hed ~~organ~~ weight, ~~and nonneoplastic macroscopic and microscopic examinations~~ could be set at 70 ppm.

7. Conclusions as to the chronic toxicity of atrazine or the oncogenic potential based on these data are limited by the study design. Only four organs were examined microscopically and other types of tests, such as clinical chemistry, urinalysis and hematological tests were not conducted.

CLASSIFICATION: SUPPLEMENTARY INFORMATION

The systemic No Observed Effect Level (NOEL) = 70 ppm

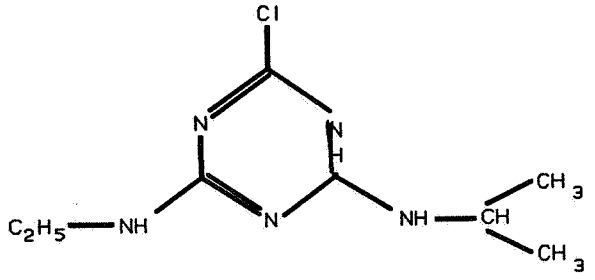
The Lowest Observed Effect Level (LOEL) = 200 ppm reduced body weights and body weight gains.

I. MATERIALS, METHODS AND RESULTS

A. Test Article Description

Name: Atrazine Technical (2-chloro-4-ethylamino-6-isopropylamino-s-triazine)

Formula:



Batch Number: D3413J10

Purity: 97%

Physical Property: white powder

Stability: 7 days at room temperature

B. Test Article Analyses for Purity and Stability

A purity of 97% atrazine technical was used in this study. Homogeneity and stability tests were conducted for the low- (10 ppm) and high- (400 ppm) diets prior to study. During the mixing of the diet, duplicate samples were taken from the top, middle, and bottom. Homogeneity and stability analyses were carried out. Stability of atrazine in the diet was slightly decreased in the 400 ppm diet at day 7 and had decreased to 15% less than the target concentration at day 21. New diets were prepared weekly.

C. Animals

Four hundred and sixteen female CDF® (F-344)/Crl BR rats were purchased from Charles River Laboratories (Raleigh, North Carolina). Following a 2-week quarantine period at 6 weeks of age, all animals were examined for condition and disease. The animals were then laboratory acclimated for 3 weeks. The animals were initially housed two per cage and randomly assigned to treatment groups by a body-weight-stratified, computerized, randomization procedure.

D. Dosing

Atrazine technical was administered at dietary concentrations of 0, 10, 70, 200, or 400 ppm to groups of 70 female rats. Diets containing atrazine technical were available *ad libitum*. Interim sacrifices of 10 animals/group were performed at months 1, 3, 9, 12, 15, and 18 months with terminal sacrifice of surviving animals at 24 months.

Table 1

RESULTS OF HOMOGENEITY ANALYSES FOR ATRAZINE TECHNICAL

Dietary level (ppm)	Blender discharge	Corrected assayed value (ppm)	Percent Target
0		+[a]	
10	Top	9.62	96.2
		9.90	99.0
	Middle	9.71	97.1
		9.90	99.0
	Bottom	9.62	96.2
		9.62	96.2
400	Top	380	95.0
		387	96.8
	Middle	382	95.5
		384	96.0
	Bottom	382	95.5
		388	97.0

[a] The positive detection in control was due to feed matrix interference. A revised dilution scheme was developed that alleviated this interference.
Data extracted from Table 1 located on Report page 37.

Table 2

STABILITY OF ATRAZINE TECHNICAL IN RODENT DIET FORMULATIONS

Dietary level (ppm)	Storage time (days)	Corrected assayed value (ppm)	Percent target
0	0–21	+[a]	0
10	0	9.65	96.5
		9.81	98.1
	7	10.4	104
		9.59	95.9
	14	9.24	92.4
		9.14	91.4
	21	9.09	90.9
		9.19	91.9

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Table 2 (continued)

STABILITY OF ATRAZINE TECHNICAL IN RODENT DIET FORMULATIONS

Dietary level (ppm)	Storage time (days)	Corrected assayed value (ppm)	Percent target
400	0	381	95.3
		386	96.6
	7	393	98.3
		380	95.0
	14	360	90.0
		375	91.9
	21	358	89.5
		358	84.5

ND - None detected
[a] The positive detection in control was due to feed matrix interference.

