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

FEB 15 2000

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Atrazine:** Twenty-eight day study evaluating the effect of atrazine on the estrous cycle and the proestrus afternoon luteinizing hormone (LH) and prolactin surges.

DP Barcode: D261630
Submission Code: S572272
Chemical: Atrazine
PC No.: 080803

FROM: Roger Hawks, Ph.D.
Health Effects Division (7509C)
Office of Pesticide Programs
Roger Hawks 2/3/00

THROUGH: Jess Rowland, Branch Chief
Health Effects Division (7509C)
Office of Pesticide Programs
Jess Rowland 2/5/00

TO: Pam Noyes
Special Review and Reregistration Division (7508C)
Office of Pesticide Programs

Purpose of Memo: The purpose of this memo is to transmit the Data Evaluation Record (DER) of a 28 day study in which the effects of atrazine exposure on the estrous cycle, and LH and prolactin proestrus afternoon surges was examined (MRID 43934406). This study was reviewed and found to be **Acceptable-Nonguideline**. The study does not satisfy a FIFRA Subdivision F or OPPTS series 870 guideline and was not submitted with the intention of doing so.

Primary Reviewer - Roger Hawks, Ph.D., RRBIII
Secondary Reviewer - Michelle Centra, Ph.D., RRBIII

The DER is attached and the executive summary follows:

EXECUTIVE SUMMARY:

In a study to evaluate the effect of atrazine exposure on the proestrus afternoon luteinizing hormone (LH) surge (MRID 43934406) atrazine, 97.1% a.i., was administered to 450 female Sprague Dawley rats in the diet. Dose levels were 0 (vehicle control), 2.5, 5, 40 and 200 mg/kg/day for 28 days. Mortality, clinical signs, gross pathology and pituitary weights were not affected in this study. Food consumption and body weights are decreased at the 40 and 200 mg/kg/day doses (body weight decreased 13% and 47% at 40 and 200 mg/kg/day respectively). The number of animals with diestrus blocks were increased at 40 and 200 mg/kg/day. The number of animals with estrus blocks were increased at 200 mg/kg/day. The prolactin surge was attenuated at 200 mg/kg/day. The LH surge was attenuated at 40 and 200 mg/kg/day.

The LOAEL is 40 mg/kg/day, based on decreases in food consumption, body weight, body weight gain, estrous cycle alterations and LH surge attenuation. The NOAEL is 5 mg/kg/day.

This special study in the rat is **Acceptable-nonguideline**. This study does not satisfy any guideline requirements and was not submitted with the intention of satisfying a guideline requirement.

EPA Reviewer: Roger Hawks Roger Hawks , Date 2/3/00
Reregistration Branch III (7509C)
EPA Secondary Reviewer: Michelle Centra Michelle Centra Date 2/3/00
Reregistration Branch III (7509C) (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Special Study (non-guideline). Evaluation of the Proestrus afternoon luteinizing hormone surge in Sprague-Dawley females exposed to atrazine for 28 days.

DP BARCODE: D261630 SUBMISSION CODE: S572272
P.C. CODE: 080803 TOX. CHEM. NO.: 063

TEST MATERIAL (PURITY): Atrazine (97.1%)

SYNONYMS: G-30027

CITATION: Morseth, S. (1996) Evaluation of luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats. Corning Hazelton Inc., Vienna, VA. Laboratory report number: CHV 2386-111. January 25, 1996. MRID:43934406. Unpublished.

SPONSOR: Novartis Corporation (formerly Ciba-Crop Protection) Greensboro, N.C.

EXECUTIVE SUMMARY:

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The LOAEL is 40 mg/kg/day, based on decreases in food consumption, body weight, body weight gain, estrous cycle alterations and LH surge attenuation. The NOAEL is 5 mg/kg/day.

This special study in the rat is **Acceptable-nonguideline**. This study does not satisfy any guideline requirements and was not submitted with the intention of satisfying a guideline requirement.

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

I. MATERIALS AND METHODS

A. MATERIALS:

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

2. Vehicle and positive control: 0.5 %
   Carboxymethylcellulose (CMC)

3. Test animals: Species: Rat
   Strain: Sprague-Dawley
   Age and weight at study initiation: 8 weeks; Weight at initiation not given
   Source: Charles River Labs, Raleigh, N.C.
   Housing: During the study the animals were individually housed in stainless steel wire-mesh cages.
   Diet: PMI® Feeds, Inc. Certified Rodent Diet #5002. ad libitum
   Water: Tap water ad libitum
   Environmental conditions:

   Temperature: 66.7 to 78.4°F.
   Humidity: 40 to 70%
   Air changes: ≥10 per hour
   Photoperiod: 14 hour light/10 hour dark

   Acclimation period: Two weeks

4. Estradiol : Beta-Estradiol 3-benzoate
   Description: white powder
   Lot#: 52H3881
   Purity: 98% a.i.
   Stability of compound: Stable at room temperature

