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

HED

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Date: 1/4/99

MEMORANDUM

SUBJECT: Atrazine - Review of a 2-year oncogenicity study, MRID 44544701.

FROM: Roger Hawks, Ph.D. Roger Hawks 1/4/99
RCAB
Health Effects Division (7509c)

THROUGH: Steve Knizner
Branch Senior Scientist, RCAB
Health Effects Division (7509c)

TO: Steve Huff
Special Review and Reregistration Division (7508w)

DP Barcode: D248083
Case: 819248
Submission: S546567
Chemical: Atrazine
Caswell No.: 063
PC Code: 080803
Registrant: Novartis Crop Protection
P.O. Box 1830
Greensboro, NC
27419-8300

Action Requested: Review a 2-year oncogenicity study in the rat (MRID 445447-01).

Response: The study has been reviewed and found to be Acceptable-Nonguideline. This does not satisfy an OPPTS 870 guideline and was not submitted with the intention of doing so.

Reviewers: Primary Reviewer - Roger Hawks, Ph.D. (RCAB)
Secondary Reviewer - Melba Morrow, D.V.M. (RRBI)

The Data Evaluation Record (DER) for this study is attached and the executive summary of the DER is included on the following page of this memo.

EXECUTIVE SUMMARY:

In a carcinogenicity study intended to provide information about the mode of action for oncogenicity in atrazine exposed Sprague-Dawley rats,(MRID 44544701), atrazine, [97.1% a.i.] was administered to 800 female Sprague-Dawley rats. The rats were divided into 2 groups of 400 each. One group was ovariectomized (OVX) while the other was left intact. Atrazine was mixed with the diet at dose levels of 0 (control) 25, 50, 70 and 400 ppm (0, 1.5, 3.1, 4.2, 24.4 mg/kg/day for intact animals and 0, 1.2, 2.5, 3.5, and 20.9 mg/kg/day for ovariectomized animals) for 2 years.

Hematology, clinical chemistry, and urinalysis were not assessed in this study. Food consumption in the dose groups compared to the controls was not altered by compound exposure.
The trend for survival was statistically significantly (SS) decreased in the dosed groups compared to the controls. Survival was as follows: 43.3% in controls; 31.7% - 25 ppm; 28.8% - 50 ppm; 31.6% - 70 ppm; 21.7% 400 ppm. Body weight was SS reduced in the first half of the study in the 400 ppm group (other groups were not significantly altered), but by the end of the study body weights were similar to control values. Organ weights for pituitary, uterus and the ovaries were taken in this study. No organ weights in either the intact or ovariectomized group were altered by compound exposure. The only gross necropsy finding which was altered by compound exposure was the occurrence of mammary masses in the intact dosed animals. Dosed animals showed a higher incidence of mammary masses (many of which were confirmed by histopathology to be tumors).
There were no non-neoplastic findings at histology that were increased in dosed OVX animals compared to controls. OVX animals
in all groups displayed very high incidences of juvenile uterus and castration cells in the pituitary which would be expected in an OVX animal. The only finding in dosed intact animals which was increased in incidence over controls were ovarian cysts which were slightly increased at 70 and 400 ppm compared to controls. There were though, many findings which were prevalent in the intact animals, yet were not seen (or were seen at lower levels) in the OVX animals. Mammary gland galactoceles were seen in anywhere from 65 to 78% of the intact animals, depending on the dose group, but the highest percentage of galactoceles in the OVX group was 24% in the 50 ppm group. Mammary gland secretory activity was seen in from 34 to 46% of the intact animals but was not seen in any OVX animals. Uterine dilation was seen in from 8-18% of the intact animals and uterine cystic endometrial hyperplasia was seen in about 50% of the intact animals. No OVX animals displayed uterine dilation and only about 20% displayed cystic endometrial hyperplasia. Intact animals also had increased incidence of pituitary findings compared to OVX animals. From 65 to 73% of the intact animals were found to have sinusoid ectasia/angiectasis but the range in OVX animals was 31 to 45%. The differences in mammary gland, uterine and pituitary findings between OVX and intact animals may provide information about the mode of action of atrazine's carcinogenicity.

Neoplastic histopathology findings were mostly limited to the pituitary and the mammary gland. Neither OVX nor intact animals showed an increase in pituitary tumors compared to their respective controls, but intact animals did show a 20-30% greater incidence of pituitary adenomas compared to OVX animals.
There were few mammary tumors in the interim sacrifice animals, which is not surprising given that these animals were sacrificed after only one-year. Excluding the interim sacrifice and looking only at those animals which were sacrificed at 24 months and those which died prematurely, there was

an increase in mammary tumor incidence at all intact dose groups compared to controls. In ascending order of dose the percentage of animals with any type of mammary tumors was: 38.3% in controls; 53.3%; 71.2%; 56.6% and 68.3% at 400 ppm. Looking at carcinomas alone values are: 18.3%; 36.7%; 33.9%; 20%; and 41.7%. Fibroadenomas alone were: 26.6%; 40%; 52.5%; 45%; and 40%. If interim sacrifice data are included the incidence rates for all types of tumors combined becomes: 30%, 42.5%; 56.4% 47.5% and 53.8%. The increased incidence is statistically significant at 50, 70 and 400 ppm.

A decrease in the time-to-tumor is also evident from exposure to atrazine. In the control group 50% of the tumors occurred in the last 6 months of the study. The percentage of tumors which appeared in the last 6 months of the study in the dose groups were: 35.8%; 37.5% 36.5% and 33.4%. The number of tumors which occurred in the first year of the study was slightly increased at 25, 50 and 760 ppm and greatly increased at 400 ppm: 9% in controls; 10.7%; 10%; 12% and 17.9% in the 400 ppm group.

An increased incidence of in mammary tumor in intact animals is determined to occur at doses as low as at 50 ppm (3.1 mg/kg/day), based on a statistically significant increase in combined fibroadenomas, carcinomas and adenomas.

The purpose of this study was to examine mammary tumor carcinogenesis in female Sprague-Dawley rats. Thus, a large part of this review focuses on carcinogenicity.

One of the more striking aspects of the study was the complete lack of mammary tumors in OVX animals. Not a single mammary tumor of any sort was seen in any OVX animal. The lack of mammary tumors in OVX animals provides evidence that the mode of action of atrazine is neither a direct genotoxic nor estrogenic effect on the mammary gland. Rather, an indirect hormonally-mediated effect involving the ovary is implied.

At the 50 ppm and above doses, there was a treatment related increase in mammary tumor incidence when compared to controls. Dosing was considered adequate based on decreases in body weight. Additionally, there was a decreased time-to-tumor at doses of 25 ppm and above.

This carcinogenicity study in the rat is **Acceptable-nonguideline**, and *does not* satisfy the guideline requirement for a carcinogenicity study (83-2a) in the rat nor was it submitted with the intention of satisfying a guideline.

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EPA Reviewer: Roger Hawks, Ph.D Roger Hawks, Date 1/4/99
Toxicology Branch II (7509C)
EPA Secondary Reviewer: Melba Morrow, DVM M Morrow, Date 1/4/99
Registration Action Branch I (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity feeding-rat; OPPTS 870.3200 [§83-2 b]

DP BARCODE: D248083 SUBMISSION CODE: S546567
P.C. CODE: 080803 TOX. CHEM. NO.: 063

TEST MATERIAL (PURITY): Atrazine (97.1%)

SYNONYMS: G-30027

CITATION: Morseth, S. (1998) Chronic (12-24 month) study in rats with atrazine technical. Covance Laboratories, Vienna, VA. Laboratory report number: 2386-108. April 15, 1998. MRID: 44544701. Unpublished.

SPONSOR: Novartis Crop Protection, Greensboro, N.C.

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The only gross necropsy finding which was altered by compound exposure was the occurrence of mammary masses in the intact dosed animals. Dosed animals showed a higher incidence of mammary masses (many of which were confirmed by histopathology to be tumors).

