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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND TOXIC
SUBSTANCES

July 23, 1996

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on Atrazine (5th)
FROM: Esther Rinde, Ph.D. *E.R.*
Manager, Carcinogenicity Peer Review
Health Effects Division (H7509c)
TO: Addressees

Attached for your review is a package on Atrazine prepared by Dr. Melba S. Morrow.

A meeting to consider the carcinogenicity classification of this chemical is scheduled for Wednesday July 31, 1996, at 10:00 am in Room 817, CM2.

Addressees

- S. Irene
- W. Burnam
- K. Baetcke
- K. Dearfield
- H. Pettigrew
- B. Fisher
- L. Brunzman
- E. Doyle
- Y. Ioannou
- M. Copley
- M. Morrow
- J. Stewart
- R. Hill
- Y. Woo
- A. Aranda
- R. Ross/L. Brennecke



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

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PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Fifth Carcinogenicity Peer Review for Atrazine

FROM: Melba S. Morrow, D.V.M. *msm 7/22/96*
Section II, Toxicology Branch I
Health Effects Division

TO: Esther Rinde, Ph.D.
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division

THRU: Joycelyn E. Stewart, Ph.D. *JS 7/23/96*
Head, Section II
Toxicology Branch I
Health Effects Division
and
Karl Baetcke, Ph.D. *KB 7/28/96*
Chief, Toxicology Branch I
Health Effects Division

Attached is a copy of the Weight of the Evidence Document for the fifth carcinogenicity peer review for Atrazine.

The purpose of this document is to present new data and supporting documentation provided by Ciba Geigy that address the mechanism of carcinogenesis.

The Peer Review Committee is asked to evaluate the new information and to classify the chemical accordingly.



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Attachments
No. 1 Review from T. Crisp
No. 2 Statistical Analysis from L. Brunsman
No. 3 DER from K. Farwell
No. 4 Figure 1, S. Triazine Herbicides

2. Background

Atrazine has been the subject of four previous HED Cancer Peer Review Committee evaluations and one evaluation by the Scientific Advisory Panel. Since the time of these reviews, the sponsor, Ciba Geigy, has proposed several theories pertaining to the mechanism of mammary carcinogenicity in female Sprague Dawley rats.

The first HED cancer peer review of atrazine was held on September 10, 1987. A rat oncogenicity study conducted at dietary levels of 0, 10, 70, 500 and 1000 ppm (0, 0.5, 3.5, 25 or 50 mg/kg) in Sprague Dawley rats was the subject of the peer review. In this study, an increase in the incidence of carcinomas of the mammary gland was reported at 3.5, 25 and 50 mg/kg/day; an increase in the incidence of fibroadenomas/adenomas was reported at 50 mg/kg and an increase in the incidence of all mammary tumors at 25 and 50 mg/kg/day. At 50 mg/kg, there was an increase in the incidence of testicular tumors in males; however, it was determined by the Committee that because of the increase in mortality in females and the 26% decrease in body weight gain, this level exceeded the MTD and tumors occurring at this dose level would not be considered in determining the cancer classification. The compound was classified as a C carcinogen based on the increased incidence of mammary adenocarcinomas reported at doses of 3.5 mg/kg and above. A Q_1 of 2.2×10^{-1} was determined for atrazine.

A second HED cancer peer review was held on June 6, 1988, to evaluate the results of an oncogenicity study conducted in CD-1 mice. Atrazine was administered at dietary levels of 0, 10, 300, 1500 or 3000 ppm (0, 1.4, 38.4, 194 or 386 mg/kg). At the doses tested, there was no evidence of carcinogenicity in this species. It was concluded by the Committee that the mouse oncogenicity study would not alter the previous Category C classification which was assigned after the first peer review.

On September 7, 1988, the Scientific Advisory Panel (SAP) met and classified atrazine as a C carcinogen. The SAP did not agree with the quantification of risk for the following reasons: 1) the tumor site was the mammary gland in Sprague Dawley rats and 2) the carcinogenicity of atrazine appeared to be complicated by the influence of secondary factors such as endocrine imbalance. The SAP decision was followed by a third HED Cancer Peer Review Committee meeting held on September 29, 1988. At that meeting, the C classification was upheld and a decision was made that the tumor response was of sufficient concern that the RfD should not become the default position for expressing long term effects.

A fourth HED cancer peer review was held on April 27, 1989. At this meeting, the HED Cancer Peer Review Committee agreed that due to a lack of hormonal data, there was insufficient justification for varying from the use of a Q_1 . The Committee recommended that the registrant generate data to support their theory that a

hormonal mechanism of carcinogenicity existed for atrazine.

The MTD issue was addressed directly in a memo from J. Hauswirth to R. Taylor dated June 6, 1988. The Committee felt that the MTD had been exceeded in both male and female rats at 1000 ppm. The MTD was reached at 500 ppm in both sexes based on body weight gain decrements. In other memos from M. Copley to R. Taylor dated November 1988 and April 1989 it was stated that malignancy occurred at doses that had few signs of toxicity.

Ciba Geigy has filed new data which include a one year study in Sprague Dawley rats and a study to assess the effects of atrazine on the luteinizing hormone (LH). In addition to the new data, several articles and papers have been provided that address strain differences, estrogenicity and hormonal mechanisms of carcinogenicity.

3. New Information Presented by the Registrant

- a. Studies Conducted to Evaluate the Effects of LH in Determining the Mechanism of Mammary Carcinogenesis in Sprague Dawley Rats
MRID 43934404, 05 and 06
January, 1996

These studies were conducted to evaluate the effects of luteinizing hormone (LH) on estrous cycle disruption and included the method validation phase of the study and the preliminary report in addition to the main study. In the main study, atrazine was administered by gavage for 28 to 31 days at dose levels of 2.5, 5.0, 40 and 200 mg/kg to ovariectomized female Sprague Dawley rats (90/dose level). The duration of the study, from initial dosing to terminal sacrifice was approximately 45 days. According to the study report, atrazine was associated with a decrease in the LH surge and persistent diestrus and prolonged estrus at the two highest dose levels. The observed effects on LH were only significant at 200 mg/kg. At 5 mg/kg, effects on LH surge were present but inconclusive and no disruption in estrus cycle patterns was noticeable. Based on these findings, the NOEL was 2.5 mg/kg.

The following Table depicts the means, ranges and standard deviations for LH concentrations on the day of analysis, at the designated sampling intervals.

Table I
Serum LH (pg/mL) Concentrations Following Atrazine Administration

Dose (mg/kg)	Sample Time (Hrs)					
	1100	1400	1600	1800	2000	2200
0						
mean	998	1122	3315	5138	2242	761
range	260/2116	497/2593	461/9717	744/13910	716/7027	455/1306
S.D.	614	564	693	4403	1850	288
2.5						
mean	943	1171	2951	4489	1118	486
range	260/1847	262/3032	992/5739	1366/18381	463/1959	260/1925
S.D.	614	802	1315	4345	412	138
5.0						
mean	1140	882	3099	2804	1554	508
range	260/2682	260/3701	420/10408	158/5068	370/5097	164/4731
S.D.	715	925	2521	1345	??	317
40.0						
mean	1219	1125	3518	3246	1740	689
range	385/1681	232/3514	583/17932	292/7181	403/4240	98/1185
S.D.	467	795	4514	1981	1157	373
200.0						
mean	873	1099	1685	2752	1853	1126
range	260/2003	168/3476	250/11844	413/10173	220/4389	374/2888
S.D.	655	863	2962	3137	1139	816

Discussion of the Data: Several of the assumptions made by the registrant in support of the LH mechanism have not been fully investigated, or the results are inconclusive (See attached Table: Ciba's LH Hypothesis...). Specific flaws in the LH study included the lack of comparison of the LH surge observed in atrazine treated rats to that observed in normal, untreated middle aged (10 -15 months) rats; failure to demonstrate unovulated or abnormal ovarian follicles either grossly or histologically; failure to provide data that demonstrated a stimulation of prolactin; large standard deviations that in some instances exceed the mean values and indicate a high degree of variability in the data; and the failure to demonstrate or correlate the LH findings to promotion of mammary tumor growth due to the short duration of the study. A critique of the study is provided in the attached May 30, 1996 memo from T.M. Crisp to E. Francis, K. Hammerstrom, K. Baetcke and M. Morrow. (The memo is Attachment 1 to this weight of the evidence document).

In addition to the flaws in the LH study, it is understood that LH is under the control of the gonadotropin releasing hormone (GnRH) that is released by the anterior pituitary. No links or assessments have been conducted to determine the role of the pituitary hypothalamic tract with regard to the hormonal perturbations that are supposedly occurring in these aging rats. Furthermore, if LH is controlled at this site (anterior pituitary) in the body, the changes in this hormone could be the result of an effect at the site of hormonal control.

CIBA'S HYPOTHESIS for HORMONAL MECHANISM of CARCINOGENESIS INVOLVING LH

Hypothesis: During mid-age, Sprague Dawley female rats develop episodes of estrous cycle disruption caused by failure of the luteinizing hormone surge to induce ovulation

**** One study, involving a 28 day dosing period does not provide information on events in mid- age Sprague Dawley rats.**

**** Attenuation of LH surge occurred but effect of triazines on GnRH was not investigated to determine whether the LH activity is an initiating event or one event in a series.**

Unovulated ovarian follicles continue to secrete estrogens, which stimulates prolactin secretion and promotes growth of mammary tumors.

**** Grossly and microscopically no ovarian lesions have been reported in long-term or short term studies that would suggest an abnormal alteration in the ovarian follicles.**

**** Stimulation of prolactin secretion has not been clearly or consistently demonstrated in the LH study or in any of the other long or short-term studies with atrazine.**

Atrazine exposure accelerates these changes, leading to precocious mammary tumor development.

**** Precocious mammary tumor development has been reported at doses of 400 ppm; however there has been no correlation or one single study to tie in the different scenarios associated with hormonal disruption and mammary tumor development.**

F-344 rats do not develop episodes of persistent estrogen secretion and do not develop mammary tumors.

**** Studies have indicated that Fisher-344 rats do not develop mammary tumors and the hormonal profile in aged Fishers is quite different from that in aged Sprague Dawleys. LH surge studies to compare differences in the two strains have not been conducted and are not necessary since the Fishers do not appear to be susceptible to the effects of atrazine.**

**** Indicate areas that have not been fully investigated to support the LH hypothesis**

b. One Year Combined Feeding/Oncogenicity Study in Female Sprague Dawley Rats
Authors: Pettersen and Turnier
December 8, 1995
(MRID 43934402)

This special toxicity study was designed to determine the effects of 12 months of dietary exposure of atrazine to female CD rats on mammary glands, pituitary glands, estrous cycle, and plasma hormones. Estrous cycle evaluation (by vaginal smear and histology) and plasma hormone (estradiol, progesterone, prolactin, and luteinizing hormone) concentrations are to be reported later.

Technical atrazine (97.1% a.i.) was administered in the diet to 55 female Crl:CD(SD)BR rats per dose group at dose levels of 0, 15, 30, 50, 70 or 400 ppm, corresponding to 0.8, 1.7, 2.8, 4.1, or 23.9 mg/kg/day. Ten females per dose group had trunk blood collection and estrous cycle evaluation made at interim sacrifices of 3, 6, 9, or 12 months. Fifteen females per dose group had interim orbital blood samples and estrous cycle evaluations made at 3, 6, 9, and 12 months followed by sacrifice at 12 months. No effects upon clinical signs or mortality were noted.