5. Estradiol Vehicle: Sesame Oil
   Description: clear, yellowish liquid
   Lot#: 1113H1212
   Stability of compound: Stable at room temperature

B. STUDY DESIGN:

1. In life dates - start of dosing: September 5, 1995
   Necropsy: October 20, 1995

2. Animal assignment

   Animals were assigned to dose groups based on body weight using a computerized weight-randomization program to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group[1]	Dose to animal (mg/kg/day)	Concentration (mg/mL)	Number of females
5	0	0	90
6	2.5	0.25	90
7	5	0.5	90
8	40	4.0	90
9	200	20.0	90

1 Test groups are referred to as 5-9 rather than 1-5 because this study was part of a separate 6-month study (MRID 44151102) in which the test groups were assigned values 1-4.

3. Dose selection rationale: This study was designed to evaluate the effects of atrazine exposure on the proestrus afternoon LH and prolactin surges. The main focus was the effect atrazine exposure on the LH surge. This hormonal surge stimulates ovulation. A delay or lack of ovulation in response to atrazine exposure has been implied in a previous study (MRID 42085001) which examined estrous cycles (through vaginal smears) in SD rats exposed to atrazine at doses of 4.23 mg/kg/day and above. SD rats in this study displayed an increased number of days in the estrus phase of the estrous cycle early in the study following atrazine exposure. Increased number of days in estrus implies a delay or lack of ovulation. A histomorphic evaluation of the ovaries from

this study (MRID 43598622) also showed evidence of a delay or absence of ovulation at the early time points in atrazine-exposed SD females.
The proestrus afternoon LH surge was investigated in this study to examine the possibility that the apparent delay or absence of ovulation seen in MRID 42085001 was due to atrazine's effects on the LH surge.
In addition to the proestrus afternoon LH surge, rats also display a proestrus afternoon prolactin surge. The effects of atrazine exposure on this surge were also investigated.

4. Analysis of prepared formulations

Homogeneity of the low and high dose formulations was measured prior to dosing.

Homogeneity. Values shown are percent of target concentration.

	2.5 mg/kg/day	200 mg/kg/day
Top	102%; 102%	103%; 103%
Middle	108%; 101%	105%; 105%
Bottom	103%; 103%	105%, 104%

Stability was measured in low and high dose formulations on samples the day they were prepared and after ten days of refrigeration.

Stability. Values shown are percent of target concentration.

	2.5 mg/kg/day	200 mg/kg/day
Day 0	103%	104%
Day 10	89.9%; 90%	102%; 101%

Samples from weeks one and seven were analyzed for concentration.

Concentration. Values shown are percent of target concentration.

	2.5 mg/kg/day	5.0 mg/kg/day	40 mg/kg/day	200 mg/kg/day
Week 1	103%	105% 107%	104% 105%	104%
Week 7	98.1% 97.1%	99.9% 97.5%	101% 99.7%	111% 105%

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 - Body weights, body weight change and food consumption were analyzed by One-Way Analysis of Variance (ANOVA). Cochran-Armitage Test for Trend followed by a Fisher-Irwin Exact Test for multiple comparisons were used to analyze estrous cycle parameters. Repeat bleed LH data were analyzed by a repeated measures ANOVA with treatment as the between factor and sampling hour as the repeated factor. When a significant treatment or time effect was seen a one-way ANOVA with t-contrasts was performed. The non-repeat bleed data was analyzed by two-way ANOVA with treatment and hour as between factors. When a significant treatment or time effect was seen a one-way ANOVA with t-contrasts was performed. All the analysis on LH data were done on rank-transformed data.

C. METHODS:

1. Pilot study and method validation study

A pilot study (MRID 43934404) and method validation study (MRID 43934405) were performed and submitted to the Agency. Separate DERs have been written for these two studies. The pilot study did not expose animals to atrazine and the method validation study exposed animals for only three days. The purpose of these studies was not to examine the effects of atrazine exposure on the rats; rather it was to evaluate the validity of the experimental methods to be used in the measurement of the LH and prolactin surges.

More detailed information about the pilot study and the method validation study can be found in their respective DERs.

2. Observations:

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

3. Body weight

Animals were weighed once prior to treatment and weekly thereafter.

4. Food consumption and compound intake

Food consumption for each animal was determined as grams of food per week. Compound intake (mg/kg/day) values were calculated from the food consumption and body weight gain data.

5. Vaginal Smears

Vaginal smears were performed for the purpose of examining the effect of atrazine on the estrous cycle. Vaginal smears were performed beginning after 7 days of treatment and continuing daily for approximately 3 weeks. Vaginal smears were prepared by performing a vaginal lavage with 0.9% saline solution and spotting the lavage material on a glass slide. The slides were stained with 1% Toluidine blue stain and allowed to dry. The slides containing the vaginal smears were sent to Dr. Lee Tyrey of Duke University for evaluation.
The criteria for classification of the vaginal smears into a particular stage of the estrous cycle is not described in this report. The reviewer assumes that the criteria for classification of vaginal smears into stage of the estrous cycle was similar to that described in a previous submission: Eldridge, J., Wetzel, L., Tisdel, M., and Luempert, L.G. (1993a) *Determination of Hormone Levels in Sprague-Dawley Rats Treated with Atrazine Technical: Revised Supplement to Final Report.* Hazleton Washington, Inc. Lab Project Number: 483-278. MRID 42743902.
The criteria used in this study were as follows:

- *Diestrus* - Characterized by few cornified or nucleated epithelial cells, and increased numbers of leucocytes.