There were no non-neoplastic findings at histology that were increased in dosed OVX animals compared to controls. OVX animals in all groups displayed very high incidences of juvenile uterus and castration cells in the pituitary which would be expected in an OVX animal. The only finding in dosed intact animals which was increased in incidence over controls were ovarian cysts which were slightly increased at 70 and 400 ppm compared to controls. There were though, many findings which were prevalent in the intact animals, yet were not seen (or were seen at lower levels) in the OVX animals. Mammary gland galactoceles were seen in anywhere from 65 to 78% of the intact animals, depending on the dose group, but the highest percentage of galactoceles in the OVX group was 24% in the 50 ppm group. Mammary gland secretory activity was seen in from 34 to 46% of the intact animals but was not seen in any OVX animals. Uterine dilation was seen in from 8-18% of the intact animals and uterine cystic endometrial hyperplasia was seen in about 50% of the intact animals. No OVX animals displayed uterine dilation and only about 20% displayed cystic endometrial hyperplasia. Intact animals also had increased incidence of pituitary findings compared to OVX animals. From 65 to 73% of the intact animals were found to have sinusoid ectasia/angiectasis but the range in OVX animals was 31 to 45%. The differences in mammary gland, uterine and pituitary findings between OVX and intact animals may provide information about the mode of action of atrazine's carcinogenicity.

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At the 50 ppm and above doses, there <u>was</u> a treatment related increase in mammary tumor incidence when compared to controls. Dosing was considered adequate based on decreases in body weight. Additionally, there was a decreased time-to-tumor at doses of 25 ppm and above.

This carcinogenicity study in the rat is **Acceptable-nonguideline**, and *does not* satisfy the guideline requirement for a carcinogenicity study (83-2a) in the rat nor was it submitted with the intention of satisfying a guideline.

<u>COMPLIANCE</u>: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Atrazine Technical
   Description: White powder
   Batch #: SG8029BA10
   Purity: 97.1% a.i.
   Stability of compound: Stable at room temperature
   CAS#: 1912-24-9

2. Vehicle: No vehicle was used, compound was mixed directly into the diet.

3. Test animals: Species: rat
   Strain: Sprague-Dawley
   Age and weight at study initiation:
   8 weeks old
   body weight range - 150 to 283g
   Source: Charles River Laboratories. Raleigh, N.C.
   Housing: one per cage in stainless steel wire cages
   Diet: PMI® #5002 ad libitum
   Water: Tap water ad libitum
   Environmental conditions: Temperature: 64 to 81° F
   Humidity: 37.5 to 84.9%
   Air changes: minimum of 1 per hour
   Photoperiod: 12 hr light/12 hr dark
   Acclimation period: 2 weeks

B. STUDY DESIGN:

1. In life dates - start: May 9, 1995 end: July 22, 1997

2. Animal assignment

   Animals were assigned using a random card draw procedure to the test groups in table 1. Only animals which had not displayed any clinical signs during the acclimation period and those who were determined by vaginal cytology to be cycling normally were included in the randomization pool.

TABLE 1: STUDY DESIGN

	Dose ppm	Mean dose mg/kg/day intact	Mean Dose mg/kg/day ovx	12 month sac.	24 month sac	Total no. animals*
con.	0	0	0	20	60	160
Low-1	25	1.5	1.2	20	60	160
Low-2	50	3.1	2.5	20	60	160
Mid	70	4.2	3.5	20	60	160
High	400	24.4	20.9	20	60	160

* Note that 20 intact and 20 OVX animals were included at each dose group for 1 yr sac and 60 intact and 60 OVX were included in each dose group for 24 month sacrifice. Thus, the total number of animals in each dose group for the entire study was 160(80 intact and 80 OVX).

3. Dose Selection: Doses were selected based on the results of several previous 2-yr oncogenicity studies (MRIDs 40629302; 42085001;42204401; and 4394402).

4. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of test substance with PMI Certified Rodent Diet #5002 and was stored at room temperature. Homogeneity was tested on prestudy high and low dose samples and stability was tested on high and low dose samples from prestudy and week three. Stability was tested at 10 and 20 days following storage. During the study, samples of all dose levels of treated food were analyzed at weeks 1, 5, 14, 26, 39, 52, 78, 91 and 104, for concentration.

Results - Homogeneity Analysis:

	Nominal conc.	Measured conc. in ppm	% of nominal
Top	25 ppm	23.09;23.05	92.2;92.4
	400 ppm	409.8;403.6	102;101
Middle	25 ppm	23.10;23.17	92.4;92.7
	400 ppm	399.2;402.1	100;101

	Nominal conc.	Measured conc. in ppm	% of nominal
Bottom	25 ppm	23.66;23.8	94.6;95.2
	400 ppm	405.6;401.4	101;100

Results from duplicate samples at the high and low dose are displayed above. The measured concentration appeared to be very similar to the expected concentration as indicated by a mean of 93.2% for al the 25 ppm samples and a mean of 101% for all 400 ppm samples.

Stability Analysis:

	Nominal conc.	Measured conc. in ppm	% of nominal
Day 0	25 ppm	25.27[a];23.31[b]	101; 93.2
	400 ppm	387.9[a];375.7[b]	97; 101
Day 10	25 ppm	21.03; 22.71	90.8; 93.2
	400 ppm	370.8; 403.6	92.7; 101
Day 20	25 ppm	22.75; 22.84	91; 91.3
	400 ppm	375.7; 371.1	93.9; 92.8

a - The original samples collected for 10-day stability analysis were inadvertently destroyed. The values for days 0 and 10 are from a batch prepared on 7/31/95 and the day 0 values represent the mean of two analyses.
b - The day 0 values are the mean of the prestudy homogeneity analyses

Results from duplicate samples at the high and low dose are displayed above.

Concentration Analysis:

Nominal conc. in ppm	Measured conc. in ppm	% of nominal
25	24.45	97.8%
50	47.98	95.9%
70	68.83	98.3%
400	397.2	99.3%

Values of all measurements taken at all dose groups at all weeks in which an analysis of concentration was conducted.

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. Animals received fresh diet weekly.

6. Statistics - From pages 19 and 20 of MRID 44544701:

"Cumulative survival data were analyzed by life table techniques using the National Cancer Institute Package (Thomas, Breslow, and Gart. *Trend and homogeneity analyses of proportions and life table data*, Comput. Biomed. Res., 10:373-381, 1977). Trend analysis of survival was evaluated at the 5% one-tailed probability level. The ovariectomized and intact female data sets were analyzed separately."
"Mean weekly body weights (intact animals), body weight change, weekly food consumption, mean food consumption (weeks 1-104 based on values collected), fasted terminal body weight, and organ weight data of the treated groups were compared statistically to the data of the control group using one-way analysis of variance (ANOVA). The ovariectomized and intact female data were analyzed separately. Since the group 5 mean body weight at week 1 was significantly different from the control mean, the body weight data for the ovariectomized animals (groups 1-5) were analyzed using one-way analysis of covariance (ANCOVA), with the week 1 body weight as the covariate."
"For the data that were analyzed by ANOVA, Levene's test (Levine, 1960) was utilized to test for variance homogeneity. If variances of untransformed data were heterogenous ($p \leq 0.05$), rank transformation of the data was performed to achieve variance homogeneity. If transformation did not achieve variance homogeneity, the analysis were performed on the rank transformed data. If the ANOVA was significant, Dunnett's multiple comparison t-test (Dunnett, 1964) was used for pairwise comparisons between the treated and control group means. Group comparisons were evaluated at the 5.0% two-tailed probability level."
"Palpable tumors noted in the mammary gland of the intact females (carcinoma, adenoma/carcinoma and fibroadenoma) were analyzed by the life table techniques (Thomas, Breslow, and Gart) using the first palpation time of any histopathologically-verified tumor as onset time. Pituitary adenomas noted in the intact and ovariectomized females were analyzed by logistic prevalence method (Dinse and Lagakos, *Regression analysis of tumor prevalence data*, J. Roy. Sts. Soc. Series C [Appl. Stat.], 32: 236-248, 1983)."

