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

014013

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Date: 2/29/00

SUBJECT: Atrazine: Review of a 26 week study evaluating the effect of atrazine exposure on the proestrus afternoon luteinizing hormone surge and the estrous cycle (MRID 44152102).

DP Barcode: D231534 and D232347
Submission Code: S514581
Chemical: Atrazine
PC No.: 080803
Caswell No.: 063

FROM: Roger Hawks, Ph.D. Roger Hawks 3/2/00
Reregistration Branch III
Health Effects Division (7509C)

THROUGH: Jess Rowland, Branch Chief 3/2/00
Reregistration Branch III
Health Effects Division (7509C)

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 26 week study in which the effects of atrazine exposure on the estrous cycle, and the proestrus afternoon luteinizing hormone and prolactin surges were examined (MRID 44152102).
This study has been reviewed and found to be Acceptable-non-guideline. The study does not satisfy a FIFRA Subdivision F or OPPTS series 870 guideline requirement 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 evaluation the effect of long-term atrazine exposure on the proestrus afternoon luteinizing hormone (LH) surge (MRID 44152102) atrazine, 97.1% a.i., was administered to 360 female sprague Dawley rats in the diet. Dose levels were 0 (negative control), 25, 50, and 400 ppm (0, 1.80, 3.65, 29.44 mg/kg/day) for 26 weeks (approximately six months).

Body weight, body weight gain and food consumption were significantly ($p \leq 0.05$) decreased in HDT animals compared to controls (body weight decreased 8.5% at the end of the study and food consumption decreased 3.75% for the entire study). The percentage of days in estrus were significantly increased ($p \leq 0.01$) during the 21-22 and 25-26 week time periods at the HDT. Percent days in estrus were also increased during the 21-22 and 25-26 week time periods at the MDT, but the increase was only significant ($p \leq 0.05$) for the 21-22 week time period. The proestrus afternoon LH surge was severely attenuated at the HDT (LH levels at most sampling time points were actually decreased compared to baseline) and less so at the MDT (maximum increase over baseline was 157% compared to maximum increase over baseline in controls of 273%). Pituitary weight were increase at the HDT (absolute weight increased 22% and weight relative to body weight was increased 28%). Pituitary weights at the other two doses were not affected. There was a slight increase at the HDT of animals displaying enlarged pituitaries (0% in controls compared to 3.4% at 29.44 mg/kg/day) and thickened mammary glands (0% in controls compared to 6.7% at 29.44 mg/kg/day). There were no other gross necropsy findings in the HDT that could be attributed to compound exposure and there were no compound-related gross pathology findings at the MDT or LDT. Selected tissues were saved for histopathology but those results have yet to be reported. There were no compound related effects in mortality or clinical signs. The proestrus afternoon prolactin surge was not affected by compound exposure at any dose. The LDT had no effects on the estrous cycle, LH or prolactin surges.

The LOAEL is 3.65 mg/kg/day, based on estrous cycle alterations and LH surge attenuation. The NOAEL is 1.8 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 3/1/00
Reregistration Branch III(7509C)
EPA Secondary Reviewer: Michelle Centra Michelle Centra Date 3/2/00
Reregistration Branch III(7509C)

014013

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 six months.

DP BARCODE:D231534 and D232347 SUBMISSION CODE:S514581
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- 6 month report. Corning Hazelton Inc., Vienna, VA. Laboratory report number: CHV 2386-111. October 25, 1996. MRID:44152102. Unpublished.

SPONSOR: Ciba-Crop Protection, Greensboro, N.C.

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proestrus afternoon LH surge was severely attenuated at the HDT (LH levels at most sampling time points were actually decreased compared to baseline) and less so at the MDT (maximum increase over baseline was 157% compared to maximum increase over baseline in controls of 273%). Pituitary weight were increase at the HDT (absolute weight increased 22% and weight relative to body weight was increased 28%). Pituitary weights at the other two doses were not affected. There was a slight increase at the HDT of animals displaying enlarged pituitaries (0% in controls compared to 3.4% at 29.44 mg/kg/day) and thickened mammary glands (0% in controls compared to 6.7% at 29.44 mg/kg/day). There were no other gross necropsy findings in the HDT that could be attributed to compound exposure and there were no compound-related gross pathology findings at the MDT or LDT. Selected tissues were saved for histopathology but those results have yet to be reported. There were no compound related effects in mortality or clinical signs. The proestrus afternoon prolactin surge was not affected by compound exposure at any dose. The LDT had no effects on the estrous cycle, LH or prolactin surges.