- *Proestrus* - Both cornified and nucleated epitheleal cells present. Frequently preceded by 1-2 days of few epitheleal cells present.

- *Estrus* - Very dense with cornified cells. Frequently followed by a day not dense in cornified cells.

6. Ovariectomy and Estradiol Administration

The ovariectomy and estradiol administration methodology were confirmed in the pilot and method validation studies.
Ovariectomy (OVX) was performed on the animals ten days prior to sacrifice. The estradiol-containing pellets were implanted three days prior to sacrifice. The estradiol containing pellets contained 4 mg of estradiol/ml dissolved in sesame oil.
The rationale behind why OVX followed by implantation of an estradiol-containing pellet was performed is described in the DER for the pilot study (MRID 43934404).

7. Hormone measurements

LH surge

The LH measurement methodology was confirmed in the pilot and method validation studies (MRID 43934404 and 43934405).
LH measurements were performed on both repeat bleed and non-repeat bleed animals. Both sets of animals were bled at six different time points. These time points are listed below. Explanations of each column from this table are shown below the table.

Table 2: Timepoints for bleeding and expected serum LH levels.

Clock time	Biologic time*	# non-repeat bleed[1]	# repeat bleed[2]	Expected serum LH levels	Equivalent to:
7:00am	1100	10	10	baseline	proestrus morning
10:00 am	1400	15	10	baseline	early afternoon proestrus
12:00 pm	1600	15	10	LH surge	mid-afternoon proestrus
2:00 pm	1800	15	10	LH surge	Late afternoon proestrus
4:00 pm	2000	15	10	LH surge	Proestrus evening
9:00 pm	2300	10	10	baseline	Proestrus evening

* There were 90 females in each dose group: 10 + 15 + 15 + 15 + 15 + 10 non-repeat bleed animals = 80 animals plus the 10 repeat bleed animals equals 90 animals per group.

[1] A different set of animals was bled at each time point

[2] The same set of animals was bled over again at each time point.

Descriptions of the information contained in each column of the above table are shown below.

a. *Clock time* - This is the actual time when the animals were sacrificed. Because the animals were on their own light cycles which did not necessarily correlate with the time of day as determined by the clock, the biologic time is the more important parameter to examine.

b. *Biologic time* - The biologic time is the time of day the animals see; the time of day as determined by the animals light cycle. This is the important parameter to consider when estimating when the proestrus afternoon LH surge should be occurring.

c. *Non-Repeat Bleed* - The non-repeat bleed animals consisted of 10 or 15 rats/group which were bled only once at a single time point. These animals were decapitated and trunk blood was collected for hormone measurements.

d. Repeat Bleed - The repeat bleed animals consisted of ten animals per group which were bleed, placed back in their cages, and then bleed again at each time point. Blood from the repeat bleed animals was obtained through the jugular vein for the first 4 timepoints, via the ocular plexus for the 5th and by decapitation for the final timepoint. The advantage of bleeding the same animal repeatedly is that variation may be reduced as the same animal is being used for each sampling time. However, the disadvantage is that the repeated bleeding every few hours may unduly stress the animals, thereby altering hormone levels (especially prolactin levels which are highly susceptible to being altered by stress).

e. *Expected serum LH levels* and *Equivalent to* - These two columns should be viewed together. These columns reflect that the proestrus afternoon LH surge can be expected to occur, as the its name suggests, on the afternoon of proestrus.

8.Estradiol measurements

Serum estradiol levels were measured by radioimmunoassay in order to confirm the success of the estradiol capsule implantation. Estradiol was measured from the same blood samples in which LH and prolactin were measured.

9. Prolactin Surge and Pituitary Prolactin

Serum prolactin was measured in order to examine the effect of atrazine exposure on the proestrus afternoon prolactin surge. Prolactin values were obtained only for the nonrepeat bleed animals (the 10 or 15 animals per group which were decapitated at each time point). The repeat bleed animals did not have their serum prolactin levels examined because it was believed that the stress of the repeat bleed procedure might alter prolactin levels.
The study report also stated that tissue pituitary prolactin levels were to be determined in the non-repeat bleed animals. This is described immediately below in section *"10. Sacrifice and Pathology"*

10. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the following tissues were collected and preserved in 10% neutral buffered formalin: mammary masses; normal mammary tissue; uterus; vagina; ovaries (saved at ovariectomy). These tissues were paraffin embedded and sent to Robert McConnell, D.V.M. (12 Cherryville Rd., Flemington, N.J.) for histopathologic evaluation. The pituitary from each non-repeat bleed animal was also collected. The pituitaries were weighed, frozen at -70° C and sent to Dr. Charles Eldridge of Wake Forest Medical School for determination of tissue hormone levels.