C. METHODS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality.

2. Body weight

Animals were weighed at the start of the study, weekly for the first 14 weeks of the study, once every fourth week up to 52 weeks and then at weeks 59, 104 and 105.

3. Food consumption and compound intake

Food consumption for each animal was determined on a weekly basis for the first 13 weeks, and once every 4 weeks thereafter. Mean daily diet consumption was calculated as g food/rat/week. Compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination

Eyes were not examined opthalmoscopically.

5. Blood work

Blood was not collected. Hematology or clinical analysis were not performed.

6. Urinalysis

Urine was not collected.

7. Vaginal Smears

Vaginal smears for the determination of estrous cycling patterns were performed on all intact animals. Smears were conducted for two consecutive weeks (including weekends) every 4 weeks. This amounts to 2 weeks of vaginal smears followed by two weeks rest throughout the study.
Smears were always done in the morning. A vaginal smear consisted of a vaginal lavage using 0.9% saline which was spotted thinly onto a glass slide. These slides were stained with 1% Toluidine Blue in H_2O solution and allowed to dry. Upon drying they were sent to Dr. Lee Tyrey at Duke University for evaluation.

8. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to a complete gross pathological examination. A full histopathological examination was not conducted in this study. Only the pituitaries, ovaries (intact animals only) uterus with vagina, mammary tissue from the inguinal area, and the brain were preserved in 10% neutral buffered formalin. All these tissues were subsequently examined histologically but only the pituitary, ovaries, and uterus with vagina were weighed.

II. **RESULTS:**

A. Observations

1. Toxicity - Other than the occurrence of palpable masses in the intact group, there were no clinical findings which were increased in dosed animals compared to controls. Few animals in the OVX group were observed to have palpable masses during the course of this study. None of the palpable masses in the OVX animals were confirmed by histopathology to be mammary tumors. Most, but not all, of the palpable masses in the intact animals were later confirmed to be tumors by histopathology.

Table 2: Palpable masses. The number of animals with palpable masses is shown along with the percentage of animals in that group which were found to have palpable masses. Each group consists of 80 animals.

	control	25 ppm	50 ppm	70 ppm	400 ppm
OVX	3 (3.8)	2 (2.5)	2 (2.5)	2 (2.5)	6 (7.5)
Intact	29 (36.2)	28 (35)	43 (53.8)	39 (48.8)	46 (57.5)

Data for this table taken from page 23, current study, MRID 44544701.

2. Mortality - There was little difference in mortality between groups at 104 weeks in the ovariectomized (OVX) animals. However, in the intact animals, mortality increased with increasing dose. The study author states that historical control data for this laboratory indicate that the mean survival rate of intact SD rats in this laboratory is 40% at 104 weeks. The examining pathologist for this study commented on what he believed the cause of death for each animal was. As is shown in table 5, the most common cause of death for both intact and OVX animals was pituitary neoplasms. In all dose groups, except the 50 ppm intact, pituitary neoplasms killed over 50% of the animals which died before sacrifice. Mammary

tumors also were attributed to the early death of many of the intact animals. In all the intact animal groups, including controls, pituitary and mammary neoplasms combined were responsible for over 80% of the early deaths. Not a single OVX animal died of a mammary tumor (in fact, not a single OVX animal was observed to have a mammary tumor). Exposure to atrazine did not appear to increase the deaths due to either type of tumor in either intact or OVX animals.

Table 3: Adjusted survival at week 104.

Adjusted survival does not include accidental deaths or interim sacrifices. The numerator is the number of animals which survived and the number in parenthesis is the percent of animals which survived.

	control	25 ppm	50 ppm	70 ppm	400 ppm	Hist. Control@
OVX	44/60 (73.3%)	42/59 (71.2%)	46/59 (80%)	45/60 (75%)	44/60 (73.3%)	---
Intact*	26/60 (43.3%)	19/60 (31.7%)	17/59 (28.8%)	19/60 (31.6%)	13/60 (21.7%)	$\bar{x}$=40% Hi.=52% Low=33%

* Trend is significant. Specific p value not indicated.
\# Significant compared to control. Specific p value not indicated. Data from page 21, current study, MRID 44544701
@ Historical controls data shown on page 80 current study. These data are from 14 separate 2 yr rat studies conducted at Covance.

Table 4: Percent survival in 3 2 yr oncogenicity studies with atrazine exposed SD rats.

	Control	400/500 ppm	% difference	MRID
Morseth	43%	22% (400 ppm)	49%	Current study
Thakur	53%	37% (400 ppm)	29%	42204401
Mayhew	50%	26% (500 ppm)	48%	00158930
Hist. Control	$\bar{x}$=40% Range= 33-52%	--	--	see Table 3

Table 5: Cause of death in unscheduled deaths as noted by the examining pathologist. Values shown are percent of unscheduled deaths which had either pituitary neoplasms or mammary neoplasm listed as the cause of death.

	control	25 ppm	50 ppm	70 ppm	400 ppm
Pituitary Neoplasm	OVX=75 In.=70.5	OVX=61.1 In.=66.6	OVX=50 In.=40.9	OVX=86.6 In.= 60.9	OVX=68.8 In.= 61.7
Mammary Neoplasm	OVX=0 In.=23.5	OVX=0 In.=23.8	OVX=0 In.=43.2	OVX=0 In.=21.9	OVX=0 In.=34
Pituitary/ mammary	In.=93.5 32/34*	In.=90.4 38/42	In.=84.1 37/44	In.=82.8 34/41	In.=95.7 45/47

Data from pages 204 and 206, current study, MRID 44544701
* - The first number represents the number of intact animals dying an unscheduled early death who were considered by the examining pathologist to have either a pituitary or mammary neoplasm as the primary cause of death. The second number is total number of animals in that dose group that died an early unscheduled death.

B. Body weight - Ovariectomized animals of all groups gained weight more rapidly and weighed more at the end of the study than intact animals from the equivalent group.

Table 6: Mean body weights at selected intervals. Values shown are body weight in grams. OVX=ovariectomized, Int=intact.

	1 wk	3 month (12 wk)	6 month (26 wk)	12 month (52 wk)	18 month (78 wk)	24month (105wk)
Con.	OVX=226 Int=208	OVX=397 Int=307	OVX=439 Int=343	OVX=523 Int=412	OVX=535 Int=452	OVX=540 Int=421
25 ppm	OVX=223 Int=209	OVX=395 Int=310	OVX=442 Int=348	OVX=528 Int=428	OVX=537 Int=462	OVX=562 Int=467
50 ppm	OVX=219 Int=211	OVX=391 Int=310	OVX=439 Int=345	OVX=524 Int=414	OVX=558* Int=451	OVX=545 Int=433
70 ppm	OVX=220 Int=212	OVX=390 Int=310	OVX=438 Int=347	OVX=526 Int=419	OVX=548 Int=477	OVX=528 Int=470
400 ppm	OVX=216* Int=214	OVX=370* Int=290*	OVX=405* Int=327	OVX=479* Int=390*	OVX=492 Int=417	OVX=488 Int=424

* significantly different from control at $p<0.05$
Data taken from pages 100-110, current study, MRID:44544701

Table 7: Mean body weights gains at selected intervals month intervals. Values shown are % increase in body weight.