Fresh diets were prepared weekly. The formulation of the test diet was not adjusted for purity. Each level of atrazine technical was premixed with feed and then added to the required amount of feed and mixed, then stored at room temperature.

The dietary atrazine levels selected for this study were based on the results of previously conducted studies, and discussions with EPA personnel. The concentration of atrazine technical in the diet was assessed at weeks 1, 4, 6, 10, 16, 18, 27, 31, 33, 35, 39, 42, 50, 57, 60, 61, 79, 80, 81, 82, 88, 89, 90, 93, 103, and 104. The diet was determined to be adequate for use if the measured concentration was within $\pm$15% of the target concentration.

E. General Observations

The authors reported that absolute body weight, body weight changes, total food consumption, and organ weight data were analyzed using one-way Analysis of Variance. Levene's test for homogeneity of variance was followed by ANOVA, and then Dunnett's test was used for groupwise comparisons. If variances of untransformed data were heterogeneous, the data were transformed to achieve variance homogeneity. When the series of transformations were unsuccessful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were routinely performed at the 5.0% two-tailed probability level. The analyses used to evaluate any other endpoints in the study were not reported.

1. Mortality/Moribundity/Survival - All rats were observed twice daily for mortality and moribundity. There did not appear to be survival problems. One to five animals per dose group (a total of 13 animals) were lost on study due to death or moribund sacrifice and the number of animals at terminal sacrifice was higher in the high-dose group than in the controls. 8

2. Clinical Observations - Cageside observations were performed once daily. Thorough physical examinations were performed weekly. All reported observations were of the type and incidence commonly observed in animals of this species and strain in the test laboratory. The number of animals displaying lacrimation was more prevalent in controls and the lowest dose, and chromodacryorrhea was reduced with increasing dose in the two highest dose groups.

3. Body Weights/Food Consumption/Test Material Intake - Individual body weight and individual food consumption values were recorded weekly from initiation of treatment to week 16 and once every fourth week thereafter. Compound consumption was calculated in the study.

   Mean body weight and body weight gain values for the 200 and 400 ppm dose groups were generally lower than respective control values. Statistically significant decreases were observed at weeks 4, 13, 40, and 64 for body weights for the periods of weeks 0-4 (14%), 0-13 (10%), 0-40 (10%), and 0-64 (8%) for body weight gains in the 200 ppm dose group. In the 400 ppm dose group, statistically significant decreases in body weights were reported for weeks 4, 13, 40, 52, 64 and 76. Body weight gain decrements were significantly decreased (10-18%) throughout the study. A decreasing number of animals were evaluated and less than 10 animals per group on average were evaluated for body weight gain over the duration of the study.

   Total food consumption for all treated groups for all intervals analyzed was within 10% of the control value. In comparison to respective control values, there were significant decreases in mean total food consumption for all treated groups at weeks 1-4, for the 200 and 400 ppm dose groups at weeks 1-13, and for the 400 ppm dose group at weeks 1-40, 1-52, 1-64, and 1-76. No significant decreases in total food consumption were observed in any treated group compared to controls by terminal sacrifice (104 weeks).

4. Ophthalmoscopic examination - No details about ophthalmoscopic examinations were included in the Materials and Methods section of this study. Ophthalmic endpoints, such as opaque eyes and chromodacryorrhea, were included in the evaluation of clinical signs. The incidences of ophthalmic endpoints were not significantly increased compared to untreated controls.

F. Clinical Pathology

1. Hematology - Trunk blood was collected at each scheduled sacrifice and was sent to another institution for analysis. Hematology results were not presented in this study.

2. Blood Chemistry - Blood chemistry analyses were not conducted in this study. Although the title of the study indicates that hormone levels would be determined, no analyses for hormone levels were conducted.