II. **RESULTS:**

A. Observations

1. Toxicity - There were no clinical signs of toxicity that could be attributed to compound exposure.

2. Mortality - There were no compound-related deaths. There was one accidental death in each of the following groups: control, 2.5, 5 and 200 mg/kg/day. The control death was believed to be due to gavage error while the other deaths occurred during the serial bleeding.

B. Body weight - Body weights and body weight gains were lower, compared to controls, in the 40 and 200 mg/kg/day dose groups. Body weight at the end of the study (week 6) was reduced 3% compared to controls at 40 mg/kg/day and was reduced 11% at 200 mg/kg/day.

Table 3: Group mean body weights and body weight changes (grams)

	Week 1	Week 4	Week 6	Grams gained Weeks 1-5
Control	$\bar{x}$= 203 SD= 13.3	$\bar{x}$= 246 SD= 19.6	$\bar{x}$= 271 SD= 20.6	$\bar{x}$= 68 SD= 13.1
2.5	$\bar{x}$= 203 SD= 12.1	$\bar{x}$= 245 SD= 18	$\bar{x}$= 269 SD= 19.9	$\bar{x}$= 66 SD= 15.1
5.0	$\bar{x}$= 203 SD= 12	$\bar{x}$= 244 SD= 18.4	$\bar{x}$= 267 SD= 18.7	$\bar{x}$= 64 SD= 12.3
40	$\bar{x}$= 205 SD= 14.6	$\bar{x}$= 239 SD= 19.8	**$\bar{x}$= 263*** SD= 19.6	**$\bar{x}$= 59*** SD= 14.2
200	$\bar{x}$= 205 SD= 13.7	**$\bar{x}$= 224*** SD= 16.5	**$\bar{x}$= 240*** SD= 19.3	**$\bar{x}$= 36*** SD= 15.9

* p≤ 0.05 compared to control. Body weight data from pages 45 and 47, current study.

C. Food consumption

1. Food consumption - Food consumption for the entire study was decreased in the 200 mg/kg/day dose group (-8.2%) compared to controls. Food consumption was decreased at 5 mg/kg/day compared to controls, but only at the first timepoint.

Table 4: Food consumption. Values shown are in grams.

	Week 1	Week 5	Weeks 1-5
Control	$\bar{x}$= 129 SD= 15.8	$\bar{x}$= 143 SD= 15.7	$\bar{x}$= 671 SD= 62.7
2.5	$\bar{x}$= 128 SD= 15.1	$\bar{x}$= 140 SD= 19.2	$\bar{x}$= 667 SD= 66.6
5.0	$\bar{x}$= 129 SD= 12.7	$\bar{x}$= 141 SD= 18	$\bar{x}$= 675 SD= 58.8
40	**$\bar{x}$= 124*** SD= 13.2	$\bar{x}$= 141 SD= 15.3	$\bar{x}$= 667 SD= 59
200	**$\bar{x}$= 102*** SD= 12.4	**$\bar{x}$= 133*** SD= 15.7	**$\bar{x}$= 620*** SD= 60.2

* p≤ 0.05 compared to control. Data from page 49, current study

D. Estrous Cycle Data (Vaginal Smears) - Estrous cycles did not appear to be altered at the two lower doses. At 40 and 200 mg/kg/day, estrous cycles were altered. Increases in animals with diestrus blocks were seen at both 40 and 200 mg/kg/day. Additionally, increases in animals with estrus blocks was seen at 200 mg/kg/day.

Table 6: Estrous cycle parameters. N= 90 in all dose groups.

	Control	2.5 mg/kg/day	5 mg/kg/day	40 mg/kg/day	200 mg/kg/day
% and # normally cycling	74.4% (67)	73.3% (66)	72.2% (65)	55.6% (50)	36.7% (33)
% and # with diestrus blocks[1]	23.3% (21)	22.2% (20)	23.3% (21)	**40*% (36)**	**56.7*%** (51)
% and # with estrus blocks[2]	3.3% (3)	3.3% (3)	4.4% (4)	6.7% (6)	**12.2*%** (11)

*= p≤0.05 Data from page 24, current study
1 Defined as ≥ 4 consecutive days in diestrus
2 Defined as ≥ 2 consecutive days in estrus

E. Hormone measurements

LH surge

Measurements of serum LH were taken from both repeat bleed and non-repeat bleed animals. Standard deviations for the group means were very high. The results are shown below in Tables 9 and 10 and as line graphs in Figures 1 and 2.

Table 7: Group Mean LH Values In *Repeat* Bleed Animals.
Values given in pg/ml.