	1 wk to 12 wk	12 wk to 25 wk	25 wk to 52 wks	52 wk to 78 wks	78 wk to 105 wk	1-104 wk
Con	OVX=76 Int=47.6	OVX=10.6 Int=11.7	OVX=19 Int=20.1	OVX=2.3 Int=9.7	OVX=0.9 Int=-6.9	OVX=138 Int=102
25 ppm	OVX=77 Int=48	OVX=11.9 Int=11.2	OVX=19.5 Int=23	OVX=1.7 Int=7.9	OVX=4.6 Int=-1.1	OVX=152 Int=123
50 ppm	OVX=78.5 Int=47	OVX=12.2 Int=11.3	OVX=19.4 Int=20	OVX=6.5 Int=8.9	OVX=-2.3 Int=-4.0	OVX=149 Int=105
70 ppm	OVX=77.2 Int=46.2	OVX=12.3 Int=11.9	OVX=20.1 Int=20.7	OVX=4.2 Int=13.8	OVX=-3.6 Int=-1.5	OVX=140 Int=122
400 ppm	OVX=71.3 Int=35.5	OVX=9.5 Int=12.8	OVX=18.3 Int=19.3	OVX=2.7 Int=6.4	OVX=-0.8 Int=1.6	OVX=126 Int=98

Data taken from pages 111-121, current study, MRID:44544701

C. Food consumption and compound intake

1. Food consumption - The ovariectomized animals consumed more food in the first few months of the study than did the intact animals in all dose groups. For the remainder of the study there was not a great difference in food consumption between the two groups.
At several time points throughout the study, food consumption for the HDT OVX animals was significantly less than controls. For the study as whole, food consumption was not significantly different in the OVX controls *vs* the OVX HDT animals. No other dose group in the OVX animals had food consumption that was consistently significantly different from controls.
Similar to the OVX animals, the intact animals in the HDT had significantly decreased food consumption at several time points - especially the first 3 months of the study- compared to controls. For the study as a whole, food consumption was not significantly different in the intact controls *vs* the intact HDT animals. The Mid dose group had significantly increased food consumption for the entire study, but was only occasionally significantly different from controls at individual week time points. No other dose group in the intact animals had food consumption that was consistently significantly different from controls.

Table 8: Mean food consumption for selected weeks. Values shown are mean group values in grams of food per animal per week.

	week 1	week 12	Week 52	Week 77	Week 104	1-104 wk
Con	OVX=176 Int=146	OVX=136 Int=130	OVX=127 Int=128	OVX=140 Int=146	OVX=125 Int=129	OVX=5141 Int=4874
25 ppm	OVX=177 Int=146	OVX=136 Int=135	OVX=131 Int=143*	OVX=137 Int=147	OVX=127 Int=138	OVX=5166 Int=5075
50 ppm	OVX=180 Int=149	OVX=140 Int=132	OVX=129 Int=144*	OVX=139 Int=156	OVX=120 Int=134	OVX=5141 Int=5118
70 ppm	OVX=172 Int=148	OVX=142 Int=131	OVX=131 Int=144*	OVX=130 Int=155	OVX=122 Int=140	OVX=5161 Int=5213*
400 ppm	OVX=160* Int=131*	OVX=135 Int=122*	OVX=128 Int=134	OVX=136 Int=141	OVX=122 Int=118	OVX=5024 Int=4830

* significantly different from control at $p<0.05$
Data taken from, pages 122 - 132, current study, MRID:44544701

2. Compound consumption (time-weighted average) - Compound consumption in both intact and OVX animals decreased as the study progressed. Food consumption during the last week of the study was about half what it was in first week of the study. This was true for all dose groups of both OVX and intact animals. Compound consumption in the intact animals was slightly higher than in the OVX animals. Mean compound consumption for the course of the study OVX/Intact: 25 ppm - 1.2/1.5; 50 ppm - 2.5/3.1; 70 ppm - 3.5/4.2; 400 ppm - 20.9/24.4.

3. Food efficiency - The study authors did not perform food efficiency calculations. Because there was not a statistically significant difference in either body weight or food consumption for any dose groups compared to controls (except the 70 ppm intact group), food efficiency calculations were not deemed relevant by this reviewer.

D. Blood work, Ophthalmoscopic exams and urinalysis: Hematology, clinical chemistry, ophthalmoscopic exams and urinalysis were not conducted.

E. Vaginal Smears: The vaginal smear data were not included in this report and will be reported at a later date.

F. Sacrifice and Pathology:

1. Organ weight - There were no differences in either absolute or relative (to body weight) organ weight in any dose group compared to controls at either 12 months or at

24 months. Although there are no differences between dose groups there are differences between the OVX animals and the intact animals. Organ weights at both time points, both absolutely and relatively, were less in the OVX animals than in the intact animals. Tables 9 and 10 display the absolute and relative organ weights for both sets of animals.

Table 9: Mean group absolute organ weights. These values do not include animals that died before their scheduled sacrifice.

	12 month intact	12 month OVX	24 month intact	24 month OVX
Con	Uterus- 1.35 Pit.-0.043 Ovaries-0.129	Uterus-0.36 Pit.-0.019	Uterus-1.52 Pit.-0.124 Ovaries-0.163	Uterus-0.47 Pit.-0.046
25 ppm	Uterus-1.36 Pit.-0.033 Ovaries-0.14	Uterus-0.34 Pit.-0.19	Uterus-1.57 Pit.-0.158 Ovaries-0.17	Uterus-0.42 Pit.-0.040
50 ppm	Uterus-1.25 Pit.-0.024 Ovaries-0.124	Uterus-0.32 Pit.-0.023	Uterus- 1.4 Pit.-0.119 Ovaries-0.162	Uterus- 0.47 Pit.-0.053
70 ppm	Uterus- 1.34 Pit.-0.028 Ovaries-0.125	Uterus-0.34 Pit.-0.018	Uterus- 1.53 Pit.-0.107 Ovaries-0.186	Uterus-0.45 Pit.-0.066
400 ppm	Uterus- 1.34 Pit.-0.029 Ovaries-0.144	Uterus-0.36 Pit.-0.019	Uterus- 1.34 Pit.-0.123 Ovaries-0.241*	Uterus-0.5 Pit.-0.048

* see footnote for table 10

Table 10: Mean group relative to the body organ weights. These values do not include animals that died before their scheduled sacrifice.

	12 month intact	12 month OVX	24 month intact	24 month OVX
Con	Uterus- 0.35 Pit.-0.0115 Ovaries-0.034	Uterus-0.065 Pit.-0.0035	Uterus-0.4 Pit.-0.0359 Ovaries-0.0423	Uterus-0.09 Pit.-0.01
25 ppm	Uterus-0.338 Pit.-0.0089 Ovaries-0.034	Uterus-0.064 Pit.-0.0035	Uterus-0.375 Pit.-0.0386 Ovaries-0.0383	Uterus-0.079 Pit.-0.0099
50 ppm	Uterus-0.31 Pit.-0.006 Ovaries-0.03	Uterus-0.062 Pit.-0.0043	Uterus- 0.348 Pit.-0.0317 Ovaries-0.0409	Uterus-0.091 Pit.-0.0156
70 ppm	Uterus- 0.359 Pit.-0.0075 Ovaries-0.003	Uterus-0.065 Pit.-0.0035	Uterus- 0.357 Pit.-0.0287 Ovaries-0.0418	Uterus- 0.09 Pit.-0.0168
400 ppm	Uterus- 0.361 Pit.-0.0077 Ovaries-0.039	Uterus-0.072 Pit.-0.0038	Uterus- 0.333 Pit.-0.0307 Ovaries-0.0669*	Uterus- 0.11 Pit.-0.0129

* Ovarian weight, both absolutly and relatively, is unusually high due to the presence of one set of ovaries which weighed 1.16 grams. This value was clearly an outlier since 1.16 grams is more than 3 times heavier than the next heaviest set of ovaries in *any* group. The mean absolute weight for this group excluding this set of ovaries is 0.165 grams.
Organ wt data from pages 189 to 200 current study

2. Gross pathology - There were few gross necropsy findings in the animals that had been ovariectomized. All animals in this group had a small uterus, which is to be expected. The incidence of thickened mammary gland tissue was increased in the two high dose groups over control, but this increase was not dose related, and occurred only in animals which died unscheduled deaths. Intact dosed animals showed an increase in ovarian cysts when compared to control animals. Dosed animals also showed increases in masses in the mammary region compared to control animals. Many of these masses were confirmed by histology to be mammary tumors. Many animals of all groups, both intact and OVX animals, showed lesions of the pituitary gland. Neither dosed OVX or dosed intact animals showed increased incidences of lesions when compared to control OVX or control intact animals. The percentage of animals with pituitary lesions did not seem to differ between OVX and intact animals regardless of dose group. Tables 11

and 12 list incidences of non-pituitary gross necropsy findings for OVX and intact animals. Table 13 lists pituitary gross necropsy findings.