The 400 ppm group had statistically significant decreases in mean body weight compared to controls beginning at week 2 (96% of controls), decreasing to 88-92% of controls for the last 10 months of the study. The 400 ppm group had statistically significantly decreased mean body weight gain from weeks 2-46 with mean values ranging from 82-89% of controls. Food consumption for the 400 ppm group ranged from 88-101% of controls, statistically significant at 6 weekly intervals in the first 11 weeks. The 400 ppm group had food consumption close to that of controls for the last 9 months of the study. Food efficiency for the 400 ppm group was decreased in comparison to controls during the first 12 weeks of the study. After 12 weeks, the 400 ppm group had similar, or occasionally increased food efficiency in comparison to controls. Mammary gland hypertrophy (11/55 vs 6/55) and skin masses (9/55 vs 4/55, apparently associated with mammary tumors) were increased in high-dose females compared to controls. Mammary gland adenocarcinomas were increased in high-dose females compared to controls (6/55 vs 1/55), mammary gland adenomas were similar in incidence in high-dose animals compared to controls (1/5 vs 0/55), and mammary gland fibroadenomas were only slightly increased in high-dose females in comparison to controls (4/55 vs 2/55). The increase in mammary gland adenocarcinomas in high-dose females was not statistically significant, however the increase of combined mammary gland adenomas, adenocarcinomas, and fibroadenomas (11/55 vs 3/55 combined) was significant ($p \leq 0.05$). There were no significant differences for onset time among treatment groups when mammary gland adenomas, adenocarcinomas, and fibroadenomas were considered separately, however, there was a significant positive trend ($p \leq 0.05$) when fibroadenomas were combined with either adenomas or

adenomas and adenocarcinomas.

The NOEL is 70 ppm (4.1 mg/kg/day) and the LOEL is 400 ppm (23.9 mg/kg/day) in females based on decreased body weight, body weight gain, and increased mammary gland adenocarcinomas. A statistical Analysis by L. Brunsmann of SAB is attached (Attachment 2) along with the DER from K. Farwell (Attachment 3).

Discussion of the Data: The special one year combined chronic/oncogenicity study in female Sprague Dawley rats was classified **supplementary**, did not satisfy the guideline requirement for a chronic oral study in the rat, and could not be upgraded. The following guideline deficiencies were noted: only females were tested; only 25 animals per dose group were planned for terminal sacrifice; no clinical chemistry, hematology, urinalysis, or ophthalmology testing; liver and kidneys were not weighed; microscopic exam only included the pituitary, ovaries, uterus, vagina, inguinal skin with mammarys, and masses; and not all results were reported (hormone analysis and estrous cycle evaluations).

c. Journal Articles Provided by Ciba Geigy to Address Mechanisms of Carcinogenicity

1. Theory of Estrogenicity

In their attempt to address the mechanism of action of atrazine in Sprague Dawley rats, Ciba Geigy proposed several mechanisms, most of which were later refuted by ancillary and specialized studies. The previously proposed mechanisms entertained the possibility that atrazine possessed estrogenic properties that in turn resulted in a hormonal imbalance in Sprague Dawley rats which led to premature senescence and finally resulted in a decreased latency and an increased incidence of mammary tumors. The journal articles provided information from studies conducted in vivo and in vitro, involving different types of assays to determine whether atrazine had estrogenic properties when compared to estradiol.

Discussion of the Data:

The results from seven studies are summarized in the following Table II. Once these studies were completed, the theory of exogenous estrogenic activity was no longer pursued. Additionally, a consensus panel, convened by Ciba Geigy to evaluate the hormonal mechanism of mammary carcinogenesis concluded that 7 out of 9 in vivo studies conducted to determine the estrogenicity of atrazine, were negative. One of the positive tests had not been validated and the other positive test was inconsistent with other published reports.

TABLE II
In Vitro and in vivo hormonal studies with atrazine

Study Type	Animal/System	Dose/Duration	Results
Uterine weight stimulation/suppression	ovariectomized SD rats	0, 20, 200 or 300 mg/kg	E2 stimulated uterine weight. Atrazine associated with uterine weight decrease at higher doses, following E2 pretreatment. W/o pretreatment, no significant difference in uterine weights when compared to controls.
Progesterone receptor	Ovariectomized SD rats	0, 50 or 300 mg/kg for 3 da., followed by 5ug/kg E2	Decr. prog. receptor binding in controls and at HL. Higher doses of atrazine over time would be needed to block E2 response.
Thymidine Incorporation	female SD rats	0, 1.0, 10, 50 100 or 300 mg/kg atrazine for 3 d, followed by 0.15 ug of E2	Thymidine incorporation reduced at the three highest dose levels but without a dose response. Elevated doses suppressed the response evoked by E2 for thymidine incorporation.
Ah receptor binding	hydroxylapatite system	≤ 10uM	No binding to the Ah receptor with atrazine at concentrations as high as 10uM
Cell Proliferation Assay	MCF-7 cells	≤ 10uM	No significant alterations in cell growth with concentrations of atrazine alone. Addition of 1nM of E2, alone increased cell growth by two-fold. Combinations of E2 and atrazine were non-competitive. Response was similar to that obtained with estradiol alone.
Assay for PR-PRE complex	MCF-7 cells	1uM	Induction of PR/PRE observed with E2 but not with atrazine. No effect on E2 response when atrazine and E2 were combined (non competitive, no antagonism)
Transfection and reporter gene assays	MCF-7 cells GAL4-HEGO receptor and GAL4 promoter	≤ 10uM	E2 caused a concentration dependent induction of luciferase activity. No significant induction of activity present with atrazine at doses up to 10 uM. ER agonistic activity of atrazine not supported by this study....

4. Previously Reviewed Information
Studies Conducted in Sprague Dawley Rats:

- a. 2-Year Combined Feeding/Oncogenicity in Sprague Dawley Rats
Author: Mayhew, et.al.
Date: April 29, 1986

(This study was the subject of the first HED Cancer Peer Review held on September 10, 1987 and was the basis of the cancer classification).

Atrazine was administered in the diet of Sprague Dawley rats at dose levels of 0, 10, 70, 500 or 1000 ppm for 24 months. This is equivalent to 0, 0.5, 3.5, 25 or 50 mg/kg/day. In males there was an increase in the incidence of testicular tumors at the highest dose tested. This finding was stated in the peer review report to have occurred at a level which exceeded the MTD and was not considered in the carcinogenicity classification. In males, the systemic NOEL was 3.5 mg/kg/day and the LOEL was 25 mg/kg/day based on decreased body weight gain (14%). At the highest dose tested of 50 mg/kg, there was decreased body weight gain (14-16%) and an increase in the incidence of degeneration of the rectus femoris muscle. Histopathological lesions were also present in males and included prostatic hyperplasia and renal calculi.

In females, there was a statistically significant increase in the incidence of mammary carcinomas at doses of 3.5 mg/kg/day and higher. A significant increase in fibroadenomas/adenomas was reported at 50 mg/kg/day and a significant increase in all mammary tumors was reported at 25 and 50 mg/kg/day. In spite of the carcinogenic response at 3.5 mg/kg/day, this dose level was determined to be the NOEL for systemic effects of atrazine during the chronic portion of the study. The LOEL for systemic effects was 25 mg/kg/day based on decreased body weight gain (18 to 23%). Females receiving 50 mg/kg/day had decreased body weight gain (26 to 34%), decreased survival, decreased red blood cell count, hematocrit and hemoglobin, and an increased incidence of retinal degeneration, liver necrosis and degeneration of the rectus femoris muscle. This study also served as the basis for the RfD. The study was classified as core minimum and satisfied the requirements for a chronic/oncogenicity study in rodents. (See Table III for incidence of mammary tumors).

Table III
Incidence of Neoplastic Lesions in Male and Female SD Rats

Tumor type	Atrazine Dose Level (ppm)				
	0	10	70	500	1000
Mammary Gland					
fibroadenoma	29/89	29/65	36/70	39/68	45/88**
(%)	(33)	(45)	(51)	(57)	(51)
adenoma	1/57	0/51	1/57	1/54	2/50
	(2)	(0)	(2)	(2)	(4)
adenocarcinoma	15/90	16/68	27/70*	27/68*	45/90**
	(17)	(23)	(39)	(40)	(50)
combined	36/90	40/68	47/70	48/68	65/90*
	(40)	(59)	(67)	(71)	(72)
Testes					
interstitial	1/58	3/59	2/59	2/60	7/64*
cell tumor	(2)	(5)	(3)	(3)	(11)

*p < 0.05, **p < 0.01. An analysis of this data was conducted by SAB for the first HED Cancer Peer Review for atrazine.

b. 2-Year Oncogenicity Study in Female Sprague Dawley Rats
 Author: A.K. Thakur
 January 27, 1992

Atrazine was administered to 60 female Sprague Dawley rats per dose group for 24 months at dietary levels of 0, 70 or 400 ppm (0, 3.8 or 23 mg/kg/day). The NOEL for systemic effects was 3.8 mg/kg/day and the LOEL was 23 mg/kg/day based on decreases in body weight gain. At this dose level, the combined incidence of fibroadenomas and carcinomas were statistically increased when the numbers were adjusted for survival. A decreased latency in the onset of mammary tumors was also reported at the highest dose level, with 6 carcinomas being reported at the high dose vs 0 in the controls at the 12 month interim sacrifice. At the 12 month interim sacrifice, the total number of mammary tumors was 2, 3 and 9 for control, 70 ppm and 400 ppm dose levels, respectively. (See Table IV below for non-adjusted incidence). At 24 months, there was no significant difference in the total tumor incidence between control and treated groups.

Table IV
 Mammary Tumor Incidence

Tumors	Dose Level (ppm)		
	0	70	400
Fibroadenoma	39/60	30/60	41/60
Adenocarcinoma	17/60	13/60	22/60
Combined	46/60	34/60	49/60

p= 0.05 using life Table tests.
 (From review by H. Spencer 2/09/94, no SAB review of data appended).

c. 2-Year Oncogenicity Study in Female Sprague Dawley Rats
 Author: A.K. Thakur
 October 1991

Atrazine was administered at dietary dose levels of 0, 70 or 400 ppm for 24 months to female Sprague Dawley rats. At 1, 3, 9, 12, 15, 18 and 24 months, 10 animals per group were sacrificed for hormonal determinations. The systemic NOEL in this study was 70 ppm (3.5 mg/kg) based on a 13% decrease in body weight gain. The ovaries, uterus, vagina, mammary glands and pituitaries were evaluated histologically and an assessment was made using vaginal cytology to determine the phase of estrous that the animals were in at the time of sacrifice.

The mammary tumor incidence is summarized in Table V. In this study, there was a decreased time to tumor at 12 months in the 400 ppm group. The number of palpable masses at 12 months that were later confirmed as mammary tumors (either adenomas or carcinomas) was also increased in the highest dose level. The incidence at 12 months was 4/50, 3/48 and 14/50 for control, 70 ppm and 400 ppm dose groups, respectively. The incidence at the end of the study was not significantly different between control and high dose groups.

Table V
Mammary Tumor Incidence

Tumors	Dose Level (ppm)		
	0	70	400
Fibroadenoma	8/50	12/48	13/50
Adenocarcinoma	9/50	4/48	11/50
Combined	16/50	16/48	22/50

Discussion of the Data: The vaginal smears used in the assessment of stage of the estrus did not provide substantial proof that animals at higher doses were more inclined to remain in proestrus. Additionally, hormonal determinations were questionable with regard to the significance of the variability in the ranges that were reported for estrogen and progesterone. Reviews conducted by T.M. Crisp under memo of March 31, 1994, outlined discrepancies in hormonal data and cited that there was a concern that the experimental design of this study may be flawed. Among the discrepancies addressed in the Crisp memo were whether the blood samples collected for hormonal assay were collected at the appropriate time; whether the vaginal smear cytology data were reliable, the confidence of reported increases in specific hormones at designated sampling intervals and the reliability of group means. These items were addressed in the response to the PD 1; however, Ciba's response did not provide proof that the data were reliable or the study design was indeed, unflawed.