Dose(mg/kg/day)▸ Biologic time▾	Control	2.5	5	40	200
1100	$\bar{x}$= 732 SD= 461	$\bar{x}$= 1101 SD= 652	$\bar{x}$= 810 SD= 519	$\bar{x}$= 765 SD= 389	$\bar{x}$= 514 SD= 503
1400	$\bar{x}$= 786 SD= 557 %= +7[1]	$\bar{x}$= 2222 SD= 1220 %= +102	$\bar{x}$= 1678 SD= 1602 %= +107	$\bar{x}$= 1037 SD= 829 %= +37	$\bar{x}$= 453 SD= 313 %= -12
1600	$\bar{x}$= 1301 SD= 1031 %= +78	$\bar{x}$= 3029 SD= 2383 %= +175	$\bar{x}$= 4971 SD= 3047 %= +513	$\bar{x}$= 1137 SD= 629 %= +51	$\bar{x}$= 552 SD= 311 %= +7
1800	$\bar{x}$= 2650 SD= 2389 %= +262	$\bar{x}$= 3016 SD= 3220 %= +174	$\bar{x}$= 2717 SD= 2542 %= +235	$\bar{x}$= 1450 SD= 857 %= +92	$\bar{x}$= 812 SD= 470 %= +58
2000	$\bar{x}$= 2606 SD= 2076 %= +256	$\bar{x}$= 1731 SD= 1447 %= +57	$\bar{x}$= 2954 SD= 3513 %= +265	$\bar{x}$= 1477 SD= 1296 %= +96	$\bar{x}$= 1140 SD= 328 %= +122
2300	$\bar{x}$= 1671 SD= 674 %= +128	$\bar{x}$= 1476 SD= 456 %= +34	$\bar{x}$= 1431 SD= 346 %= +77	$\bar{x}$= 1362 SD= 329 %= +80	$\bar{x}$= 1080 SD= 301 %= +110

1 This value represents the percent change relative to each groups respective baseline (1100 value).
Data in table from pages 597 to 601 ,current study
* Specific p value not given in study report. Assumed to be <0.05

Table 8: Group Mean LH Values In *Non-Repeat* Bleed Animals
Values given in pg/ml

Dose (mg/kg/day) ▸ Biologic time ▾	Control	2.5	5	40	200
1100	$\bar{x}$= 998 SD= 614	$\bar{x}$= 943 SD= 614	$\bar{x}$= 1140 SD= 715	$\bar{x}$= 1219 SD= 467	$\bar{x}$= 873 SD= 656
1400	$\bar{x}$= 1122 SD= 564 %= +12[1]	$\bar{x}$= 1171 SD= 802 %= +24	$\bar{x}$= 882 SD=926 %= -23	$\bar{x}$= 1125 SD= 795 %= -8	$\bar{x}$= 1099 SD= 863 %= +26
1600	$\bar{x}$= 3315 SD= 2684 %= +132	$\bar{x}$= 2961 SD= 1315 %= +214	$\bar{x}$= 3099 SD= 2521 %= +172	$\bar{x}$= 3518 SD= 4514 %= +189	$\bar{x}$= 1685 SD= 2962 %= +93
1800	$\bar{x}$= 5138 SD= 4403 %= +414	$\bar{x}$= 4489 SD= 4345 %= +376	$\bar{x}$= 2804 SD= 1346 %= +146	$\bar{x}$= 3246 SD= 1981 %= +166	$\bar{x}$= 2752 SD= 3137 %= +215
2000	$\bar{x}$= 2242 SD= 1850 %= +125	$\bar{x}$= 1118 SD= 412 %= +19	$\bar{x}$= 1554 SD= 14 %= +36	$\bar{x}$= 1740 SD= 1157 %= +43	$\bar{x}$= 1853 SD= 1139 %= +112
2300	$\bar{x}$= 761 SD= 283 %= -24	$\bar{x}$= 486 SD= 138 %= +48	$\bar{x}$= 508 SD=317 %= -55	$\bar{x}$= 689 SD= 373 %= -43	$\bar{x}$= 1126 SD= 816 %= +29

1 This value represents the percent change relative to each groups respective baseline (1100 value).
Data in table from pages 597 to 601 ,current study
* Specific p value not given in study report. Assumed to be <0.05