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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/or positive control: None. Test article was mixed directly into the diet.

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: 64.4 to 78.4°F.
   Humidity: 28 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: September 18, 1995 end: March 29, 1996

2. Animal assignment

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

TABLE 1: STUDY DESIGN

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg/day)	Number of females
Control	0	0	90
Low Dose	25	1.8	90
Mid Dose	50	3.65	90
High Dose	400	29.4	90

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 and 26.63 mg/kg/day. 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. Diet preparation and analysis

Diet was prepared weekly. A premix for each dose was prepared by mixing appropriate amounts of test substance with 200 grams of PMI® Feeds, Inc. Certified Rodent Diet #5002 in a Waring blender. These premixes were then diluted to the appropriate concentration by adding additional diet. Homogeneity and stability were tested prior to initiation of dosing. Samples were also tested for stability at week 10. Samples of treated food were analyzed for concentration at weeks 1, 13 and 28.

Results - Homogeneity Analysis:

	Nominal conc.	Measured conc. in ppm	% of nominal
Top	25 ppm	25.16; 25.63	101; 103
	400 ppm	400.9; 412.5	100; 103
Middle	25 ppm	24.88; 24.74	99.5; 99
	400 ppm	412.5; 400.9	103; 100
Bottom	25 ppm	25.35; 25.27	101; 101
	400 ppm	413.5; 404.8	103; 101

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.

Stability Analysis:

	Nominal conc.	Measured conc. in ppm	% of nominal
Day 0	25 ppm	25.2	101
	400 ppm	407.5	102
Day 10	25 ppm	22.6	90.5
	400 ppm	366.3	91.8

Values shown above are the mean of the replicate samples.

Concentration Analysis:

Nominal conc. in ppm	Measured conc. in ppm	% of nominal
25	week 1= 25.17	week 1= 101
50	week 1= 47.72	week 1= 95.4
400	week 1= 407.5	week 1= 102
25	week 13= 27.8	week 13= 111.5
50	week 13= 49.2	week 13= 98.4
400	week 13= 399.3	week 13= 99.7
25	week 28= 25.5	week 28= 102
50	week 28= 48.65	week 28= 97.4
400	week 28= 402.2	week 28= 101

Values shown above are the mean of the replicate samples. Values on the above three tables from pages 39 to 41, current study

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 for 14 consecutive days followed by 14 days without smears (2 weeks on/2 weeks off). Smears were begun on the first day of treatment and were stopped when ovariectomies were performed (10 days prior to sacrifice).
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 was as follows:

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

- *Proestrus* - Both cornified and nucleated epitheal cells present. Frequently preceded by 1-2 days of few epitheal 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: Experimental design for LH measurements

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 its name suggest, 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 the 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 attributed to compound exposure.

2. Mortality - One animal in each group died during the study. None of these deaths are attributable to compound exposure.

B. Body weight - Atazine exposure at 400 ppm resulted in decreased body weight gains during the first half of the study. Table 3 shows that animals in this group could not keep pace with the weight gains seen in the other three groups. During the second half of the study the high dose animals matched the weight gain of the other groups, but they could not make up for the reduced gain during the first half and ended the study weighing less than their peers.

Table 3: Body weights and body weight changes
Values shown are in grams.

	Week 1	Week 13	Week 27	Gain weeks 1-13	Gain weeks 13-27	Gain weeks 1-27
Control	$\bar{x}$=188 SD=12.6	$\bar{x}$=287 SD=24.1	$\bar{x}$=321 SD=31.6	$\bar{x}$=99	$\bar{x}$=34	$\bar{x}$= 133
25 ppm	$\bar{x}$=190 SD=12.3	$\bar{x}$=290 SD=26.8	$\bar{x}$=328 SD=36.3	$\bar{x}$=100 +1%[1]	$\bar{x}$=38 +11.8%	$\bar{x}$= 138 -3.8%
50 ppm	$\bar{x}$=188 SD=13.5	$\bar{x}$=282 SD=25.9	$\bar{x}$=319 SD=37.2	$\bar{x}$=94 -5.1%	$\bar{x}$=37 +8.8%	$\bar{x}$= 131 +1.5%
400 ppm	$\bar{x}$=187 SD=11	**$\bar{x}$=265* SD=23**	**$\bar{x}$=300* SD=30.9**	$\bar{x}$=78 -21.2%	$\bar{x}$=35 +2.9%	$\bar{x}$= 114 -14.3%

* $p \leq 0.05$ compared to control; [1] percent difference compared to control
Body weight data from pages 47 to 50, current study.
Body weight gains calculated by reviewer from body weight data

C. Food consumption and compound intake

1. Food consumption - Although there were occasional statistically significant differences in all the dose groups compared to controls, only at the high dose group was food consumption consistently reduced compared to controls. This is evident by the statistically significant reduction in food consumption seen for weeks 1-25 in Table 4. The decreases in food consumption seen at the high dose seemed to be most dramatic in the first half of the study where food consumption was significantly reduced at 10 out of the 13 time points. Food consumption was significantly reduced in only 2 out the 12 time points in the second half of the study.