5. Additional Information Provided by the Registrant

a. Comparative Studies in Fisher 344 and Sprague Dawley Rats

Carcinogenicity studies were conducted in Fisher 344 rats to demonstrate the strain differences in the carcinogenic response. Studies were also conducted in females, only to compare hormonal activity and perturbations in cyclicity to findings in Sprague Dawley rats.

1. Two-Year Oncogenicity in Fisher 344 Rats

Author: A.K. Thakur

February 18, 1992

Atrazine was administered for 24 months to male and female Fisher 344 rats at dietary levels of 0, 10, 70, 200 or 400 ppm. The NOEL in both sexes was 70 ppm (3.4 mg/kg/day in males and 4.4 mg/kg/day in females). At 400 ppm there was an 11% decrease in body weight gain in both males and females. At this dose level, there was also a significant increase in leukemia associated hepatopathy in females. The leukemia associated hepatopathy was not believed to be associated with the administration of atrazine. At 200 ppm, there was also a significant decrease in body weight gain (8%) in both sexes from weeks 0 through 76. The NOEL for this study was 70 ppm and the LOEL was 200 ppm.

Atrazine was not associated with carcinogenicity in this strain of rats. Incidences of mammary and pituitary neoplasia were not statistically different from controls.

The study was classified as core guideline and satisfied the data requirements for an oncogenicity study.

2. Two Year Oncogenicity Study in Female Fisher-344 Rats

Author: A.K.Thakur

November 8, 1991

Atrazine was administered to female Fisher 344 rats at dietary dose levels of 0, 10, 70, 200 or 400 ppm (0, 0.5, 3.5, 10 or 20 mg/kg/day). Animals were sacrificed at 1, 3, 9, 15 and 18 months for estrous cycle staging and hormonal evaluation. This strain of rats was negative for carcinogenicity. The NOEL was 3.5 mg/kg/day and the LOEL was 10 mg/kg/day based on decreased body weight gain.

The results from the two studies in Fisher rats were compared to the results obtained in Sprague Dawley with regard to hormonal data and cycle staging to evaluate the patterns of reproductive aging that may be responsible for the differences observed in the development of mammary tumors in both strains.

The registrant concluded that in Sprague Dawley rats, there was an increase in serum estrogen and prolactin as the animals age and in Fisher 344 rats there was a decrease in estrogen and an increase in progesterone and prolactin. Sprague Dawley rats were reported to spend more time in estrus as they age, whereas, Fisher 344 rats were reported to spend more time in proestrus. The purpose of the strain comparison was to determine, using vaginal cytology and hormonal aging patterns, which strain would be more closely compared to humans.

b. Articles and Papers Addressing Strain Differences

(The articles below provides additional comparison of results following the administration of atrazine to both Fisher and Sprague Dawley strains).

1. Chronic Effects of Atrazine on Estrus and Mammary Tumor Formation in Female Sprague Dawley and Fisher 344 Rats
MRID 43598615

This article provided a comparison of the results obtained in long term studies in which atrazine was administered at similar dietary dose levels to two different strains of rats (0,10, 70, 200 or 400 ppm in Fisher rats and 0, 70 or 400 ppm in Sprague Dawley rats). The authors concluded that the levels of atrazine were at or exceeded the MTD in Sprague Dawley rats, which in turn led to lengthening of the estrous cycle primarily based on the increased number of days in estrus. Earlier onset of galactoceles formation was also demonstrated at the high dose level (400 ppm) and an earlier onset of mammary tumors with no increase in the overall incidence at study termination was reported. A hormonal profile revealed that plasma estradiol levels were significantly elevated at three months, only. Other hormonal data were unremarkable and the prolactin data during the first two sampling intervals were hemolyzed.

Based on this article, Sprague Dawley rats develop mammary tumors spontaneously as a part of the aging process and the response at the dose levels administered in this study was expected. The reproductive aging process is different in Fisher rats and much of the endocrine control of ovarian function as observed in Sprague Dawley rats is not clearly evident in the Fisher strain.

Discussion of the Data: The MTD issue and its relevance to mammary tumor formation is discussed in this article and has been addressed elsewhere in this document and in previous Cancer Peer Review documents.

In these studies, body weight gain appears to be the only parameter affected and only at the highest dose level in Sprague Dawley rats (15% decrease at 400 ppm). Other studies conducted with higher

doses (500 ppm) did not demonstrate an exacerbation or increase in the clinical signs; however, tumor incidence, specifically adenocarcinomas, was significantly increased above concurrent and historical controls.

The hormonal data are inconsistent, with no patterns being established throughout the study. Additionally, at terminal sacrifice, there was an inadequate number of animals to make a meaningful assessment of the results.

2. Short Term Effects of Chlorotriazines on Estrus in Female Sprague Dawley and Fisher 344 Rats
MRID 43598614

This paper summarized the results of a 2 week study in which atrazine and simazine were administered daily by gavage for two weeks to groups of Sprague Dawley and Fisher rats. The dose levels were 0, 100 or 300 mg/kg. The study was conducted to examine the effects of the compounds on ovaries, uterus, and adrenals, estrous cycling, vaginal cytology and hormonal levels. The results discussed in this entry will pertain to atrazine only.

Significant effects (reductions) in body weight were present in both strains at 100 (14%, Sprague Dawley) and 300 mg/kg (26%, Sprague Dawley) along with significant reductions in absolute and relative ovarian and uterine weights ($p < 0.05$). Absolute and relative adrenal weights were increased. Plasma hormone levels were assessed in proestrus and revealed a marked decrease in estradiol at both dose levels, significant increases in progesterone at 300 mg/kg, slight and insignificant increases in prolactin at 300 mg/kg and a decrease in mean corticosterone levels at 300 mg/kg in Sprague dawley rats. In Fischer 344 rats, none of the hormones measured, estrogen, progesterone, prolactin or corticosterone, were altered by the administration of atrazine.

When both atrazine groups of Sprague Dawley rats were compared to the control groups, there appeared to be a significant increase in cycle length. This was characterized by an increase in the length of time spent in estrus and a decrease in the amount of time spent in diestrus. The time spent in estrus was confirmed by an increase in the number of cornified and nucleated cells at both 100 and 300 mg/kg. No changes in cycle phases were reported for Fischer 344 rats.

Discussion of the Data: It is noted that this study examined the effects of both atrazine and simazine. Interestingly, when the control groups are compared to each other, there is a degree of variation that suggests that some of the findings and conclusions made in this study did not take into account the variability within the species. For instance, the control value for the number of

days spent in a estrus was 4.2 ± 0.4 days for the concurrent atrazine control and 4.7 ± 1.0 day for the concurrent simazine control. Furthermore, with a study of only 2 weeks duration, conclusions pertaining to an increase in cycle length and hormone levels based on a single proestrus sampling, would be premature. The duration of the study would be insufficient to determine whether the results represent an inconsequential finding in the data or a real pattern associated with the administration of the compound.

3. Rat Tumorigenesis: Relevance of Hormonal Imbalance to Dose Selection (a paper, presented by Jim Stevens, Ciba Geigy)
MRID 43598613

Jim Stevens, a toxicologist with Ciba Geigy Corporation, presented a paper in which he discussed the mechanisms of hormonal imbalance and the mechanisms which may be involved in mammary tumor pathogenesis in rats. The paper suggested that imbalances in hormonal levels involve either blockade of hormone synthesis or secretion, interference with enzyme systems or competition with receptor sites. Hormones are under the control of the hypothalamus and the pituitary and may serve as switches that bring about a response. These hormones may also increase tissue susceptibility to compounds which act as initiators. The paper touched on the importance of binding for hormonal responsiveness and mentioned that prolactin levels influence the number of estrogen receptors in mammary tissue and estrogen levels affect the number of progesterone receptors in the liver.

The paper discusses and highlights the differences in the reproductive hormonal make-up of aging Fisher 344 rats as compared to aging Sprague Dawley rats. In Sprague Dawley rats, senescence is marked by a decrease in FSH, estradiol and progesterone and an increase in prolactin. An increase in the length of the estrous cycle followed by acyclicity also occurs with aging in this strain. In Fisher rats, reproductive aging is characterized by an increase in progesterone levels and the existence of a state of pseudopregnancy. Galactocoeles, which are considered as markers of prolactin exposure, are more prevalent in aged Sprague Dawley strain than in the aged Fisher strain.

In his paper, Stevens asserts that the mammary tumors observed with atrazine in Sprague Dawley rats are an over-dose phenomenon and occur only when the MTD is exceeded.

Discussion of Data: It should be pointed that the only clinical signs of excessive dosing with atrazine were a reduction in body weight and an increase in the incidence of mammary tumors at the

dose levels examined. Nothing else suggests an overt toxicity that would bring about other physiological changes and result in tumor formation. The paucity of clinical signs of toxicity in Sprague Dawley rats at dietary doses up to and including 500 ppm have been demonstrated in the four long-term studies cited on pages 9 through 12 of this weight of the evidence document.

If hormones are, in fact switches, further examination of effects on hormonal imbalances such as a disruption in the ratios of one hormone to another should be examined. Some of Steven's proposals have already been refuted, specifically, the blockade of the synthesis of estradiol. The effects of other hormones on the carcinogenicity of atrazine have not been fully investigated nor have the effects on, or the role of the hypothalamus been sufficiently examined. Interference with transforming or metabolizing systems alluded to in the Stevens paper as a possible mechanism for the production of mammary tumors, has also not been investigated.

4. Evaluation of a Hormonal Mechanism for Mammary Carcinogenesis:
Consensus Panel Report
MRID 43598620

This document provided a basis for conclusions reached by an independent group of scientists, convened by the registrant, with regard to a mechanism for carcinogenesis and the additional areas where studies would be needed to account for the influence of sex, species and strain. The panel addressed the suitability of the Sprague Dawley rat as a model for evaluating mammary carcinogens and concluded that the strain was inappropriate because of the endocrine processes that take place in the strain as it ages.

With regard to an age based mechanism, the panel concluded that in the Sprague Dawley, reproductive senescence is characterized by a persistent elevation in the hormones prolactin and estrogen. In affected animals, a persistent estrus exists and is due to an ovulation failure. The panel also believes that atrazine disrupts the LH secretory mechanism in Sprague Dawley rats resulting in an acceleration of age related endocrine problems and a high background incidence of mammary tumors.

Based on their proposed mechanism(s) of action, the panel suggested additional studies, primarily to determine the effect of LH on tumorigenic response in general and the effect on mammary tissue, specifically. References are made within the document to the high level of exposure, not just to atrazine, but to other chlorotriazines and to the neuroendocrine regulation of reproductive aging.

Discussion of the Data: Many of the assumptions made by the technical panel have not been demonstrated (LH secretory disruption, increases in prolactin, persistent estrus); others have been refuted (induction of estrogenic responses, initially believed to be the mechanism of carcinogenicity). With regard to neuroendocrine aging, no studies providing a comparison or contrast of events in normal aging animals and in those receiving atrazine have been conducted. Suggestions that a threshold mechanism exists are discredited by the fact that in at least one study in Sprague Dawley rats, an increase in mammary tumor incidence was present at 10 ppm and in another study an increase was apparent at 70 ppm. Furthermore, the lack of other clinical signs of toxicity that would suggest that the MTD had been exceeded were conspicuously absent in studies conducted with dose levels as high as 400 ppm. The panel has focused on dose levels but has neglected to examine the different windows of susceptibility and how they may be different in humans and in rats.