Figure 1: LH measurements repeat bleed

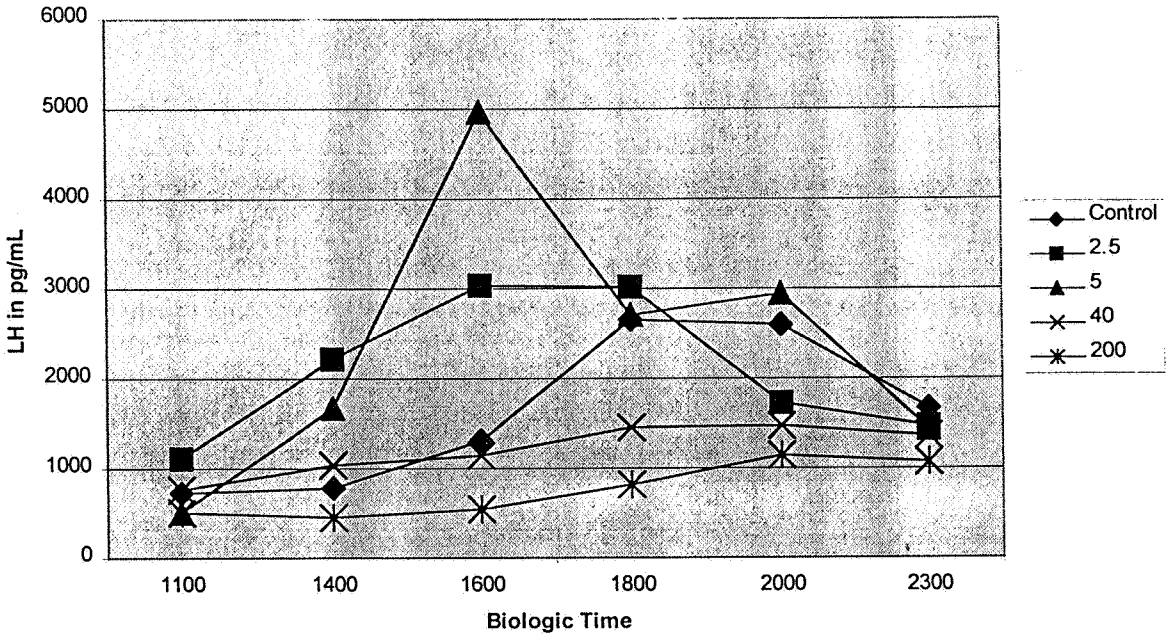
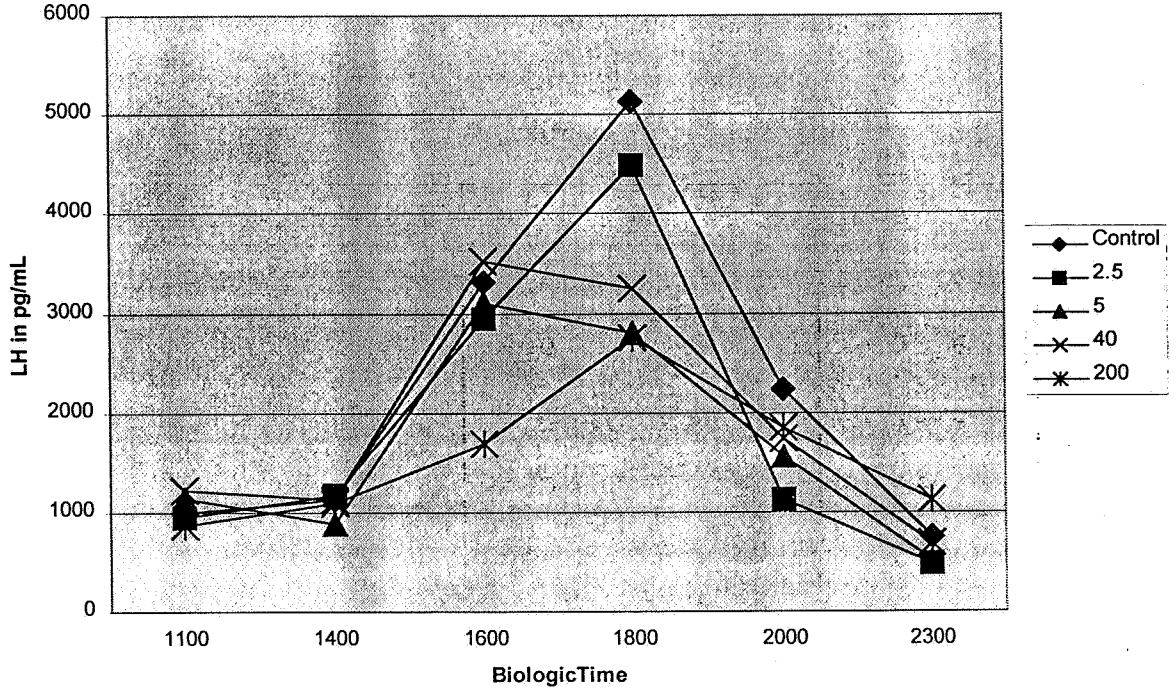


Figure 2: LH measurements in non-repeat bleed animals



Estradiol

Group mean values or raw numbers for the estradiol measurements are not given. The study states on page 26 that estradiol levels within an acceptable range to induce an LH surge were obtained.