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

	Week 1	Week 13	Week 25	Weeks 1-25
Control	$\bar{x}$=124 SD=17.4	$\bar{x}$=138 SD=14.4	$\bar{x}$=137 SD=16	$\bar{x}$=3438 SD=255.1
25 ppm	**$\bar{x}$=131*** SD=16.6	$\bar{x}$=141 SD=14.8	$\bar{x}$=143 SD=16.1	$\bar{x}$=3462 SD=259.6
50 ppm	$\bar{x}$=127 SD=16.7	**$\bar{x}$=131*** SD=15.6	$\bar{x}$=135 SD=19	$\bar{x}$=3455 SD=302.9
400 ppm	**$\bar{x}$=114*** SD=11.1	**$\bar{x}$=127*** SD=12	$\bar{x}$=135 SD=14.9	**$\bar{x}$=3309*** SD=293.7

* $p \leq 0.05$ compared to control
Data from pages 57 to 60, current study

2. Compound consumption - Compound consumption tended to decrease in all dose groups as the study progressed. Such a pattern is typical and is expected.

Table 5: Compound consumption. Values shown are in mg/kg/day.

	Week 1	Week 13	Week 25	Weeks 1-25
25 ppm	$\bar{x}$=2.3 SD=0.3	$\bar{x}$=1.7 SD=0.2	$\bar{x}$=1.6 SD=0.2	$\bar{x}$=1.80
50 ppm	$\bar{x}$=4.6 SD=0.6	$\bar{x}$=3.3 SD=0.3	$\bar{x}$=3.1 SD=0.3	$\bar{x}$=3.65
400 ppm	$\bar{x}$=33.7 SD=2.8	$\bar{x}$=27.2 SD=2.2	$\bar{x}$=25.8 SD=2.4	$\bar{x}$=29.44

Data from pages 62 to 65, current study

D. Estrous Cycle Data (Vaginal Smears)

A statistically significant increase in percent days in estrus was seen in both the high and mid dose groups. Increases in the high dose group were seen as early as 13-14 weeks following initiation of compound exposure and increases at the mid-dose group were seen as early as 21-22 weeks following initiation of compound exposure. Increases in animals displaying estrus blocks were seen at all time points from 13-14 weeks and up in both the MDT and

HDT. Increases in percent days in diestrus were not seen at any time point though there was an increase in number of animals with diestrus blocks in the high dose group in the 1-2, 21-22 and 25-26 week time periods. There was a statistically significant *decrease* in percent days in diestrus during weeks 21-22 and 25-26 in the high dose group (data not shown). This decrease in days in diestrus would be expected given the increase in days in estrus seen at these time points.

Tables 6, 7 and 8 display percent days in estrus, estrus blocks, and diestrus blocks. An estrus block is defined here as ≥ 2 consecutive days in estrus. A diestrus block is defined here as ≥ 4 consecutive days in diestrus.

Table 6: Percent Days In Estrus

Dose▸ Weeks▾	Control	25 ppm	50 ppm	400 ppm
1-2	$\bar{x}$= 22% SD= 5.2%	$\bar{x}$= 22% SD= 5.6%	$\bar{x}$= 22% SD= 5.4%	$\bar{x}$= 21% SD= 7%
5-6	$\bar{x}$= 23% SD= 5.1%	$\bar{x}$= 23% SD= 5.1%	$\bar{x}$= 25% SD= 10%	$\bar{x}$= 24% SD= 7.4%
9-10	$\bar{x}$= 25% SD= 9.4%	$\bar{x}$= 25% SD= 9.4%	$\bar{x}$= 26% SD= 10.2%	$\bar{x}$= 26% SD= 9.3%
13-14	$\bar{x}$= 31% SD= 22.4%	$\bar{x}$= 28% SD= 18%	$\bar{x}$= 31% SD= 21.1%	**$\bar{x}$= 40%*** SD= 27.6%
17-18	$\bar{x}$= 31% SD= 25.4%	$\bar{x}$= 33% SD= 24.7%	$\bar{x}$= 36% SD= 25.1%	**$\bar{x}$= 45%*** SD= 32.1%
21-22	$\bar{x}$= 32% SD= 25.4%	$\bar{x}$= 41% SD= 31.9%	**$\bar{x}$= 45%*** SD= 32.2%	**$\bar{x}$= 51%**** SD= 34.8%
25-26	$\bar{x}$= 47% SD= 33.2%	$\bar{x}$= 48% SD= 35.5%	$\bar{x}$= 54% SD= 35.1%	**$\bar{x}$= 63%**** SD= 37%