3. Urinalysis - No urinalyses were conducted in this study.

4. Estrous Cycle Determination - Estrous cycle determination for 10 animals per group was established 2 weeks prior to the start of each scheduled sacrifice. A daily vaginal smear was made for each animal scheduled for sacrifice. The concentration of cells was scored as 0 (sparse), 1 (medium), or 2 (heavy) for cornified (C), nucleated (N), and leukocytes (L). Each animal was sacrificed at the first proestrus phase observed after a minimum 14 days of vaginal smears. The proestrus phase was indicated by the first appearance of well-defined cornified cells, a medium (C-1) or heavy (C-2) concentration of cornified cells, with possible nucleated cells present. All animals scheduled for sacrifice that had not reached the proestrus phase by 21 days of lavaging were sacrificed. This would indicate that the animals scheduled for sacrifice, for example at one month, were not all sacrificed at one scheduled time, but that the actual sacrifices were performed over a 6-day period (days 15-21 of the estrous cycle determination period). These results of the smears, which included the concentrations of cells present, were reported; however, the authors indicated that analysis and interpretation of these data were the responsibility of the Sponsor.

G. Sacrifice and Pathology

Sacrifices were performed on 10 (9 for Month 18 sacrifice) rats/group at the completion (approximately) of Months 1, 3, 9, 12, 15, 18 and on all remaining animals (5-9 per dose group remained; 7 animals remained in the control group and 9 remained in the high-dose group) after Month 24. On the day of scheduled sacrifice, each designated animal was weighed. Necropsies were performed on all animals following death (scheduled or unscheduled) by trained personnel using procedures approved by board-certified pathologists. Only the pituitary, mammary glands, uterus, and ovaries were examined microscopically.

1. Macroscopic - The necropsy included a gross examination of the external surface of the body, all orifices, the cranial cavity, the carcass, the external surface of the brain, the external surface of the spinal cord and cut surfaces of the brain and spinal cord, the nasal cavity and paranasal sinuses, the thoracic, abdominal, and pelvic cavities and their viscera, and the cervical tissues and organs.

   No statistically significant increases in the incidences of any macroscopic lesion were observed in the animals examined at each interim sacrifice or at terminal sacrifice (Fisher's exact, $p<0.05$). However, 10 animals or less could be evaluated at each time interval. The macroscopic findings observed were typically reported at this laboratory for this strain of rats, but no data to support this statement were provided. The gross examination findings noted were primarily in the uterus and pituitary, and the observed incidence of any lesion across all time periods was comparable between control and treated groups.

2. Organ weights and body weight ratios - Significant decreases in some absolute and relative organ weights were observed in the organs of the 10 animals per dose group evaluated at each scheduled interim sacrifice. However, no significant changes in the absolute or relative organ weights of the remaining animals (5-9 per dose group) were observed at terminal sacrifice, when compared to controls.

3. Microscopic

   There were no dose-related increases in the incidence of any microscopic lesion at any of the interim sacrifices or at terminal sacrifice in the organ systems examined (see Table 4). The first tumor observed was a pituitary adenoma at the 9 month interim sacrifice in the 200 ppm dose group. No statistically significant or dose-related increase in the incidence of this tumor type was observed at terminal sacrifice; therefore, the incidence of this tumor type does not appear to be related to atrazine administration. In animals evaluated at terminal sacrifice, pituitary adenomas, ovarian theca cell tumors, uterine leiomyosarcomas, and mammary fibroadenomas were observed. The incidences of these tumors did not increase in a dose-related fashion.

A brief description of the statistical analysis employed was included in the report.

A Good Laboratory Practice Compliance Statement, a Quality Assurance Statement, and a list of Quality Assurance inspections were included.