Prolactin Surge and Pituitary Prolactin

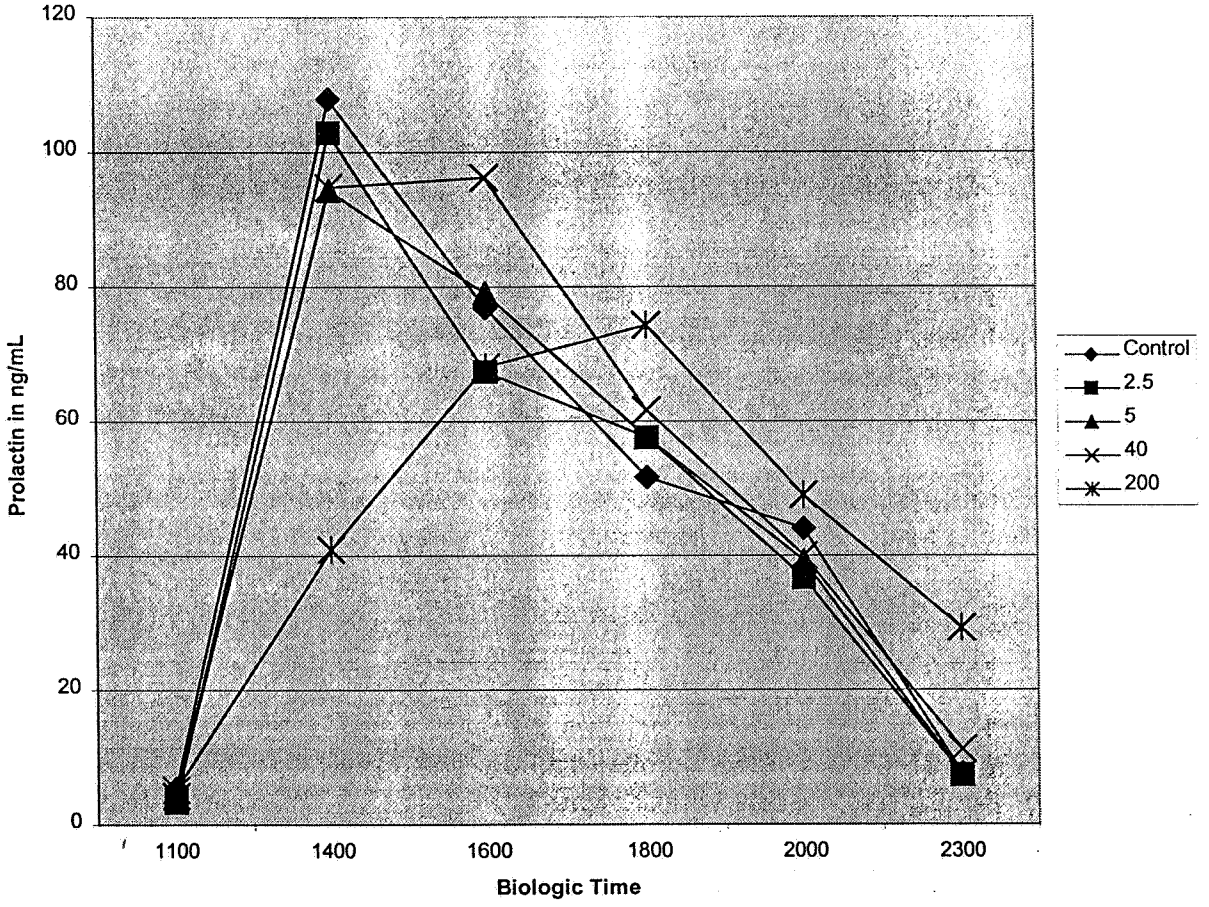
Atrazine exposure appeared to attenuate the proestrus afternoon prolactin surge at the high dose of 200 mg/kg/day only. The study states that prolactin levels in the pituitary of non-repeat bleed animals was to be measured and submitted at a later time. This data has yet to be submitted.

Table 9: Group Mean Prolactin Values In Non-Repeat Bleed Animals.
Values given in ng/ml

Dose(mg/kg/day) ▸ Biologic time▾	**Control**	**2.5**	**5**	**40**	**200**
1100	$\bar{x}$= 5.4 SD= 5.3	$\bar{x}$= 3.3 SD= 3.1	$\bar{x}$=5 SD= 4.4	$\bar{x}$= 4.4 SD=3.8	$\bar{x}$= 5.2 SD= 4.4
1400	$\bar{x}$= 107.8 SD= 57.6 %= +1900	$\bar{x}$= 102.8 SD= 47 %= +3000	$\bar{x}$= 94.4 SD= 34.5 %= +1888	$\bar{x}$= 94.7 SD= 47.7 %= +2052	$\bar{x}$= 40.9 SD= 47.6 %= +839
1600	$\bar{x}$= 76.9 SD= 30.3 %= +1324	$\bar{x}$= 67.4 SD= 31.2 %= +1942	$\bar{x}$= 79.1 SD= 27.7 %= +1582	$\bar{x}$= 96.2 SD= 48.2 %= +2086	$\bar{x}$= 63.2 SD= 37 %= +1115
1800	$\bar{x}$= 51.6 SD= 17.6 %= +856	$\bar{x}$= 57.7 SD= 29.3 %= +1648	$\bar{x}$= 57.6 SD= 23.6 %= +1152	$\bar{x}$= 61.7 SD= 30.5 %= +1302	$\bar{x}$= 74.6 SD= 27.1 %= +1335
2000	$\bar{x}$= 44 SD= 19.7 %= +715	$\bar{x}$= 36.6 SD= 12.8 %= +1009	$\bar{x}$= 39.3 SD= 13.8 %= +786	$\bar{x}$= 40.1 SD= 23.7 %= +811	$\bar{x}$= 49.1 SD= 26 %= +844
2300	$\bar{x}$= 7.6 SD= 6 %= +40	$\bar{x}$= 7.4 SD= 7.3 %= +124	$\bar{x}$= 7.5 SD= 6.3 %= +50	$\bar{x}$= 11.1 SD=9.3 %= +152	$\bar{x}$= 29.2 SD= 18.2 %= +462