As indicated in the report, atrazine does not appear to have a significant stimulatory effect on estrogen secretion; effects on prolactin remain obscure. With regard to the studies that were suggested by the panel, the results are now in and do not add clarity to the panel's proposed mechanism with regard to the effects of LH on premature aging and mammary tumorigenesis.

c. Other Proposed Mechanisms

1. Hypothesized Pituitary- Hypothalamic Effects

Ultrastructural Changes in the Rat Hypothalamus Arcuate Nucleus following DACT Feeding
MRID 43598616

Sprague Dawley rats, 20 weeks of age were fed DACT, a metabolite of atrazine, at dose levels of 0 or 1000 ppm. The study was conducted to test the theory that estrogens enhance hypothalamic structural gliosis. In cases of neuronal degeneration (etiology undefined), it has been proposed that the astrocytes and microglial cells enlarge and accumulate dense bodies. The degree of enlargement and granular content are used as indices of hypothalamic damage. Glial activity has been observed in the arcuate nucleus of the hypothalamus, the region that plays a role in the secretion of gonadotropins. It is also speculated in this report that the ultrastructural pathology reported in the hypothalamus may possibly be related to stimulation of endogenous peroxidase activity which has been reported to occur during periods of prolonged estrus.

In this study the granular content in astrocytes was increased by 4% at week 32 and by 36% at week 48. The microglial cells were increased in number at both weeks 32 and 48; however, the increases

were not significant and the microglial cells were not classified as reactive (meaning they were not enlarged and did not contain dense bodies).

Discussion of the Data: An evaluation of the results showed that the granular content of the astrocytes was not homogeneous and that the microglial cells, although increased in number, were not considered reactive cells analogous to those which have been reported in cases of neuronal degeneration. The results of this study as they pertain to the effects of the atrazine metabolite, DACT, are ambiguous and the contribution of the changes observed in the hypothalamus to the increased incidence of mammary tumors in Sprague Dawley rats would need to be more thoroughly probed and correlated.

2. Additional Information on the Oncogenic Potential of Atrazine

Weight of the Evidence on the Oncogenic Potential of Atrazine
Consensus Panel Report
43598624

The consensus panel report from a group of independent scientists who were convened by Ciba Geigy concluded the following with regard to the carcinogenic potential of Atrazine:

Atrazine induces adenocarcinoma in Sprague Dawley rats and accelerates the onset at doses greater than or equal to the MTD, with the exception of one study where increases in mammary tumors were observed below the MTD. This could not be reproduced in additional studies conducted at the same dietary level.

Atrazine is negative for oncogenicity in male Sprague Dawley rats, in both sexes of Fisher rats and in both sexes of CD-1 mice.

Atrazine accelerates age related reproductive changes in Sprague Dawley rats causing a state of constant estrus (prolonged estrogen exposure) albeit atrazine has not been demonstrated to be estrogenic by in vivo and in vitro tests. The female Sprague Dawley rat is not considered to be a good model for mammary tumor induction in humans because of the differences in reproductive cyclicity between the SD rat and human females.

Atrazine was not considered to be genotoxic in 31/37 mutagenicity studies. The remaining 6/37 studies were considered either positive or equivocal.

Atrazine is completely absorbed and rapidly eliminated via the urine. Dealkylation is the major route of metabolism and the

primary metabolite is DACT.

Atrazine is structurally related to simazine in that it contains a 2-chloro-4,6-bis-(alkylamino)-s-triazine ring and both have been associated with the formation of mammary tumors (increased incidence and early onset). The panel report states that atrazine at doses of 400 ppm or greater accelerates senescence in female Sprague Dawley rats and is characterized by an increase in serum estradiol levels, early onset of constant estrus and ovulatory failure. Changes in mammary histomorphology appear to be associated with an imbalance of endogenous estrogen and not caused by an exogenous source of estrogen.

Discussion of the Data: The consensus panel suggested a mechanism for the development of mammary tumors in Sprague Dawley rats; however, the possible influences of the hypothalamus are not investigated thoroughly or followed in this report. The effect of atrazine on the ability of LHRF (GnRF) to evoke a neuronal response has not been addressed. Furthermore, it has been stated that there is an age related decline in estrogen receptors; however, this phenomenon has not been correlated with the decline in neuroendocrine control of the estrous cycle. The panel report has alluded to the possibility that a neuroendocrine component exists in the development of mammary tumors.

In the neuroendocrine scheme of aging the following series of events are believed to take place:

Under neuroendocrine control animals lose their ability to ovulate. This leads to an increase in the secretion of estradiol. New follicles are formed but, because ovulation is impaired, there is a decrease in the number of corpora lutea formed and a decrease in the progesterone level. The balance between the progesterone/estrogen activity is shifted, with estrogen becoming the most persistent hormone. This estrogenic persistence leads to overstimulation of estrogen responsive tissues such as the uterus, mammary gland and anterior pituitary. Estrogenic effects on the anterior pituitary would lead to prolactin secretion that would in turn stimulate the rate of growth of existing mammary tumors.

While portions of the different mechanisms of carcinogenic activity have been examined, they have not been proven, correlated or reproduced with a level of confidence that would rule out all other feasible mechanisms. It is possible that the carcinogenic effect of atrazine may lie in its ability to promote phenotypic expression of mammary cancer in a sensitive strain or species. It is also possible that the mechanism is not a singular, triggering incident, but a series of events.

Within the consensus panel report there was a comparison of reproductive senescence in humans and rats. The emphasis should

not be on events related to aging across species, but rather the potential for this compound to cause or be associated with the development of cancer over the lifetime of a human. A factor for consideration in the risk equation, when extrapolating from rats to humans with regard to breast cancer, is the paucity of information on hormonal disruption and the impact of any such disruption during various stages of development in humans. Additionally, the etiology of human breast cancer is for the most part unknown. Mammary cancer is not a concern solely in aging humans, but a concern over the lifetime of an individual and specifically during periods when hormonal equilibrium is disturbed (i.e. menarche, pregnancy, lactation and menopause).

Finally, the proof that the tumor response suggests that an MTD has been exceeded has not been provided. The studies conducted in Sprague Dawley rats at dose levels up to and including 400 ppm have not demonstrated that atrazine has overt systemic toxicity. Furthermore, doses between 70 ppm and 400 ppm were not investigated in Sprague Dawley rats as they were in Fisher 344 rats so it is not clearly established that the signs of toxicity (weight loss, decreased tumor latency) observed at 400 ppm would not have occurred at dose levels between 70 and 400 ppm. It appears that from the long term the studies that have been conducted to support Ciba's theory of excessive dosing, levels between 70 and 400 ppm have been intentionally omitted. A table providing dose response information from long-term and intermediate term studies is attached.

6. Metabolism

When C14 labelled atrazine was administered to rats, the distribution was found to be dose dependent and to follow first order kinetics. Red blood cells stored the highest level of atrazine, followed in decreasing order by the liver, kidneys, ovaries, pituitary, brain, and the pectoral region of the mammarys. The half life of atrazine in the body was 1.61 days, with 95% of the administered dose being excreted within 7 days. Atrazine is metabolized primarily by N-dealkylation. The compound is eliminated by both the urinary and fecal routes.

7. Structure Activity Relationship (SAR)

Atrazine is an s-triazine pesticide and is related to simazine, cyanazine, propazine, terbuthylazine and terbutryn, all of which have been associated with the induction of mammary gland adenomas and/or adenocarcinomas in Sprague Dawley rats. Terbutryn has also been associated with carcinogenicity involving the thyroid and the liver. The structures for the chemicals are attached (Attachment 4, Figure 1).

In a report dated January 17, 1991 which addressed the SAR analysis of s-triazine pesticides and related compounds, it was stated that the carcinogenic activity of any given s-triazine was dependent on the nature of the substituent at the 2, 4 and 6 position on the ring. It was further stated that the presence of N-alkyl group(s) is crucial for carcinogenic activity of s-triazine compounds.

8. Weight of the Evidence Considerations

Atrazine when administered to Sprague Dawley rats by gavage for 28 to 31 days at dose levels of 0, 2.5, 5.0, 40, or 200 mg/kg was said to be associated with a decrease in the LH surge and persistent diestrus and prolonged estrus. It was concluded by a reviewer within the Agency that the LH study provided by Ciba was flawed and contained deficiencies that would not support the LH suppression as a mechanism of mammary carcinogenicity.

A one-year study in Sprague Dawley rats demonstrated that atrazine, when administered at dietary levels of 0, 15, 30, 50, 70 or 400 ppm, was associated with an increase ($p \leq 0.05$) in the combined incidence of mammary adenomas, adenocarcinomas and fibroadenomas at the highest dose tested.

Other studies conducted in Sprague Dawley rats demonstrated that at dietary doses of 400 ppm, atrazine was associated with a decreased latency period for mammary tumors. In another study in Sprague Dawley rats, which served as the basis for the classification as a category C carcinogen, atrazine was associated with a significant increase in the incidence of mammary tumors at 70 ppm.

Articles have been provided by Ciba Geigy to support current and previous theories on the hormonal mechanisms of carcinogenesis. Many of the earlier assumptions on the estrogenic activity have been abandoned by the registrant. Other mechanisms, such as pituitary-hypothalamic activity have been suggested as having possible relevance to the hormonal activity and mammary carcinogenesis; however, they have not been thoroughly investigated.

Atrazine is rapidly metabolized by N-dealkylation and is eliminated by both the urinary and fecal routes. It is structurally related to simazine, cyanazine, propazine and terbutryn, all of which have been associated with the induction of mammary gland adenomas and/or adenocarcinomas in Sprague Dawley rats.

Based on the information presented, the HED CPRC is asked to address the following:

1. Has a hormonal mechanism for mammary carcinogenesis been adequately described for atrazine in Sprague Dawley rats?
2. In the studies in which a positive carcinogenic response has been demonstrated has the MTD been exceeded?
3. Is it likely that carcinogenic effects observed in Sprague Dawley rats would not be expected in humans?
4. With the theories and data that have been presented on the possible hormonal mechanism of carcinogenesis can other mechanisms/modes of action be ruled out?



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

May 30, 1996

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Review of an Atrazine Rat Study

FROM: Thomas M. Crisp, Ph.D.
Biologist
NCEA-WO (8623)

Thomas M Crisp

TO: Elaine Z. Francis, Ph.D.
Director, Toxics/Pesticides Staff
Office of Research and Science Integration (8104)

Karen Hammerstrom
Assistant Center Director
NCEA- Washington (8603)

Karl P. Baetcke, Ph.D.
Chief, Toxicology Branch I
Health Effects Division (7509C)
Office of Pesticide Programs

Melba Morrow, D.V.M.
Toxicology Branch I
Health Effects Division (7509C)
Office of Pesticide Programs

I have reviewed the Atrazine rat study submitted by Ciba-Geigy entitled "Evaluation of the Luteinizing Hormone (LH) Surge in Atrazine-Exposed Female Sprague-Dawley Rats", along with the three accessory supporting documents entitled: "Failure of Chloro-s-triazine-derived compounds to induce estrogenic responses *in vivo* and *in vitro*", by Connor, K., et al.; "Evaluation of the luteinizing hormone (LH) in female Sprague-Dawley rats - pilot study" and

“Evaluation of the luteinizing hormone surge in female Sprague Dawley rats - method validation”
by Morseth, S.L.

Critique of Supporting Documents:

1. “Failure of Chloro-s-triazine-derived compounds to induce estrogenic responses *in vivo* and *in vitro*”, by Connor, K., et al.:

This report presents both *in vivo* and *in vitro* data that the chloro-s-triazine herbicides, Atrazine and Simazine, do not exhibit estrogenic activity via an estrogen receptor mechanism. This is supported by the observation that at the concentrations tested (50, 150 and 300 mg/kg) for 3 days, the triazines do not increase uterine wet weight in immature female Sprague-Dawley rats or induce the uterine cytosolic progesterone receptor (classic validated bioassays for estrogens). Furthermore, the triazines failed to induce cell proliferation and nuclear progesterone receptor levels in MCF-7 cells or luciferase activity in transfected cells possessing the Gal4 estrogen receptor and a Gal4-regulated luciferase reporter gene. In addition, the authors show that cells co-treated with Atrazine or Simazine and estradiol (E_2) do not inhibit or enhance E_2 -induced responses. The document is well written, clear and the interpretation of the data is objective and reasonable. The only error identified in the manuscript by this reviewer is the failure of the authors to include the text reference of Lyttle and DeSombre (1977), in the middle of page 10, in the reference section. All in all, this manuscript presents a well-designed, carefully-executed and well-controlled study and this reviewer has high confidence in the results and conclusions of the study.