Table 11: Non-pituitary gross pathology findings in *OVX* animals, scheduled and unscheduled deaths (12 month sacrifice data excluded). Sch.= scheduled; Unsh.= Unscheduled; Comb.=combined deaths.

	Control	25 ppm	50 ppm	70 ppm	400 ppm
Small Uterus	Sch.= 95% Unsh.=75% Comb.=90%	Sch.=100% Unsh.=83% Comb.=95%	Sch.=96% Unsh.=50% Comb.=88%	Sch.=98% Unsh.=67% Comb.=90%	Sch.=100% Unsh.=75% Comb.=93%
Thick-ened mammary gland	Sch.=0% Unsh.=13% Comb.=3%	Sch.=0% Unsh.=17% Comb.=5%	Sch.=0% Unsh.=0% Comb.=0%	Sch.=0% Unsh.=40% Comb.=10%	Sch.=2% Unsh.=25% Comb.=8%

Table 12: Non-pituitary gross pathology findings in *intact* animals, scheduled, unscheduled deaths (12 month sacrifice data excluded). VFR=ventral front right; VFL=ventral front left; VHM=ventral hind mid. Sch.= scheduled; Unsh.= Unscheduled

	Control	25 ppm	50 ppm	70 ppm	400 ppm
Ovarian cyst	Sch.=15% Unsh.=6% Comb.=10%	Sch.=22% Unsh.=17% Comb.=18%	Sch.=31% Unsh.=11% Comb.=17%	Sch.=37% Unsh.=15% Comb.=22%	Sch.=15% Unsh.=28% Comb.=25%
Mass-VFR	Sch.=12% Unsh.=15% Comb.=13%	Sch.=22% Unsh.=17% Comb.=18%	Sch.=38% Unsh.=25% Comb.=28%	Sch.=11% Unsh.=15% Comb.=13%	Sch.=23% Unsh.=49% Comb.=32%
Mass-VFL	Sch.=4% Unsh.=15% Comb.=10%	Sch.=28% Unsh.=7% Comb.=13%	Sch.=25% Unsh.=14% Comb.=17%	Sch.=16% Unsh.=22% Comb.=20%	Sch.=23% Unsh.=21% Comb.=22%
Mass-VHM	Sch.=0% Unsh.=6% Comb.=3%	Sch.=11% Unsh.=7% Comb.=8%	Sch.=13% Unsh.=16% Comb.=15%	Sch.=0% Unsh.=10% Comb.=7%	Sch.=31% Unsh.=9% Comb.=13%
Thick-ened mammary gland	Sch.=77% Unsh.=59% Comb.=67%	Sch.=72% Unsh.=67% Comb.=68%	Sch.=50% Unsh.=52% Comb.=52%	Sch.=84% Unsh.=56% Comb.=65%	Sch.=100% Unsh.=57% Comb.=83%

Table 13: Pituitary gross pathology findings in intact animals compared to OVX animals, scheduled and unscheduled deaths (12 month sacrifice data excluded). Sch.= scheduled; Unsh.= Unscheduled. Values shown are % of animals which displayed that finding.

	Enlarged	Mottled	Irregularly shaped
Control-Intact/OVX	Sch.=69/16 Unsh.=85/81 Comb.=78/33	Sch.=38/1 Unsh.=38/38 Comb.=38/12	Sch.=23/5 Unsh.=50/38 Comb.=38/13
25 ppm-Intact/OVX	Sch.=89/24 Unsh.=88/56 Comb.=88/33	Sch.=50/2 Unsh.=33/22 Comb.=38/8	Sch.=34/0 Unsh.=31/39 Comb.=33/12
50 ppm-Intact/OVX	Sch.=81/24 Unsh.=66/43 Comb.=87/28	Sch.=56/0 Unsh.=41/29 Comb.=45/7	Sch.=25/4 Unsh.=20/14 Comb.=22/7
70 ppm-Intact/OVX	Sch.=95/24 Unsh.=83/93 Comb.=87/42	Sch.=37/9 Unsh.=41/38 Comb.=14/15	Sch.=26/4 Unsh.=24/33 Comb.=25/12
400 ppm-Intact/OVX	Sch.=77/23 Unsh.=85/69 Comb.=88/35	Sch.=54/2 Unsh.=43/31 Comb.=15/10	Sch.=62/0 Unsh.=30/31 Comb.=37/8

Data for tables 11, 12 and 13 taken from current study, MRID 44544701, pages 144 to 188.

3. Microscopic pathology -

a) Non-neoplastic - With the exception of ovarian cysts in the intact HDT, there were no non-neoplastic histopathology findings in either OVX or intact dosed animals which were increased in incidence over controls. The small uterus and pituitary findings in the OVX animals at gross pathology were confirmed by histology. The ovarian cysts and pituitary findings were confirmed by histology. Histology revealed that intact animals had galactoceles (retention cyst in the mammary caused by occlusion of a lactiferous gland), which were not seen in the OVX animals. Tables 14 and 15 display non-neoplastic histology findings which occurred at a high incidence rate in OVX or intact animals.

Table 14: Non-neoplastic histology findings in *OVX* animals scheduled, unscheduled deaths and 12 month sacrifice combined. Values shown are percentage of animals which were observed to have that finding.

	Control	25 ppm	50 ppm	70 ppm	400 ppm
Juvenile Uterus	100%	100%	100%	100%	100%
Mammary-Galactocele	19%	15%	24%	20%	17%
Pituitary-Castration Cells	81%	90%	88%	83%	83%
Pituitary-Focal Hyperplasia	19%	19%	21%	23%	18%
Pituitary-Sinusoid Ectasia/ Angiectasis	39%	44%	31%	45%	44%

Table 15: Non-neoplastic histology findings in *intact* animals scheduled, unscheduled deaths and 12 month sacrifice combined. Values shown are percentage of animals which were observed to have that finding.

	Control	25 ppm	50 ppm	70 ppm	400 ppm
Mammary-Focal Hyperplasia	13%	14%	4%	9%	8%
Mammary-Secretory Activity	34%	39%	42%	43%	46%
Mammary-Chronic Inflammation	15%	15%	8%	11%	21%
Mammary-Galactocele	70%	73%	65%	75%	78%
Uterus-Cystic Endometrial Hyperplasia	48%	52%	46%	45%	39%
Uterus-Dilation	8%	13%	18%	18%	13%
Ovary-cyst	19%	16%	15%	24%	25%
Ovary-Sertoliform Tubule Hyperplasia	75%	81%	81%	79%	75%
Pituitary-Focal Hyperplasia	8%	5%	11%	9%	8%
Pituitary-Sinusoid Ectasia/ Angiectasis	73%	73%	65%	68%	70%

Data in tables 13 and 14 from pages 221 to 225, current study, MRID 44544701

b) Neoplastic - Table 16 displays pituitary tumor incident data in both OVX and intact animals as well as mammary tumor incident data in intact animals. The pituitary tumor incident data is shown because these tumors occur at high rates and because the pathologist

concluded that many of the unscheduled deaths were caused by pituitary tumors. The mammary tumor data is included in this table because the increase in mammary tumors in some dose groups is likely to be compound-related. There were occasional incidences of other types of tumors, but these tumors were few and did not appear in a dose-related manner. Table 17 displays tumor incidences for animals which were sacrificed at terminal (104) weeks sacrifice. Table 18 displays data for animals that were sacrificed before terminal sacrifice but were not interim sacrifice animals (unscheduled sacrifice). Table 19 displays combined data for scheduled and unscheduled sacrifices. Table 20 displays mammary tumor incidence in the interim sacrifice (12 months) animals. Table 21 shows the percentage increase in tumor incidence over control. Table 22 displays historical control data from a variety of sources along with concurrent control data from this study. Table 23 displays time-to-tumor data. The time-to-tumor data was calculated by looking at time of appearance of each palpable mass which was confirmed by histopathology to be a mammary tumor.