Table 4

HISTOPATHOLOGY INCIDENCE SUMMARY IN FEMALE RATS
TREATED WITH ATRAZINE TECHNICAL

Months	0 ppm	10 ppm	70 ppm	200 ppm	400 ppm
Tumor: Pituitary Adenoma					
1	0/10	0/10	0/10	0/10	0/10
3	0/10	0/10	0/10	0/10	0/10
9	0/10	0/10	0/10	1/10	0/10
12	1/10	0/10	1/9	1/10	1/10
15	2/10	1/10	1/10	0/10	0/10
18	1/10	1/10	2/10	2/10	0/9
24	5/7	2/9	1/5	1/8	4/8
Tumor: Ovary Malignant and Benign Granulosa/Thick Cell					
1	0/10	0/10	0/10	0/10	0/10
3	0/10	0/10	0/10	0/10	0/10
9	0/10	0/10	0/10	0/10	0/10
12	0/10	0/10	0/9	0/10	0/10
15	0/10	0/10	0/10	0/10	0/10
18	0/10	0/10	0/10	0/10	0/9
24	1/7	1/9	0/5	0/8	0/9
Tumor: Uterine Leiomyosarcoma					
1	0/10	0/10	0/10	0/10	0/10
3	0/10	0/10	0/10	0/10	0/10
9	0/10	0/10	0/10	0/10	0/10
12	0/10	0/10	0/10	0/10	0/10
15	0/10	0/10	0/10	0/10	0/10
18	1/10	0/10	0/10	0/10	0/9
24	0/7	1/9	0/5	0/8	0/9
Tumor: Mammary Fibroadenoma					
1	0/6	0/9	0/10	0/9	0/8
3	0/10	0/10	0/10	0/10	0/10
9	0/10	0/9	0/8	0/8	0/10
12	0/9	0/10	0/9	0/9	0/10
15	0/10	0/10	0/9	0/10	0/10
18	0/10	1/10	0/10	0/10	0/9
24	3/7	1/9	1/5	2/8	0/9

II. DISCUSSION

This study was conducted to evaluate whether atrazine administration had any impact on tumor development in the mammary gland, ovaries, pituitary, or uterus, along with the determination of hormone levels and estrous cycles in female rats. Ten animals/dose group were sacrificed at six selected time intervals, leaving approximately 10 animals/dose group remaining at terminal sacrifice. The incidence of tumors in the organs that were microscopically examined did not increase with time and were not statistically significantly increased compared to controls at any time period (tests done by reviewer using Fisher's exact $p<0.05$). In some instances, the incidences of tumors in control animals were greater than or equal to the incidence in treated animals. Therefore, it does not appear that atrazine had an effect on tumor development in these organs in treated animals.

In evaluating the chronic toxicity of atrazine, no hematology or clinical chemistry tests were conducted. In addition, many organ systems were not examined microscopically so toxic or oncogenic effects could not be assessed for these other organs. Therefore, it is difficult to determine the chronic toxicity of atrazine based on the information reported in this study. Decreases in body weights and body weight gains were observed in the 200 ppm and 400 ppm dose groups, indicating that adequate dosage levels were administered.

III. CONCLUSIONS

This study was conducted to determine the effect of atrazine technical on tumor development in the pituitary gland, uterus, ovaries, and specifically the mammary glands. Atrazine technical was administered in the diet to groups of 70 female CDF®(F-344)/ CrlBR rats at concentrations of 0, 10, 70, 200, and 400 ppm. Interim sacrifices were conducted on ten animals/dose group at approximately 1, 3, 9, 13, 15, and 18 months, with terminal sacrifice at 24 months. At terminal sacrifice, only 5 to 9 animals per dose group remained for examination, due to the large number of interim sacrifices. Since this study was not conducted to meet the requirements of any Guidelines (§83-1, Chronic Toxicity, §83-2 Oncogenicity, or §83-5 Combined Toxicity/Oncogenicity), it was evaluated as supplementary information. Atrazine did not appear to have any effect on the development of tumors in the organ systems evaluated. Neither the tumor type nor frequency in these organs increased with increasing dose at any time period evaluated. A NOEL for chronic toxicity, based on decreases in body weight and body weight gains ~~, organ weight, and nonneoplastic macroscopic and microscopic examinations~~ could be set at 70 ppm. Conclusions as to the chronic toxicity of atrazine or the oncogenic potential based on these data are limited by the study design. Only four organs were examined microscopically and other types of tests, such as clinical chemistry, urinalysis and hematological tests were not conducted.

CLASSIFICATION: SUPPLEMENTARY INFORMATION

The systemic No Observed Effect Level (NOEL) = 70 ppm

The Lowest Observed Effect Level (LOEL) = 200 ppm reduced body weights and body weight gains.