2. “Evaluation of the luteinizing hormone (LH) in female Sprague-Dawley rats - pilot study”, by Morseth, S.L.

The purpose of this second supporting document was to determine the optimal procedures to be employed for testing the integrity of the LH surge in female Sprague-Dawley rats in a 4-week study. With some exceptions, the study design for evaluating blood levels of LH, PRL and estradiol (E_2) seems reasonable. However, this reviewer does have some questions and concerns about the execution of the protocol. First, why were the hormones measured in plasma rather than serum? What was the amount of heparin in collection tubes and was this volume subtracted from the volume of plasma used in calculating concentration of LH, PRL and estradiol? Does heparin interfere in the assays? If not, how was this determined to be the case? The concentration of estradiol reported seems high, since the 75-150 pg/ml values for estradiol reported in this study are 2-4 times greater than that normally reported elsewhere (Smith, Freeman and Neill, “The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: Prolactin, gonadotropin and steroid levels associated with the rescue of the corpus luteum of pseudopregnancy”. *Endocrinology* 96:219-226, 1975). Why wasn't progesterone measured? Would this not provide useful information on whether LH surges were sufficient to elicit ovulation and corpus luteum formation? This reviewer thinks that this failure was a serious oversight. Details of the radioimmunoassays (RIA) used are missing. What are the

mean gonadotropin and sex steroid values for normally cycling rats in the laboratory of the investigator undertaking the RIAs? This information is important in order to compare values with those in this study and with other published data. What was the range for the standard curves for each assay? What was the volume of plasma used in the assays? What was the intra- and interassay % error? How were the kits employed validated in the laboratory of the investigator? Prior to (RIA) for (E_2), was the plasma extracted with organic solvents to rule out cross-reactivity with other competing endogenous steroids? Was diethyl ether used? Was the extracting solvent redistilled just prior to extraction? What was the % recovery following extraction? Was this taken into consideration in determining final concentration?

There is also some confusion in the document. For example, on pg 13 of the "methods" section, it was not clear when the estradiol implants were done with respect to ovariectomy. It was not until pg 29, that the answer was found and that they were done on the day of ovariectomy. This reviewer also found the use of "clock time" and "biological time" confusing (pgs 11 and 14).

With respect to the submitted data in Figure 1, why do the peaks of PRL and LH rise before the usual peak at 1700 hrs? Finally, what is the explanation for the considerable range in values for the individual hormone concentrations at the different time points measured (appendix 1)?

Without answers and clarification to the above questions and concerns, this reviewer has considerable reservations as to whether this project provides sufficient optimal conditions for supporting the definitive study.

3. "Evaluation of the luteinizing hormone surge in female Sprague Dawley rats - method validation", by Morseth, S.L.

The objective of this project was to determine the optimal methods for testing the integrity of the LH surge in Atrazine-treated female Sprague-Dawley rats. It is not clear to me how this study could support the definitive study, since the Atrazine treatment schedule was started after ovariectomy and E_2 implantation and was only 3 days in duration. In the larger, definitive study, the animals were dosed with Atrazine for 28-31 days and then ovariectomized and implanted with E_2 . Next, repeat bleeds (5) from the same animal (0.8-1 ml/bleed) over the short time frame is inadvisable for monitoring changes in hormone levels, particularly PRL. Such severe depletion of total blood volume is stressful and compromises the animal. Finally, the high standard deviations from the mean concentrations of LH and PRL for control and treated animals leave room for considerable reservation as to the usefulness of the results. In some cases the standard deviations were greater than the mean (Appendix 1).

All in all, this reviewer thinks that this document provides less than adequate support for the following study.

Critique of "Evaluation of the Luteinizing Hormone (LH) Surge in Atrazine-Exposed Female Sprague-Dawley Rats" Document:

This interim study was designed to evaluate the effects of Atrazine administered by oral gavage for four weeks in female Sprague-Dawley rats upon the preovulatory surges of pituitary hormones and on the estrous cycle.

First, there are several editorial and scientific errors in the document. These are as follows:

- 1) Pg 8, Table of contents, Appendices 6 and 7 are reversed.
- 2) Pg 19, Penultimate sentence of page is incomplete.
- 3) Pg 24, 2 nd sentence of 2 nd paragraph, concentration should be 200 mg/kg instead of 400 mg/kg.
- 4) Pg 29, 8 lines down, the conversion for 40 mg/kg is not 400 ppm. 40 mg/kg equates to 800 ppm.

There is also some confusion in the expression of the data. For example it is not clear what is meant by the heading of No. of normal animals in Table 1, Summary of Vaginal Cytology Data. This needs clarification. If the author means the number of animals that presented normal 4-5 day regular estrous cycles routinely, then this should be indicated in the methods and results sections of the text and as a footnote of the table. Figures 1, 2, and 3 need standard deviation or error bars for mean points to indicate the degree of variability of the data. In figure 2, why are the mean values for 2.5 and 5 mg/kg above control values for the 1400 and 1600 hr time slots?

Finally, there is some serious misinterpretations of the data. Of paramount importance is the classification of "extended estrous blocks". In the thinking of this reviewer, the recording of an estrous smear for two days, does not represent an extended period of estrus. Table 1 and the text (last paragraph pg 24, and the top of pg 25), and in the Discussion and Conclusion section (pgs 28-30) refer to number of animals with extended estrous blocks. At 0 and 2.5 mg/kg doses, all of the animals designated as having estrous blocks exhibited, in actuality, only 2 days of estrus. For the three higher concentrations, only 2, 3 and 5% of the animals in these groups, respectively exhibited extended estrous periods. Therefore, the characterization of these observations should not be one of extended estrous periods, but rather one of extended diestrous periods. This mischaracterization of the data is significant, since an early working hypothesis for a mechanism, of Atrazine-induced, mammary gland tumorigenesis in the Sprague-Dawley rat attributed precocious estrogen exposure and persistence estrous blocks as a contributing factor. This is now an unlikely scientific explanation!

In summary, this reviewer thinks that the data submitted fails to provide adequate scientific and creditable evidence for mammary gland carcinogenesis in the Sprague-Dawley rat based upon an estrogen-induced mechanism of action. Furthermore, this reviewer is aware of another Atrazine study entitled: "Effect of Atrazine on Ovarian Function in the Rat" by R.L. Cooper, et al., that has been accepted for publication in Reproductive Toxicology, July, 1996

issue. In this unpublished study, the authors report that Atrazine does not increase the number of days in vaginal estrus. In contrast, they report that Atrazine induces prolonged periods of vaginal diestrus, which were associated with elevated levels of progesterone and low levels of estradiol; further documenting the fact that Atrazine-induced changes in the endocrine milieu are **not conducive** to the development of mammary gland tumors.

cc: Mike Callahan (8623)
Bob Sonawane (8623)



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

JUL 03 1996

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Atrazine Qualitative Risk Assessment Based On Sprague-Dawley Crl:CD(SD)BR Female Rat Dietary Study

P.C. Code 80803

TO: Melba Morrow, Veterinary Medical Officer
Review Section II
Toxicology Branch I
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Lori L. Brunsman
7/3/96

THROUGH: Hugh M. Pettigrew, Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Hugh M. Pettigrew

Background

A one-year chronic toxicity study with Atrazine Technical in Sprague-Dawley Crl:CD(SD)BR female rats was conducted by Ciba-Geigy Corporation, Ciba Crop Protection, Environmental Health Center, Farmington, Connecticut, for Ciba Crop Protection, Ciba-Geigy Corporation, Greensboro, North Carolina, and completed December 8, 1995 (Study No. F-00171; MRID No. 439344-02).

The study design allocated groups of 25 female rats to dose levels of 0, 15, 30, 50, 70, or 400 ppm of Atrazine Technical for 52 weeks. An additional 30 female rats per dose were designated for interim sacrifice, 10 each at weeks 13, 26, and 39.

Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Atrazine Technical in female rats. See Table 1 for mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

In the analyses of all animals in the study, female rats had significant increasing trends in mammary gland adenocarcinomas and adenomas, fibroadenomas and adenocarcinomas combined, both at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 400 ppm dose group with the controls for mammary gland adenomas, fibroadenomas and adenocarcinomas combined at $p < 0.05$.

When the animals that died or were sacrificed before week 39 (the time at which the first tumor was observed) were excluded from the analyses, the trends, pair-wise comparisons, significance levels and tumors of significance were virtually the same as those for all animals in the study.

The statistical analyses of the female rats were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. See Tables 2 and 3 for tumor analysis results.

Table 1. Atrazine - Sprague-Dawley Crl:CD(SD)BR Rat Study
Female Mortality Rates^{*} and Cox or Generalized K/W Test Results

Dose (ppm)	<u>Weeks</u>							Total
	1-13	13 ⁱ	14-26	26 ⁱ	27-39	39 ⁱ	40-54 ^f	
0	0/55	10/55	1/45	10/44	0/34	10/34	0/24	1/25 (4)
15	0/55	10/55	0/45	10/45	1/35	9/34	1/25	2/26 (8)
30	0/55	10/55	0/45	10/45	0/35	10/35	0/25	0/25 (0)
50	0/55	10/55	0/45	10/45	0/35	10/35	1/25	1/25 (4)
70	2/55	10/53	0/43	10/43	0/33	9/33	0/24	2/26 (8)
400	0/55	10/55	0/44 ^a	10/44	1/34	10/33	0/23	1/24 (4)

^{*}Number of animals that died during interval/Number of animals alive at the beginning of the interval.

ⁱInterim sacrifices at weeks 13, 26 and 39.

^fFinal sacrifice at week 52.

^aOne accidental death at week 14, dose 400 ppm.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Atrazine - Sprague-Dawley Crl:CD(SD)BR Rat Study

Female Mammary Gland Tumor Rates* and Exact Trend Test and Fisher's Exact Test Results (p values)

	All Animals					
	<u>Dose (ppm)</u>					
	0	15	30	50	70	400
Adenomas (%)	0/55 (0)	0/55 (0)	1/55 (2)	0/55 (0)	1 ^a /55 (2)	1/54 (2)
p =	0.1539	1.000	0.500	1.000	0.500	0.495
Fibro-adenomas (%)	2 ^b /55 (4)	2/55 (4)	2/55 (4)	1/55 (2)	4/55 (7)	4/54 (7)
p =	0.1150	0.691	0.691	0.500 ⁿ	0.339	0.331
Adeno-carcinomas (%)	1/55 (2)	2/55 (4)	0/55 (0)	1/55 (2)	1/55 (2)	6 ^c /54 (11)
p =	0.0024 ^{**}	0.500	0.500 ⁿ	0.752	0.752	0.054
Combined (%)	3/55 (5)	4/55 (7)	3/55 (5)	2/55 (4)	6/55 (11)	10 ^d /54 (19)
p =	0.0034 ^{**}	0.500	0.661	0.500 ⁿ	0.245	0.034 [*]

*Number of tumor bearing animals/Number of animals examined.

^aFirst adenoma observed at week 52, dose 70 ppm.

^bFirst fibroadenoma observed at week 39, dose 0 ppm.

^cFirst adenocarcinoma observed at week 39, dose 400 ppm.

^dOne animal in the 400 ppm dose group had both a fibroadenoma and an adenocarcinoma.