Table 16: Neoplastic histology findings in OVX and intact animals scheduled, unscheduled deaths and 12 month sacrifice combined.
The ratio shown is the number of animals which had that type of tumor over the total number of animals examined in that group. All the mammary tumors listed occurred in intact animals.

	Controls	25 ppm	50 ppm	70 ppm	400 ppm
Pituitary (OVX) - B-Adenoma	42/80 (53%)	39/80 (49%)	35/80 (44%)	42/80 (53%)	41/79 (52%)
Pituitary (intact) - B-Adenoma	56/80 (70%)	60/80 (76%)	52/80 (65%)	56/80 (70%)	52/80 (68%)
Mammary-fibroadenoma	16/80 (20%)	25/80 (31%)	33/78 (42%)	29/80 (36%)	25/80 (31%)
Mammary-Carcinoma	12/80 (15%)	18/80 (23%)	20/78 (26%)	14/80 (18%)	27/80 (34%)
Mammary-Adenoma	0%	0%	1/78 (1%)	0%	0%
Any mammary neoplasia	24/80 (30%)	34/80 (42.5%)	44/78 (56.4%)	38/80 (47.5%)	43/80 (53.8%)

Data from pages 221 to 225, current study, MRID 44544701

Table 17: Neoplastic histology findings in OVX and intact animals *scheduled* deaths (24 month sacrifice) only. The ratios shown in tables 17, 18, 19 and 20 is the number of animals which had that the of tumor over the total number of animals examined in that group. All the mammary tumors listed occurred in intact animals.

	Controls	25 ppm	50 ppm	70 ppm	400 ppm
Pituitary (OVX) - B-Adenoma	28/44 (64%)	26/42 (62%)	26/46 (56%)	26/45 (58%)	28/44 (64%)
Pituitary (intact) - B-Adenoma	23/26 (88%)	18/18 (100%)	15/16 (94%)	16/19 (84%)	10/13 (77%)
Mammary-fibroadenoma	4/26 (15%)	10/18 (56%)	9/15 (60%)	8/19 (42%)	6/13 (46%)
Mammary-Carcinoma	3/26 (12%)	12/18 (66%)	5/15 (33%)	3/19 (16%)	6/13 (46%)
Mammary-Adenoma	0	0	0	0	0
Any mammary neoplasia	7/26 (27%)	12/18 (67%)	11/15 (73%)	10/19 (53%)	10/13 (77%)

Data from pages 214 to 218, current study, MRID 44544701

Table 18: Neoplastic histology findings in OVX and intact animals *unscheduled* deaths only.

	Controls	25 ppm	50 ppm	70 ppm	400 ppm
Pituitary (OVX) - B-Adenoma	14/16 (88%)	13/18 (72%)	7/14 (50%)	14/15 (93%)	11/15 (73%)
Pituitary (intact) - B-Adenoma	30/34 (88%)	37/42 (88%)	34/44 (77%)	37/41 (90%)	41/47 (87%)
Mammary-fibroadenoma	12/34 (35%)	14/42 (33%)	22/44 (50%)	19/41 (46%)	18/47 (38%)
Mammary-Carcinoma	8/34 (24%)	10/42 (24%)	15/44 (34%)	9/41 (22%)	19/47 (40%)
Mammary-Adenoma	0/34	0/42	1/44 (2%)	0/41	0/47
Any mammary neoplasia	16/34 (47%)	20/42 (48%)	31/44 (70%)	24/41 (59%)	31/47 (66%)

Data from pages 202 to 207 current study, MRID 44544701

Table 19: Neoplastic mammary histology findings in intact animals *Scheduled and unscheduled* deaths combined.

	Controls	25 ppm	50 ppm	70 ppm	400 ppm
Fibroadenoma	16/60 (26.6%)	24/60 (40%)	31/59 (52.5%)	27/60 (45%)	24/60 (40%)
Carcinoma	11/60 (18.3%)	22/60 (36.7%)	20/59 (33.9%)	12/60 (20%)	25/60 (41.7%)
Adenoma	0/60	0/60	1/59 (1.7%)	0/60	0/60
Any mammary neoplasia	23/60 (38.3%)	32/60 (53.3%)	42/59 (71.2%)	34/60 (56.6%)	41/60 (68.3%)

Data calculated by reviewer using data from tables 17 and 18.

Table 20: Mammary tumor incidence in the interim sacrifice group

	Controls	25 ppm	50 ppm	70 ppm	400 ppm
Fibroadenoma	0	1/20	2/19	2/20	1/20
Carcinoma	1/20	1/20	0/19	2/20	2/20
Adenoma	0	0	0	0	0
Any mammary neoplasia	1/20	2/20	2/20	4/20	2/20

Data from page 45, current study.

Table 21: Percentage increase in tumor incidence over control

	25 ppm	50 ppm	70 ppm	400 ppm
All	42	83	58	79
Scheduled+ Unscheduled	39	83	48	78
Scheduled Only	48	170	96	185
Unscheduled Only	2	49	26	40

Data from page 45, current study.

Table 22: Historical control data for Sprague-Dawley females.

	Fibroadenoma	Carcinoma
Pooled Charles River Data	$\bar{x}$= 31.44% Range= 13.7-49.0%	$\bar{x}$= 17.68% Range= 7.1-31.4%
Pooled Ciba-Geigy, Summit, N.J. Data	$\bar{x}$= 31.3% Range= 20-43.3%	$\bar{x}$= 16.8% Range= 6.7-30%
Pooled Hazelton Data Pre-1984	$\bar{x}$= 38.5% Range= 22-55%	$\bar{x}$= 17.1% Range= 10-30%
Pooled Covance Data 1989-1994	$\bar{x}$= 47.8% Range=35.3-65%	$\bar{x}$= 21.5% Range= 6.0-32.8%
Concurrent controls (All animals)	$\bar{x}$= 20%	$\bar{x}$= 15%
Concurrent controls (Scheduled deaths)	$\bar{x}$= 15%	$\bar{x}$= 12%
Concurrent controls (Unscheduled deaths)	$\bar{x}$= 35%	$\bar{x}$= 24%
Concurrent controls (Scheduled + unscheduled deaths)	$\bar{x}$= 27%	$\bar{x}$= 18%

Ciba-Geigy Data, Summit, N.J. data from: McMartin, D., *et al.*, "Neoplasms and Related Proliferative Lesions in Control Sprague-Dawley Rats from Carcinogenicity Studies. Historical Data and Diagnostic considerations." Tox. Path: 20(2), 212-225.

Charles River Data from: Lang. P. . "Spontaneous Neoplastic Lesions and Selected Non-Neoplastic Lesions in the Crl:CD®BR Rat" Feb. 1992. Charles River Laboratories.

Hazelton Data from: Hazelton Labs. "Representative Historical Control Data". March 1994. Hazelton Laboratories.

Covance Data from: pooled results of 14 studies as shown on page 36, current study.

Concurrent control data from: current study.

Table 23: Time to tumor data using palpable masses which were confirmed by histopathology to be mammary tumors.