1 Percent change relative to each groups respective baseline (1100 value). Data from pages 597 to 601 ,current study

Figure 3: Prolactin measurements in non repeat bleed animals



F. Sacrifice and Pathology

1. Organ weight - The pituitary was the only organ weighed. Absolute and relative (to body) pituitary weights were similar among all groups.

2. Gross pathology - There were no findings at necropsy which could be attributed to compound exposure.

3. Microscopic pathology - The mammary tissues, uterus, vagina and ovaries from all animals were fixed in 10% NBF, embedded in paraffin, sectioned, stained, and shipped to a pathologist for evaluation. The results of that evaluation have not yet been reported.

III. DISCUSSION

A. The main purpose of this study was to examine the effect of atrazine exposure on the proestrus afternoon LH surge, and to a lesser extent, the effect of atrazine exposure on the proestrus afternoon prolactin surge. This study also yielded information concerning the effects of atrazine exposure on the estrous cycle and on pituitary weights.

Study author conclusions

The study author found that atrazine exposure in this study did not result in an increase in mortality or clinical observations. Pituitary weights were not affected and there were increases in gross pathology observations. Food consumption and body weights were reduced at 40 and 200 mg/kg/day. The study author concluded that estrous cycles were affected at the two higher dose groups. The LH surge was attenuated at 40 and 200 mg/kg/day. The study author notes that the results are inconclusive in regards to the effect of 5 mg/kg/day on the LH surge. The prolactin surge was delayed at 40 and 200 mg/kg/day and was also suppressed at 200 mg/kg/day.

Reviewer conclusions

The reviewer found that mortality, clinical signs, gross pathology and pituitary weights were not affected in this study. The reviewer agrees that food consumption and body weights are decreased at the 40 and 200 mg/kg/day doses. The reviewer agrees with the study author that the prolactin surge is delayed and perhaps attenuated somewhat at 200 mg/kg/day. The delay in the prolactin surge at 40 mg/kg/day commented on by the study author is less clear to the reviewer. Doses of 2.5 and 5 mg/kg/day clearly did not affect the prolactin surge. An attenuation of the LH surge at 40 and 200 mg/kg/day is evident. Both the repeat bleed and non-repeat bleed animals show a clear attenuation. A lack of attenuation in both the repeat bleed and non-repeat bleed animals at 2.5 mg/kg/day is evident. The results, as noted by the study author, at 5 mg/kg/day, are inconclusive. The repeat bleed animals at 5 mg/kg/day show a clear surge, while the non-repeat bleed animals at 5 mg/kg/day show evidence of an attenuated surge. The LH measurements themselves are inconclusive in relation to the effect of 5 mg/kg/day of atrazine on the LH surge. Because estrus cycles, as indicated by vaginal smears, do not appear to be affected, the reviewer concludes that atrazine exposure for one month at 5 mg/kg/day did not alter the proestrus afternoon LH surge.

To summarize:

- **Increases in animals with diestrus blocks were seen at 40 and 200 mg/kg/day;**
- **Increases in animals with estrus blocks were seen at 200 mg/kg/day**
- **Attenuation of the LH surge was seen at 40 and 200 mg/kg/day;**
- **The prolactin surge was attenuated at 200 mg/kg/day;**
- **Pituitary weights were not altered at any dose.**
- **Decreases in body weight, body weight gain and food consumption.**

Thus, the LOAELs/NOAELs for the three primary effects

examined in this study (LH surge, prolactin surge and estrous cycle alterations) are:-

LH surge LOAEL/NOAEL = 40/5 mg/kg/day;

Prolactin Surge LOAEL/NOAEL = 200/40 mg/kg/day

Estrous cycle alterations = LOAEL/NOAEL = 40/5 mg/kg/day.

This study is classified **Acceptable-non-guideline.**

B. Study deficiencies

This study contained several deficiencies. Measurements of pituitary tissue hormones levels have yet to be provided. Histopathology of the tissues saved has yet to be provided. Estradiol values, neither group mean nor individual values, are not given.

None of these deficiencies altered the classification of this study as Acceptable-nonguideline.