ⁿNegative change from control.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Atrazine - Sprague-Dawley Crl:CD(SD)BR Rat Study

Female Mammary Gland Tumor Rates[†] and Exact Trend Test and Fisher's Exact Test Results (p values)

Excludes Animals That Died or Were Sacrificed Before Week 39

	<u>Dose (ppm)</u>					
	0	15	30	50	70	400
Adenomas	0/34	0/34	1/35	0/35	1 ^a /33	1/34
(%)	(0)	(0)	(3)	(0)	(3)	(3)
p =	0.1545	1.000	0.507	1.000	0.493	0.500
<hr/>						
Fibro-adenomas	2 ^b /34	2/34	2/35	1/35	4/33	4/34
(%)	(6)	(6)	(6)	(3)	(12)	(12)
p =	0.1145	0.693	0.682	0.489 ⁿ	0.322	0.336
<hr/>						
Adeno-carcinomas	1/34	2/34	0/35	1/35	1/33	6 ^c /34
(%)	(3)	(6)	(0)	(3)	(3)	(18)
p =	0.0022 ^{**}	0.500	0.493 ⁿ	0.746	0.746	0.053
<hr/>						
Combined	3/34	4/34	3/35	2/35	6/33	10 ^d /34
(%)	(9)	(12)	(9)	(6)	(18)	(29)
p =	0.0027 ^{**}	0.500	0.649	0.486 ⁿ	0.223	0.031 [*]

[†]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 39.

^aFirst adenoma observed at week 52, dose 70 ppm.

^bFirst fibroadenoma observed at week 39, dose 0 ppm.

^cFirst adenocarcinoma observed at week 39, dose 400 ppm.

^dOne animal in the 400 ppm dose group had both a fibroadenoma and an adenocarcinoma.

ⁿNegative change from control.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.

References

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- Cox, D.R. (1972) Regression Models and Life Tables (with discussion). J. Royal Stat. Soc. Ser. B. 34, 187-220.
- Gart, J.J., D. Krewski, P.N. Lee, R.E. Tarone, and J. Wahrendorf (1986) The Design and Analysis of Long-Term Animal Experiments. In: Statistical Methods in Cancer Research, Volume III. IARC Scientific Publications No. 79. Lyon, France: International Agency for Research on Cancer, p. 18.
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ATTACHMENT 3



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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Review of Special 1-year Study with Atrazine in Female Rats (MRID 43934402).

DP Barcode: D224157
PC Code: 080803

Submission: S501421
Tox Chem No: 063

TO: Kathryn Boyle
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

FROM: Kit Farwell
Section 3, Toxicology Branch I
Health Effects Division (7509C)

Kit Farwell 7-22-96

THRU: Edwin Budd, Acting Section Head
Section 3, Toxicology Branch I
Health Effects Division (7509C)

*Budd
7/22/96*

Attached is the Data Evaluation Report (DER) for a special 1-year dietary study with atrazine in female rats. This special study was designed by the registrant to collect data related to possible mechanisms of mammary tumor development in female rats.

Dietary levels of 0, 15, 30, 50, 70 or 400 ppm corresponded to 0.8, 1.7, 2.8, 4.1, or 23.9 mg/kg/day. No effects upon clinical signs or mortality were noted. Body weight and body weight gain were significantly decreased in the 400 ppm group beginning at week 2 and continuing throughout the study. Mammary gland adenocarcinomas were increased in high-dose females compared to controls (6/55 vs 1/55), mammary gland adenomas were similar in incidence in high-dose animals compared to controls (1/5 vs 0/55), and mammary gland fibroadenomas were only slightly increased in high-dose females in comparison to controls (4/55 vs 2/55). The increase in mammary neoplasms was statistically significant ($p \leq 0.05$) only when the 3 types of neoplasms were combined. There were no significant differences for onset time among treatment groups when the mammary neoplasms were considered separately, however, there was a significant positive trend ($p \leq 0.05$) when fibroadenomas were combined with either adenomas or

cc: Melba Morrow, Toxicology Branch I
Lori Brunzman, Science Analysis Branch

adenomas and adenocarcinomas. The NOEL is 70 ppm (4.1 mg/kg/day) and the LOEL is 400 ppm (23.9 mg/kg/day) in females based on decreased body weight, body weight gain, and increased mammary gland adenocarcinomas.

The study is classified supplementary, does not satisfy the 83-1 Guideline requirements for a chronic study in rats, and cannot be upgraded because only female rats were used, not all required tissues were examined microscopically, liver and kidneys were not weighed, and no clinical chemistry, urinalysis, or hematology testing was performed. Blood samples for hormone analysis were taken but results were not reported in the study report. Vaginal smears for estrous cycle evaluation were taken with results for individual animals reported but no summary tables were provided. An analysis of the plasma hormone and estrous cycle data are to be reported at a later date.

This study will be considered at the Carcinogenicity Peer Review Meeting presently scheduled for 7/31/96.

ATTACHMENT

Atrazine

Special 1-Year Dietary Study in Female Rats

EPA Reviewer: Kit Farwell

Review Section 3, Toxicology Branch 1 (7509C)

EPA Secondary Reviewer: Edwin Budd

Review Section 3, Toxicology Branch 1 (7509C)

K. J. Full

Date 7-18-96

Edwin Budd

Date 7/22/96

DATA EVALUATION RECORD

STUDY TYPE: Special 1-Year Dietary Study in Female Rats

DP BARCODE: D224157

SUBMISSION CODE: S501421

P.C. CODE: 080803

TOX. CHEM. NO.: 063

TEST MATERIAL (PURITY): Atrazine (97.1%)

SYNONYMS:

CITATION: Pettersen, JC; Turnier, JC (1995). 1-year chronic toxicity study with atrazine technical in rats. Ciba-Geigy Corp. Environmental Health Center (Farmington, CT). Study No. F-00171. Study Date: 12/8/95. MRID 43934402. Unpublished.

SPONSOR: Ciba-Geigy Corporation, Greensboro, NC

EXECUTIVE SUMMARY: This special toxicity study (MRID 43934402) was designed to determine the effects of 12 months of dietary exposure of atrazine to female CD rats on mammary glands, pituitary glands, estrous cycle, and plasma hormones. Estrous cycle evaluation (by vaginal smear and histology) and plasma hormone (estradiol, progesterone, prolactin, and luteinizing hormone) concentrations are to be reported later.

Technical atrazine (97.1% a.i.) was administered in the diet to 55 female Crl:CD(SD)BR rats per dose group at dose levels of 0, 15, 30, 50, 70 or 400 ppm, corresponding to 0.8, 1.7, 2.8, 4.1, or 23.9 mg/kg/day. Ten females per dose group had trunk blood collection and estrous cycle evaluation made at interim sacrifices of 3, 6, 9, or 12 months. Fifteen females per dose group had interim orbital blood samples and estrous cycle evaluations made at 3, 6, 9, and 12 months followed by sacrifice at 12 months. No effects upon clinical signs or mortality were noted. The 400 ppm group had statistically significant decreases in mean body weight compared to controls beginning at week 2 (96% of controls), decreasing to 88-92% of controls for the last 10 months of the study. The 400 ppm group had statistically significantly decreased mean body weight gain from weeks 2-46 with mean values ranging from 82-89% of controls. Food consumption for the 400 ppm group ranged from 88-101% of controls, statistically significant at 6 weekly intervals in the first 11 weeks. The 400 ppm group had food consumption close to that of controls for the last 9 months of the study. Food efficiency for the 400 ppm group was decreased in comparison to controls during the first 12 weeks of the study. After 12 weeks, the 400 ppm group had similar, or occasionally increased food

efficiency in comparison to controls. Mammary gland hypertrophy (11/55 vs 6/55) and skin masses (9/55 vs 4/55, apparently associated with mammary tumors) were increased in high-dose females compared to controls. Mammary gland adenocarcinomas were increased in high-dose females compared to controls (6/55 vs 1/55), mammary gland adenomas were similar in incidence in high-dose animals compared to controls (1/5 vs 0/55), and mammary gland fibroadenomas were only slightly increased in high-dose females in comparison to controls (4/55 vs 2/55). The increase in mammary gland adenocarcinomas in high-dose females was not statistically significant, however the increase of combined mammary gland adenomas, adenocarcinomas, and fibroadenomas (11/55 vs 3/55 combined) was significant ($p \leq 0.05$). There were no significant differences for onset time among treatment groups when mammary gland adenomas, adenocarcinomas, and fibroadenomas were considered separately, however, there was a significant positive trend ($p \leq 0.05$) when fibroadenomas were combined with either adenomas or adenomas and adenocarcinomas.

The NOEL is 70 ppm (4.1 mg/kg/day) and the LOEL is 400 ppm (23.9 mg/kg/day) in females based on decreased body weight, body weight gain, and increased mammary gland adenocarcinomas.

This special study is classified **supplementary**, does not satisfy the guideline requirement for a chronic oral study in the rat, and cannot be upgraded. The following guideline deficiencies were noted: only females were tested; no clinical chemistry, hematology, urinalysis, or ophthalmology testing; liver and kidneys were not weighed; microscopic exam only included the pituitary, ovaries, uterus, vagina, inguinal skin with mammaries, and masses; and not all results were reported (hormone analysis and estrous cycle evaluations).

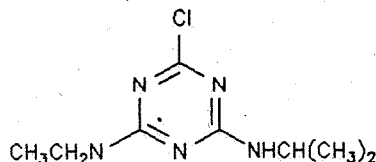
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Atrazine Technical
Description: white powder
Lot #: FL-881692, EHC Lot # 0173-10
Purity: 97.1% a.i.
Expiration date: 3/6/95
CAS #:
Supplied by Ciba Geigy Crop Protection,
Greensboro, NC.
Stored at room temperature.

Structure:

2. Vehicle and/or positive control: N/A3. Test animals:

Species: Female Rats

Strain: Crl:CD(SD)BR

Age and wt. at study initiation: 6 weeks, 146 g

Source: Charles River Laboratories, Raleigh, NC

Housing: individual polycarbonate cages

Diet: Purina (PMI) Rodent Chow 5002 ad lib, ground meal.Water: ad lib

Acclimation: 14 days

Environment: Temperature: 19-24°C

Humidity: 40-60%

Air changes: 15/hr

Photoperiod: 12 hr cycle

NOTE: There were also 14 male rats housed in the animal room to support maintenance of regular estrous cycling.

B. STUDY DESIGN: The stated purpose of this special study with female rats was described in the study protocol as follows: "This study is designed to determine the effects of chronic (12 month) dietary administration of atrazine Technical to female CD rats on the mammary and pituitary glands, the estrous cycle, and plasma levels of estradiol, luteinizing hormone (LH), progesterone and prolactin. An attempt will be made to correlate the plasma hormone levels and estrous cycle effects with the onset of mammary tumor development (if any). This study is also designed to provide a no-observable-effect level and does not unnecessarily duplicate a previous study."

1. In life dates - start: 3/16/93 end: 4/1/942. Animal assignment

Animals were assigned to study groups with similar mean weights as shown in Table 1.

TABLE 1: STUDY DESIGN

Conc. in Diet (ppm)	Dose to animal mg/kg/day	# of Females/Month of Sacrifice ¹			
		3	6	9	12
0	0	10	10	10	25
15	0.8	10	10	10	25
30	1.7	10	10	10	25
50	2.8	10	10	10	25
70	4.1	10	10	10	25
400	23.9	10	10	10	25

¹Ten rats in each dose group had vaginal smears made near the time of sacrifice and trunk blood samples taken for hormone analysis at time of sacrifice (months 3, 6, 9, or 12). An additional 15 rats in each dose group had orbital blood samples and vaginal smears taken at 3, 6, and 9 months, and when sacrificed at 12 months.