Dose Group ▸	Control	25 ppm	50 ppm	70 ppm	400 ppm
Mean wk of tumor appearance	$\bar{x}$=73.8	$\bar{x}$=70.3	$\bar{x}$=71.2	$\bar{x}$=68.3	$\bar{x}$=63.95
% of tumors which appeared ≤ 52 wks	9	10.7	10	12	17.9
% of tumors which appeared between 53 and 78 wks	41	53.5	52.5	51.5	48.7
% of tumors which appeared ≥ 79 wks	50	35.8	37.5	36.5	33.4
# of palpable masses confirmed by histopathology to be mammary tumors	22	28	40	33	39

Data calculated by reviewer using information from Appendix 5 and 10, current study, MRID 44544701.

III. **DISCUSSION**

A. This study was not submitted with the intention of satisfying a guideline requirement for atrazine. Rather, this study was submitted to provide further information regarding the incidence and onset of female mammary tumors in atrazine-treated Sprague-Dawley rats. A major difference between this study and previously submitted 2-year oncogenicity studies is that this study used ovariectomized animals and compared their responses to atrazine exposure to responses of intact animals. Also, since the intention of this study was to examine incidence and onset of mammary tumors in female rats, only female rats were used.

The study author concludes that the NOEL for cancer effects in this study was 70 ppm. The authors state that only at the HDT of 400 ppm was there an increased incidence and an earlier onset of mammary tumors in the intact animals.

> "Based on these results, the no-observed-effect-level (NOEL) for atrazine in the intact animals is considered to be at a dietary level of 70 ppm. This conclusion is based on the lack of a monotonic dose response for the fibroadenomas or carcinomas and the fact that the mammary tumor incidences observed at

> feeding levels of ≤ 70 ppm fall below the pooled control rate for this laboratory. The response observed for carcinomas in the 400 ppm group is mostly attributable to the earlier onset and partly due to increased incidence rate observed in this group." *Current study, pg 39*

The study author also concludes that the lack of mammary tumors in the OVX animals indicates that atrazine is neither genotoxic nor estrogenic. Lastly, the study author concludes that there is an earlier onset of mammary tumors at 400 ppm.

The reviewer agrees with the study author that this study provides evidence that atrazine is neither mutagenic nor estrogenic. The evidence provided by this study in regards to mutagenicity and estrogenicity will be considered along with information from previously submitted mutagenicity and estrogenicity assays to reach a determination of atrazine's genotoxic and estrogenic potential.

The NOEL and LOEL for mammary tumors in this study are less clear. While the study author states a LOEL and NOEL of 400 and 70 ppm, the reviewer believes an argument can made for a lower LOEL and NOEL.

The study author notes on page 36 that: "the incidence of animals with any mammary tumor at levels ≥50 ppm was statistically signifcant relative to the concurrent control group...".

The reviewer selects the 50 ppm as the lowest dose level at which mammary cancer is increased in incidence based on the statistically significant increase in tumors described in the quotation above.

The reviewer understands that the study author has interpreted the data differently in relation to the cancer LOEL. Much of the discussion below centers on how the reviewer perceives he has interpreted the data in this study differently than the study author has.

The reviewer's interpretation of the data differs from the study author's in three main areas:

1. The most appropriate animals to include in the tumor incidence analysis;
2. The use of historical control data;
3. The time-to-tumor analysis.

A discussion of each of these three points follows.

1. The most appropriate animals to include in the tumor incidence analysis

The study author states:

> "The percent of intact animals with carcinomas is contrasted by group with the pooled historical control incidence data for 14 Covance studies (discussion Text Table 1) . As this table shows, only the 400 ppm group carcinoma incidence rate is higher than the Covance pooled historical control rate. The other atrazine treated groups were within the Covance historical control range." *Current study, pg 34 and 35.*

The "Discussion Text Table 1" (DTT1) that the study author refers to in the quotes above is a table showing mammary tumor incidence data for all 80 animals in both segments of the study and also historical control data for 14 recent Covance 2-yr oncogenicity studies. The tumor incident data shown in this table is identical to the data shown in Table 16 in this review, and the historical control data is identical to the data shown in Table 22 in the column labeled "Pooled Covance Data 1989-1994".

This reviewer notes that DTT1 represents only one way of looking at this data. Tables 17, 18, 19 and 21 in this document show other ways of displaying this data. Tables 17 to 19 list tumor incident data for the intact animals while Table 21 displays the percent increase in mammary tumors in dosed rats *vs* controls grouped in 4 groups: 1. All animals combined (interim sac., scheduled-24 month sac., and unscheduled sac.); 2. Scheduled sac. and unscheduled sac. combined; 3. Scheduled sac. only; and, 4. Unscheduled sac. only. Table 21 shows that the tumor incidence data can appear very different depending on which specific group of animals is being examined. The percentage increase in tumors if all animals are considered or if only scheduled and unscheduled sacrifices are considered, is very similar. This similarity is not surprising since tumors were rare in the interim sacrifice group compared to the other animals in the study (percentage of intact interim sacrifice animals which had mammary tumors - 11%; percentage of intact main study animals which had mammary tumors - 57%). The data for either the scheduled or unscheduled sacrifice animals tells quite a different story. If only those animals which were sacrificed on schedule (at 24 months) are examined, an increase in tumor incidence, compared to controls, at 50 ppm and above is evident. Tumor incidence is almost doubled at 70 ppm and

is quite a bit more than doubled at 50 and 400 ppm. On the other hand, if only data from animals who died an unscheduled death is considered, the increase in tumor incidence is much smaller. An increase at doses of 50 ppm and above is still present, but the increase ranges from 26 to 49% rather than 96 to 185%. The explanation for this is likely the low tumor incidence in controls sacrificed on schedule and the high tumor incidence in controls that died an unscheduled death (shown in Table 22). With the exception of the control values, tumor incidence rates between scheduled and unscheduled deaths are similar (Tables 17 and 18).

Which of these 4 ways of examining the data is most appropriate? The study author combines all the animals in DTT1. This corresponds to Table 16 and the "All" row in Table 21 of this review. The inclusion of interim sacrifices with sacrifices done at 24 months seems inappropriate for a 2-yr oncogenicity study. An increase in tumor incidence, after only one year of compound exposure in animals only half-way through their expected life span, would not be expected. In fact, as described above and displayed in Table 20, very few of the interim sacrifice animals were found to have mammary tumors of any type. The data from the interim sacrifice is useful for an examination of the hypothesis that atrazine exposure causes an early onset of mammary tumors. However, combining interim sacrifice data with 24 month sacrifice data for the examination of the hypothesis that atrazine causes an increased tumor incidence over the course of a two year study, may not be appropriate.

The reviewer believes the most appropriate way to examine this data is to combine data from scheduled and unscheduled deaths while excluding interim sacrifice data.

2. <u>The use of historical control data</u>

The study author states:

> "The incidence of fibroadenomas in all groups in this study (especially the control group) is as much as 45% below that of historical control data for 14 studies recently conducted by Covance. (Discussion Text Table 1). ... Carcinoma rates of the intact study groups, including control, are less than the current Covance historical control rates except in the 400 ppm atrazine treated group." *Current study, pg 35.*

The use of historical control data is important to the case the study author makes that an increase in cancer incidence is not seen at 70 ppm in this study. There are, however, some concerns this reviewer has about the

historical control data which was used in this study.

The study author supports her case that no increase in tumors is seen at 70 ppm by comparing tumor incidence in this study to tumor incidence in a pool of 14 recently conducted Covance studies. The comparison the study author makes may not be appropriate because not all the 14 studies included in the Covance pool included interim sacrifice animals, as the current study did. As shown in tables 16 and 19 in this review, and described above in the discussion, tumor incidences can be different depending on whether or not an interim sacrifice group was included in the calculatiuons. The study labeled "Study code 1" on page 233 appears to be a study by Thakur which was submitted to the agency as MRID 42204401. This conclusion is based on the fact the studies have identical initiation and termination dates and identical tumor incidences. MRID 42201101 did not include an interim sacrifice group. Details are not given about any of the 14 studies included in the Covance pool, but it clearly cannot be assumed that each of these studies included (as the current study does) an interim sacrifice group.