*= p≤0.05; ** p≤0.01

Data from page 27, current study

Table 7: Number of Animals With Estrus Blocks. n= 90 in all groups at all time points. An estrus block is defined here as ≥ 2 consecutive days in estrus.

Dose▸ Weeks▾	Control	25 ppm	50 ppm	400 ppm
1-2	4	2	1	9
5-6	3	3	6	4
9-10	4	5	5	9
13-14	11	12	15	35
17-18	18	18	23	39
21-22	21	31	38	50
25-26	42	40	49	61

Data from page 67, current study

Table 8: Number of Animals With Diestrus Blocks. n= 90 in all groups at all time points. A diestrus block is defined here as ≥ 4 consecutive days in diestrus.

Dose▸ Weeks▾	Control	25 ppm	50 ppm	400 ppm
1-2	22	17	20	33
5-6	16	7	9	9
9-10	5	5	3	7
13-14	3	6	7	9
17-18	8	10	8	7
21-22	13	9	6	21
25-26	11	14	11	16

Data from page 67, current study

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 (the figures are found at the end of this DER).

A statistically significant attenuation of the LH surge was seen in both the repeat and non-repeat bleed animals at 29.44 mg/kg/day. A non-significant attenuation of the LH surge was seen in the repeat bleed animals only at 3.65 mg/kg/day.

Table 9: Group Mean LH Values In *Repeat* Bleed Animals.
Values given in pg/ml

Biologic time	Control	25 ppm	50 ppm	400 ppm
1100	$\bar{x}$= 909 SD= 410	$\bar{x}$= 1075 SD= 621	$\bar{x}$= 972 SD= 353	$\bar{x}$= 1005 SD= 482
1400	$\bar{x}$= 1136 SD= 554 %= +25%	$\bar{x}$= 1468 SD= 977 %= +37%	$\bar{x}$= 984 SD= 466 %= +1.2%	$\bar{x}$= 1155 SD= 620 %= +15%
1600	$\bar{x}$= 2213 SD= 2562 %= +143%	$\bar{x}$= 1603 SD= 682 %= +49%	$\bar{x}$= 2277 SD= 1470 %= +134%	**$\bar{x}$= 850*** SD= 352 %= -15%
1800	$\bar{x}$= 3336 SD= 3138 %= +267%	$\bar{x}$= 3631 SD= 2732 %= +237%	$\bar{x}$= 2500 SD= 1897 %= +157%	**$\bar{x}$= 858*** SD= 416 %= -15%
2000	$\bar{x}$= 3388 SD= 3344 %= +273%	$\bar{x}$= 2510 SD= 1138 %= +133%	$\bar{x}$= 2409 SD= 1525 %= +148%	**$\bar{x}$= 1042*** SD= 627 %= +4%
2300	$\bar{x}$= 1672 SD= 426 %= +84%	$\bar{x}$= 1229 SD= 492 %= +14%	$\bar{x}$= 1271 SD= 559 %= +31%	**$\bar{x}$= 953*** SD= 549 %= -5%

1 This value represents the percent change relative to each groups respective baseline (1100 value).
Data in table from page 75, current study
* Specific p value not given in study report. Assumed to be <0.05

Table 10: Group Mean LH Values In *Non-Repeat* Bleed Animals
Values given in pg/ml