3. Dose selection rationale: In two studies with CD rats (MRID 4220444-01 and 420850-01, 425471-07, 427439-02), decreased body weight gain, increased mortality, and mammary tumors in females were reported by the study authors at a dietary concentration of 400 ppm.

4. Diet preparation and analysis: Diet was prepared every 2 weeks by mixing appropriate amounts of test material with Purina (PMI) Certified Rodent Chow 5002 and was stored at 4°C. No adjustment was made for purity. Concentration and homogeneity were tested at least monthly. Animals were given fresh feed jars weekly.

Results:

Concentration and Homogeneity: 99-103%

Stability: 94.4-109.9% when stored at 4°C for up to 41 days and 90.1-100.8% when stored at room temperature in open feed jars for up to 14 days.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics - A copy of statistical methods is attached.

Atrazine

RIN 3084-96

Page 44 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
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- The product confidential statement of formula.
- Information about a pending registration action.
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- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

C. METHODS:

1. Observations: Animals were inspected at least twice daily for signs of toxicity and mortality.
2. Body weight: Animals were weighed weekly for the first 13 weeks and every 4 weeks thereafter. Final body weights were determined at necropsy.
3. Food consumption and compound intake: Food consumption for each animal was determined and mean weekly food consumption was reported as g/day. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) and compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight gain data.
4. Ophthalmoscopic examination was NOT done.
5. Urinalysis was NOT performed in this study.
6. Vaginal smears and estrous cycle evaluation: Vaginal smears to determine the stage of estrous were made at 3, 6, 9, and 12 months. Smears were made for 14-22 days for each animal near the time of sacrifice or interim blood sampling. A protocol amendment says that estrous cycle analysis will be reported at a later date.
7. Blood was NOT collected for hematology and clinical analysis.

Blood samples for hormone analysis were taken from 10 rats in each dose group at time of sacrifice (months 3, 6, 9, or 12). The females were sacrificed and trunk blood samples taken during the first proestrus after at least 14 days of daily vaginal smears or on the last day of the 22 day vaginal smearing interval if proestrus was not seen. Blood samples taken at sacrifice were obtained by decapitation without anesthesia so as to minimize effects on hormone concentrations. The trunk blood samples were collected between 11:00 a.m. and 1:00 p.m. using an oxalate rinsed funnel with heparinized centrifuge tubes.

An additional 15 rats in each dose group had orbital blood samples taken at 3, 6, 9, and 12 months. These blood samples were taken without anesthesia from the right eye if possible, or, if necessary, from the left eye. The orbital blood samples were taken between 11:00 a.m. and 1:00 p.m. using heparinized centrifuge tubes. Blood samples were taken during the first

proestrus after at least 14 days of daily vaginal smears or on the last day of the 22 day vaginal smearing interval if proestrus was not seen. Terminal sacrifice of the animals bled from the orbital plexus was by i.p. injection of sodium pentobarbital.

The female rats were removed one at a time from their home cage and carried to a separate room for sacrifice or blood sampling. Plasma hormones to be tested were estradiol, luteinizing hormone, progesterone, and prolactin. A protocol amendment says that results of hormone analysis will be reported at a later date.

8. Sacrifice and Pathology:

All animals that died and those sacrificed on schedule were subjected to gross pathological examination. The checked (X) tissues were collected for histological examination and the (XX) organs were also weighed, as shown in Table 2. Ovaries, uterus, and vagina were only evaluated for stage of estrous and senescent changes.

TABLE 2 HISTOPATHOLOGY AND ORGAN WEIGHTS

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*		Brain*
	Salivary glands*		Heart*		Periph.nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)*
	Stomach*		Lymph nodes*	XX	Pituitary*
	Duodenum*		Spleen*		Eyes (optic n.)*
	Jejunum*		Thymus*		
	Ileum*				
	Cecum*		UROGENITAL		GLANDULAR
	Colon*		Kidneys**		Adrenal gland*
	Rectum*		Urinary bladder*		Lacrimal gland
	Liver**		Testes**	X	Mammary gland*
	Gall bladder*		Epididymides		Parathyroids*
	Pancreas*		Prostate		Thyroids*
			Seminal vesicle		
	RESPIRATORY	XX	Ovaries**		
	Trachea*	XX	Uterus*		
	Lung*	X	Vagina		
	Nose				OTHER
	Pharynx				Bone*
	Larynx			X	Skeletal muscle*
					All gross lesions and masses*

* Required for chronic studies based on Subdivision F Guidelines.

+ Organ weight required in chronic studies.

NOTE: Brain was weighed but not examined microscopically.

II. RESULTS

A. Observations:

1. Toxicity - No clinical signs of toxicity attributed to treatment were seen. Frequency of clinical signs (chromodachryorrhea, dehydration, hair thinning, ocular opacity, stained hair coat) was similar between control and treatment groups.

2. Mortality - Mortality was similar between control and treatment groups. There were 1, 2, 0, 1, 2, and 2 unscheduled deaths in the control, 15, 30, 50, 70, and 400 ppm groups respectively. Kaplan-Meier adjusted survival rates were reported to be 98%, 93%, 100%, 96%, 96%, and 96% for the 0, 15, 30, 50, 70, and 400 ppm groups, respectively.

B. Body weight: Body weights are shown in Table 3. Mean body weights for the 15, 30, and 50 ppm groups were similar to control weights.

The 70 ppm group had slight decreases in mean body weight compared to controls (statistically significant only from weeks 9-12). Mean body weights for the 70 ppm group were 95% of controls at week 9 decreasing to approximately 91% of controls during the last 2 months of the study. Mean body weights for the 70 ppm group were decreased only slightly (5-7% less than controls) for the first 10 months of the study, were not statistically significant except for weeks 9-12, and are not considered toxicologically significant.

The 400 ppm group had statistically significant decreases in mean body weight compared to controls from weeks 2-42. Mean body weights for the 400 ppm group were 96% of controls at week 2, decreasing to 91% of controls by week 9, and were 89-91% of controls for the remainder of the study. The NOEL/LOEL for decreased body weight in females is 70/400 ppm.

TABLE 3 MEAN WEEKLY BODY WEIGHTS (grams)

WEEK	0 ppm	15 ppm	30 ppm	50 ppm	70 ppm	400 ppm
initial	146.31	148.50	146.88	145.95	145.37	145.34
1	171.63	176.76	173.60	172.08	166.88 (97.2%)	167.23 (97.4%)
2	196.34	200.66	196.73	198.06	193.16 (98.4%)	189.10* (96.3%)
3	218.93	223.52	218.82	218.62	214.05 (97.8%)	209.75* (95.8%)
4	237.54	243.02	235.74	236.51	232.02 (97.7%)	225.97** (95.1%)
5	251.44	255.95	250.63	251.93	245.03 (97.4%)	236.55** (94.1%)
6	264.17	267.44	262.11	264.26	257.87 (97.6%)	249.10** (94.3%)
7	273.69	276.91	271.40	273.28	265.29 (96.9%)	254.66** (93.0%)
8	283.32	285.25	281.10	279.78	271.89 (96.0%)	260.72** (92.0%)
9	293.35	293.75	288.04	284.84	279.40* (95.2%)	266.33** (90.8%)
10	299.76	300.36	295.63	293.80	285.04* (95.1%)	273.26** (91.2%)
11	306.85	303.81	299.40	297.13	289.15** (94.2%)	276.45** (90.1%)
12	309.40	309.07	304.94	302.13	295.36* (95.4%)	280.65** (90.7%)
13	312.34	314.79	310.41	304.75	301.46 (96.5%)	285.85** (91.5%)
14	320.10	320.60	315.90	312.12	307.10 (95.9%)	293.71** (91.8%)
18	339.77	338.30	335.02	329.44	325.54 (95.8%)	310.57** (91.4%)
22	357.01	352.56	350.48	341.49	337.74 (94.6%)	322.60** (90.4%)
26	364.66	367.39	357.77	349.82	343.00 (94.0%)	327.62** (89.8%)
30	382.99	384.74	374.24	373.45	356.88 (93.2%)	340.67** (90.0%)
34	394.69	399.44	387.74	386.39	367.97 (93.2%)	359.73* (91.1%)
38	409.70	418.59	403.69	400.46	383.65 (93.6%)	372.71* (91.0%)

TABLE 3 MEAN WEEKLY BODY WEIGHTS (grams)

WEEK	0 ppm	15 ppm	30 ppm	50 ppm	70 ppm	400 ppm
42	431.38	432.88	415.78	418.56	394.11 (91.4%)	381.40* (88.4%)
46	447.24	450.99	432.53	438.87	404.14 (90.4%)	398.32 (89.1%)
50	460.03	464.54	456.65	453.58	423.44 (92.0%)	408.27 (88.7%)

From Table 9.6, pages 48-50 of study report, MRID 43934402.

*p≤0.05 **p≤0.01

NOTE: Numbers in parentheses are % of control value as calculated by reviewer.

Body weight gains are shown in Table 9.7, copied from the study report. Mean body weight gains for the 15, 30, and 50 ppm groups were similar to controls. The 70 ppm group had statistically significantly decreased mean body weight gain from weeks 8-12 (90-92% of controls). Weight gains were not statistically significant for the 70 ppm group after week 12, values ranging between 87-94% of control weights for the remainder of the study.

The 400 ppm group had statistically significantly decreased mean body weight gain from week 2 until the end of the study. Mean values for body weight gain ranged from 82-89% of controls for the 400 ppm group. The NOEL/LOEL for decreased body weight gain in females is 70/400 ppm.

C. Food consumption and compound intake:

1. Food consumption - Mean weekly food consumption is shown in Table 9.8, copied from the study report. Food consumption for the 15, 30, and 50 ppm groups was similar to controls. Food consumption in the 70 ppm group ranged from 93-100% of controls. Food consumption for the 400 ppm group ranged from 88-101% of controls and was statistically significant principally from weeks 6-11. The 70 and 400 ppm groups had food consumption close to that of controls for the last 9 months of the study.

2. Food efficiency: Mean weekly food efficiency is shown in Table 9.9, copied from the study report. Food efficiency for the 400 ppm was decreased in comparison to controls during the first 12 weeks of the study. After 12 weeks, the 400 ppm group had similar, or occasionally increased food efficiency in comparison to controls.

Atrazine

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Pages 50 through 55 are not included.

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- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
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- A draft product label.
- The product confidential statement of formula.
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- The document is a duplicate of page(s) .
- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

3. Compound consumption: Compound consumption is shown in Table 1. Dietary concentrations of 30, 50, 70, and 400 ppm were calculated to be equivalent to 0.8, 1.7, 2.8, 4.1, and 23.9 mg/kg/day.

D. Ophthalmoscopic examination: Ophthalmoscopic examination was NOT performed.

E. Blood work:

1. Hematology - Hematological parameters were NOT determined.

2. Clinical chemistry - Clinical chemistry parameters were NOT determined.

3. Hormone analysis - Blood samples were taken to measure plasma hormone levels (estradiol, progesterone, prolactin, and luteinizing hormone). A protocol amendment says results will be reported at a later date.

F. Urinalysis - Urinalysis was NOT performed.

G. Sacrifice and Pathology:

1. Organ weight - There was no evidence of a treatment-related effect upon brain, ovarian, pituitary, or uterine weights, absolute or relative. Liver and kidneys were not weighed.

2. Gross pathology - Table 4 shows gross necropsy lesions. Mammary gland hypertrophy (11/55 vs 6/55) and skin masses (9/55 vs 4/55) were increased in high-dose females compared to controls. Although not explicitly stated, the skin masses were apparently associated with mammary tumors as the only skin samples reported taken were inguinal skin with mammary gland. Also increased in high-dose animals in comparison to controls were mammary gland cysts (19/55 vs 14/55) and abnormal uterine contents (4/55 vs 1/55), the latter lesions are not believed to be of toxicological significance. Although enlarged pituitary was reported increased in the 400 ppm group compared to controls (9/55 vs 5/55), this is not believed to be treatment related due to lack of a dose-response relationship.