In addition to the lack of details about interim sacrifice groups in the 14 pooled studies, there is a general lack of details about these studies. Why were these studies chosen for the pool? Are these every 2-yr oncogenicity study using SD rats that was conducted at Covance between 1989 and 1996, or are these studies a random selection of Covance studies conducted in that period?

The lack of details about these 14 studies is especially worrisome when one considers how different the results of these studies are from the results of other studies. The historical control incidences for these 14 studies are well above the Charles River control values, Hazelton pre-1984 control values, and Ciba-Geigy, Summit, N.J. control values (shown in Table 22). These three sources show similar control values and the pooled Covance values appear to be outliers. Because of this discrepancy, details about how these 14 studies were chosen for inclusion into the pool and becomes especially relevant. Information about the inclusion or exclusion of interim sacrifice data should be included simply to maintain consistency between the historical control values and the values from the current study (*i.e* don't include interim sacrifice data in the current study analysis if it isn't in the historical control data base.).

The issue of inclusion of interim sacrifice data into control pools and the issue of the dissimilarity of the 14-study Covance pool to the three other pools, weakens the argument that no increase in tumor incidence is seen at 70 ppm.

The statements in the above quotations from pages 34 and 35, above are no longer valid if interim sacrifice

data is not included in the tumor analysis. If only data from unscheduled and scheduled deaths are used then carcinomas in the 25, 50 as well as 400 ppm groups exceed the high end of the range for the pooled Covance data. Fibroadenoma incidence rates, excluding the interim sacrifice, are still below the pooled control data from the 14 Covance studies. The difference is less extreme if interim sacrifice data is excluded though.

Comparing tumor incidences in the current study with either the Charles River, Ciba-Geigy or Hazelton control data shown in Table 22 gives different results than comparisons to the Covance pooled data. Excluding interim sacrifice data, control fibroadenoma values are within the range of historical controls from Charles River, Ciba and Hazelton. Fibroadenoma incidences in dose groups exceed the mean from Charles River, Ciba and Hazelton histrical control and, in some cases, exceed the upper end of the range also. An increase in tumor incidences is seen when carcinoma tumor rates (excluding interim sacrifice) are compared to historical controls from Charles River, Ciba and Hazelton. While control carcinoma rates are very similar to Charles River, Ciba and Hazelton rates, carcinomas rates in the 25, 50 and 400 ppm groups exceed the range of these studies.

Much of the support for a lack of oncogenicity of atrazine at 70 ppm comes from comparisons to the pooled data from 14 Covance studies. More information about these studies is needed in order to lend stronger support to these comparisons. Further information about the studies in this pool is especially desirable when one considers the discrepancies between the tumor incidences seen in the Covance pool and the much lower incidences seen in the other pools.

3. The time-to-tumor analysis.

The study author notes that an earlier onset of carcinomas, compared to historical controls and concurrent controls, occurs only for carcinomas and only at the 400 ppm dose. The study author displays a time-to-tumor analysis in figures 8 and 9 on pages 37 and 38 of the current study. Figure 8 examines fibroadenomas and figure 9 examines carcinomas. The reviewer notes that another way to examine this data is to combine the tumor types and examine incidence by time period. This is similar to the analysis conducted by Thakur in Text Table 4a of MRID 42204401. The analysis the reviewer has done is described in this review under "Microscopic Pathology - neoplastic" and the results are shown in Table 23.

The analysis displayed in Table 23 shows that as the dose of atrazine rises, the percent of mammary tumors which first

appear in the first year of the study steadily increases while the percent of tumors which occurs in the last six months of the study steadily decreases. This can be noted even at the LDT. Fifty percent of the palpable masses in control animals which were later confirmed by histopathology to be tumors, appeared in the last 6 months of the study. This percentage in the 25 ppm animals dropped to 35.8% while the percentage of tumors appearing in the first 6 months of the first year rose from 41% in controls to 53.5% in the 25 ppm group. This pattern is prevalent in all the dose groups and is especially prevalent in the 400 ppm group. In this group 17.9% of the tumors were first palpated in the first year of the study, compared to only 9% in the control group.

A final issue to be addressed in this discussion is the issue of Maximum Tolerated Dose (MTD). The study author has stated that she believes that the MTD was exceeded at 400 ppm in the intact group based on significantly decreased survival in that group. The reviewer believes that a dose of 400 ppm was an appropriate high dose in this study and that 400 ppm approaches, but likely does not exceed the MTD for atrazine in this strain of rat. This point is important because the reviewer would like to emphasize that the increased incidence of tumors at 400 ppm should not be discounted based on a belief that 400 ppm exceeded the MTD. The decreased survival the study author refers to is likely due, in fact, to the increased tumor incidence and earlier onset time of mammary tumors seen at 400 ppm. This can be demonstrated by an examination of Table 5 in this review. When the cause of death, as determined by the examining pathologist, is examined it can be seen that 23.5% of the unscheduled deaths in the controls can be attributed to mammary tumors while 34% of the unscheduled deaths in the 400 ppm could be attributed to mammary tumors. The survival rate at 400 ppm is significantly lower than controls, however, this appears to be due to an increased death rate from mammary tumors rather than an increase in non-oncogenic toxicity. The only indication that 400 ppm may exceed the MTD is given by the increase in mortality seen at 400 ppm. However, the increase is mortality is due to oncogenic effects; specifically, an increase in death from mammary tumors. There are, in fact, fewer deaths from non-oncogenic causes in the 400 ppm group than in the controls (4.3% vs 6.5%, see Table 5, page 11). Furthermore, mean body weights at the termination of the study were almost identical between the controls and 400 ppm groups. Body weights throughout the study are similar between these two groups. Even at those timepoints where body weight in the 400 ppm was reduced, it was rarely reduced more than 10% compared to controls. The same can be said of percent body weight gains and food consumption. These values in the 400 ppm group are

similar to controls and even at the timepoints where they vary, the variation is never more than 10%.

The MTD was not likely exceeded at 400 ppm. The increase in mortality is due largely to the increased incidence of mammary tumors seen at this dose which serves to further emphasize the carcinogenic effect that atrazine exposure has on Sprague-Dawley female rats.

Figures 8 and 9 in the study report use historical control values from the 14 Covance studies. As mentioned above, information about these studies is required to strengthen any discussion relying on these 14 studies.

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To summarize:

- The reviewer agrees with the study author that this study provides evidence that atrazine is neither genotoxic nor estrogenic. This evidence will be considered along with information from previously submitted mutagenicity and estrogenicity assays to reach a determination of atrazine's genotoxic and estrogenic potential.

- The reviewer believes that further support is needed to buttress the study author's conclusion that no increase in oncogenicity is seen at ≤70 ppm in this study. Specifically - 1. Interim sacrifice data should be excluded from the analysis; 2. Details about the 14 studies that were included in the Covance historical data pool are needed, and; 3 Possibly, an explanation of why the historical control values for the Covance pool are so much higher than the 3 other sources of pooled data shown above in Table 22.

- The study author states that there is a decrease in the time-to-tumor at 400 ppm. The reviewer notes the method of examining time-to-tumor data that was used in Table 23 of this review to be an appropriate way to analyze this type of data. When analyzed in this fashion, the earlier onset of mammary tumors following atrazine exposure is evident at all doses and not just at 400 ppm.

- The reviewer finds that an increased incidence of mammary tumors was seen at 50 ppm in this study based on the statistically significant increase in combined tumors at this dose and above. The reviewer believes early onset of tumors may be occurring at the 25 ppm dose.

The study report is well written. This study is classified as **Acceptable-nonguideline**.

B. Study deficiencies There were no deficiencies which would alter the classification of this study.