TABLE 4 NECROPSY OBSERVATIONS - ALL ANIMALS

CONDITION	DIETARY CONCENTRATION (ppm)					
	0	15	30	50	70	400
NUMBER OF FEMALES	55	55	55	55	55	55
SKIN Mass(es)	4	4	4	4	6	9
MAMMARY GLAND(S) Cysts	14	11	11	14	12	19
Hypertrophy	6	2	7	6	6	11
PITUITARY Enlarged	5	6	7	2	4	9
UTERUS Abnormal contents	1	1	1	2	0	4

From Table 9.12.6 pages 70-73 of the study report, MRID 43934402.

3. Microscopic pathology: Table 5 shows selected histopathological findings. Only results for histological exam of pituitary, inguinal skin, and mammary gland were reported in this study.

a) Non-neoplastic - No increase in non-neoplastic lesions associated with treatment was seen microscopically.

b) Neoplastic - Mammary gland adenocarcinomas were increased in high-dose females compared to controls (6/55 vs 1/55) while mammary gland adenomas were similar in incidence in high-dose animals compared to controls (1/5 vs 0/55). Mammary gland fibroadenomas were only slightly increased in high-dose females in comparison to controls (4/55 vs 2/55). A peer review to verify mammary gland and pituitary neoplasm diagnoses confirmed the initial report.

The increase in mammary gland adenocarcinomas in high-dose females was reported not statistically significant. The increase of combined mammary gland adenomas, adenocarcinomas, and fibroadenomas was significant ($p \leq 0.05$, 3/55 vs 11/55 combined).

There were no significant differences for onset time among treatment groups when mammary gland

adenomas, adenocarcinomas, and fibroadenomas were considered separately. However, there was a significant positive trend ($p \leq 0.05$) when fibroadenomas were combined with either adenomas or adenomas and adenocarcinomas.

The study author points out that there was no evidence of pituitary neoplasms alone or when combined with telangiectasis which may be associated with pituitary adenomas.

As noted in the study deficiencies section, not all required tissues were examined microscopically.

TABLE 5 HISTOPATHOLOGICAL FINDINGS - ALL ANIMALS

CONDITION	DIETARY CONCENTRATION (ppm)					
	0	15	30	50	70	400
NUMBER OF FEMALES	55	55	55	55	55	55
MAMMARY GLAND						
Adenoma	0	0	1	0	1	1
Adenocarcinoma	1	2	0	1	1	6
Fibroadenoma	2	2	2	1	4	4
COMBINED TUMORS	3→	4	3	2	6	11*
NUMBER OF FEMALES	54	55	53	54	55	54
PITUITARY GLAND						
Adenoma	2	5	6	4	1	5
Telangiectasis	0	0	1	1	2	2

*different from control group, $p \leq 0.05$

→positive trend, $p \leq 0.05$

From Table 9.13.6, pages 82-84 and Table 6.2, page 568 of the study report, MRID 43934402.

H. Vaginal Smears and Estrous Cycle Evaluation - Estrous cycle evaluations were not reported in this report but will be presented at a later date. Vaginal smear data for individual animals were reported, but no summary tables were presented in this report. According to the Materials and Methods section (attached), after a preliminary evaluation

of the original estrous cycle data, it was decided that the slides should be re-evaluated. Although mentioned in a protocol amendment, it was not clear why the smears had to be re-evaluated.

III. DISCUSSION AND STUDY DEFICIENCIES:

A. Discussion: The blood samples and time of sacrifice were intended to be in the proestrus phase of the estrous cycle as determined by the vaginal smears. However, the estrous cycle evaluations and plasma hormone concentrations were not reported. According to a protocol amendment, estrous cycle evaluation (by vaginal smear and histology) and plasma hormone concentrations are to be reported later. The principal treatment-related effects reported in this study were decreased body weight and gains in 400 ppm females and increased adenocarcinomas in 400 ppm females.

Mean body weights for the 70 ppm group were close to those of the 400 ppm group for the last 2 months of the study. However, body weights for the 400 ppm group were statistically significant from weeks 2-42 and were decreased as much as 12% below controls. In contrast, body weights for the 70 ppm group were statistically significant only from weeks 9-12, were decreased less than 10% of controls, and are not considered toxicologically significant. The NOEL/LOEL for decreased body weight is 70/400 ppm. Similarly, body weight gain was significant from weeks 2-42 for the 400 ppm females and was significant only from weeks 8-12 in the 70 ppm group. The NOEL/LOEL for decreased body weight gain is 70/400 ppm.

Mammary gland adenocarcinomas were increased in high-dose females compared to controls (6/55 vs 1/55), mammary gland adenomas were similar in incidence in high-dose animals compared to controls (1/5 vs 0/55), and mammary gland fibroadenomas were only slightly increased in high-dose females (4/55 vs 2/55). The increased mammary adenocarcinomas in the 400 ppm group were not statistically significant while the incidence of the combined 3 tumor types in the 400 ppm group was significantly increased ($p \leq 0.05$) compared to controls. Onset time for mammary gland adenomas, adenocarcinomas, and fibroadenomas was not decreased. However, there was a significant positive trend for onset time ($p \leq 0.05$) when fibroadenomas were combined with either adenomas or adenomas and adenocarcinomas.

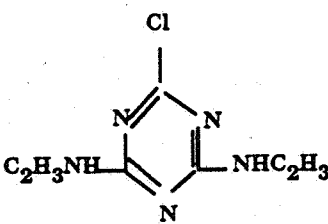
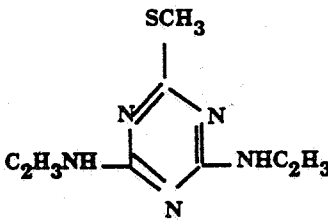
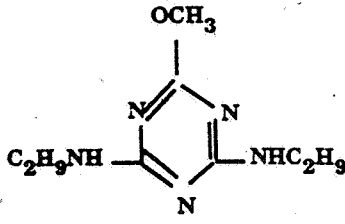
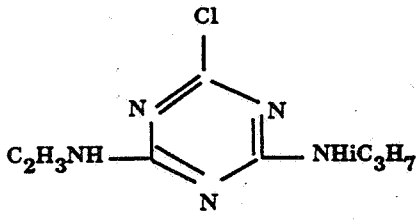
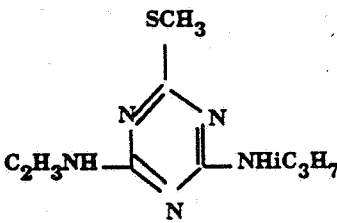
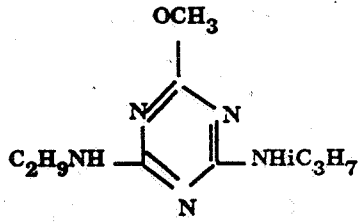
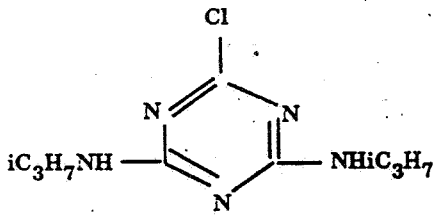
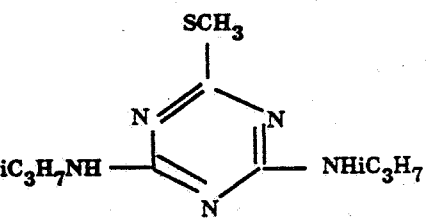
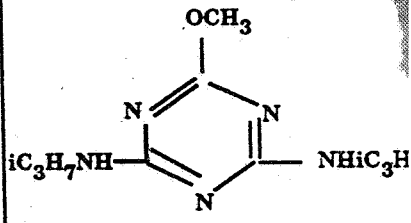
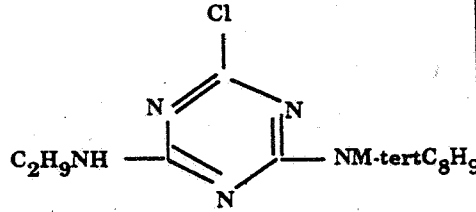
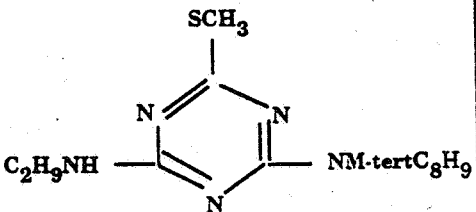
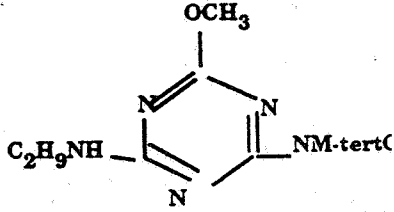
The NOEL is 70 ppm (4.1 mg/kg/day) and the LOEL is 400 ppm (23.9 mg/kg/day) in females based on decreased body weight, body weight gain, and increased mammary gland

adenocarcinomas. Although this study does not satisfy the guideline requirement for a chronic oral study in the rat due to the numerous deficiencies noted below, it does provide supplementary information about the toxicity and carcinogenicity of atrazine in female Crl:CD(SD)BR rats.

B. Study deficiencies: This special toxicity study in the female rat is classified supplementary and cannot be upgraded due to numerous guideline deficiencies: Only females were tested; no clinical chemistry, hematology, urinalysis, or ophthalmology testing; liver and kidneys were not weighed; not all results were reported (hormone analysis and estrous cycle evaluations); and limited microscopic exam (not examined microscopically were salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, gall bladder, pancreas, trachea, lung, aorta, heart, bone marrow, lymph nodes, spleen, thymus, kidneys, urinary bladder, brain, peripheral nerves, spinal cord, eyes, adrenal gland, parathyroids, thyroids, bone, and skeletal muscle). The reported skin masses were apparently associated with mammary tumors, but were not described nor were "abnormal" uterine contents described. The report says that after a preliminary evaluation of the estrous cycle data, it was decided to re-evaluate the vaginal smear slides. Although mentioned in a protocol amendment, it was not clear why the smears had to be re-evaluated.

One animal mistakenly received 15 ppm diet for 1 week rather than 30 ppm. At the 3-month interim sacrifice, 13 females in different dose groups did not have terminal body weights recorded. Terminal body weights for another 10 females at the 3-month interim sacrifice were not used because weights were mistakenly recorded post-exsanguination. The incorrect diet for 1 week and the omissions in body weight at the 3-month sacrifice did not alter the conclusions of the study.

FIGURE 1
MAMMARY TUMORIGENESIS FOR S. TRIAZINE HERBICIDES

2-SUBSTITUENTS		
Chloro-S-Triazines	Thio-methyl-S-Triazines	Methoxy-S-Triazines
<p><u>SIMAZINE</u> (+)</p> 	<p><u>SIMETRYN</u> (-)</p> 	<p><u>SIMETON</u> (NE)</p> 
<p><u>ATRAZINE</u> (+)</p> 	<p><u>AMETRYN</u> (-)</p> 	<p><u>ATRATON</u> (NE)</p> 
<p><u>PROPАЗINE</u> (+)</p> 	<p><u>PROMETRYN</u> (-)</p> 	<p><u>PROMETON</u> (-)</p> 
<p><u>TERBUTHYLАЗINE</u> (+)</p> 	<p><u>TERBUTRYN</u> (+)</p> 	<p><u>TERBUMETON</u> (+)</p> 

(+) Increased incidence of benign and/or malignant mammary tumors found in female Sprague-Dawley rats in at least one chronic bioassay.
 (-) Mammary tumor incidence in female Sprague-Dawley rat unaffected by treatment.
 (NE) Not evaluated IP - Study in progress