Biologic time	Control	25 ppm	50 ppm	400 ppm
1100	$\bar{x}$= 1900 SD= 775	$\bar{x}$= 1816 SD= 543	$\bar{x}$= 1581 SD= 791	$\bar{x}$= 1863 SD= 788
1400	$\bar{x}$= 2326 SD= 1082 %[1]= +22%	**$\bar{x}$= 1606*** SD= 926 %= -12%	$\bar{x}$= 1799 SD= 933 %= +14%	**$\bar{x}$= 1420*** SD= 622 %= -24%
1600	$\bar{x}$= 2669 SD= 1464 %= +40%	$\bar{x}$= 2507 SD= 1008 %= +38%	$\bar{x}$= 2463 SD= 1201 %= +56%	$\bar{x}$= 1913 SD= 799 %= +3%
1800	$\bar{x}$= 3456 SD= 2310 %= +82%	$\bar{x}$= 3235 SD= 2751 %= +78%	$\bar{x}$= 3175 SD= 1685 %= +101%	**$\bar{x}$= 1358*** SD= 760 %= -27%
2000	$\bar{x}$= 2327 SD= 1668 %= +22%	$\bar{x}$= 2249 SD= 1498 %= +24%	$\bar{x}$= 1899 SD= 752 %= +20%	**$\bar{x}$= 1308*** SD= 477 %= -30%
2300	$\bar{x}$= 1178 SD= 337 %= -38%	$\bar{x}$= 1258 SD= 428 %= -31%	$\bar{x}$= 1063 SD= 383 %= -33%	$\bar{x}$= 1129 SD= 350 %= -39%

1 This value represents the percent change relative to each groups respective baseline (1100 value).
Data in table from page 73, current study
* Specific p value not given in study report. Assumed to be <0.05

Estradiol

Group mean values or raw numbers for the estradiol measurements are not given. The study states on page 27 that estradiol levels within an acceptable range to induce an LH surge were obtained.

Prolactin Surge and Pituitary Prolactin

Atrazine exposure did not appear to affect the proestrus afternoon prolactin surge. Table 11 displays the prolactin data. The text of the study, on page 28, states that all groups, both treated and controls, showed statistically significant increases in plasma prolactin over the sampling

interval when compared to their own baseline values. However, neither specific p values could be found for prolactin measures nor were the specific time points at which the significant increases seen identified. Only nonrepeat animals had their serum prolactin measured. Figure 3 (found at the end of this DER) displays a line graph of the data from Table 11.
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 11: Group Mean Prolactin Values In Non-Repeat Bleed Animals. Values given in ng/ml

Biologic time	Control	25 ppm	50 ppm	400 ppm
1100	$\bar{x}$= 11.5 SD= 7.8	$\bar{x}$= 16.7 SD= 15.6	$\bar{x}$= 13.4 SD= 14	$\bar{x}$= 8.7 SD= 7.3
1400	$\bar{x}$= 51.5 SD= 32 %[1] = +348	$\bar{x}$= 39.8 SD= 29.7 %= +138	$\bar{x}$= 50.9 SD= 33.2 %= +280	$\bar{x}$= 33.1 SD= 22 %= +280
1600	$\bar{x}$= 54.2 SD= 29.7 %= +371	$\bar{x}$= 48.3 SD= 20.3 %= +189	$\bar{x}$= 66 SD= 30.1 %= +393	$\bar{x}$= 44.4 SD= 36.4 %= +410
1800	$\bar{x}$= 30 SD= 14.5 %= +161	$\bar{x}$= 31.9 SD= 15.7 %= +91	$\bar{x}$= 28.9 SD= 11.2 %= +157	$\bar{x}$= 23.9 SD= 8.6 %= +175
2000	$\bar{x}$= 19.8 SD= 10.4 %= +72	$\bar{x}$= 15.8 SD= 9.3 %= -5	$\bar{x}$= 19.8 SD= 10.7 %= +48	$\bar{x}$= 17 SD=9.7 %= +95
2300	$\bar{x}$= 7 SD= 5.4 %= -53	$\bar{x}$= 7.6 SD= 7.2 %= -55	$\bar{x}$= 8.8 SD= 11.7 %= -34	$\bar{x}$= 16.2 SD=14.5 %= +86

1 This value represents the percent change relative to each groups respective baseline (1100 value).
Data in table from page 73, current study

F. Sacrifice and Pathology

1. Organ weight - The only organ weighed was the pituitary. Table 12 below displays pituitary weights. Absolute weights are given in grams and relative weights are given as percentage of body weight.

Table 12: Absolute and Relative Pituitary Weights

	Absolute	Relative
Control	$\bar{x}$= 0.023 SD= 0.005	$\bar{x}$= 0.0075 SD= 0.0017
25 ppm	$\bar{x}$= 0.024 (+4%) SD= 0.005	$\bar{x}$= 0.0078 (+4%) SD= 0.0015
50 ppm	$\bar{x}$= 0.024 (+4%) SD= 0.005	$\bar{x}$= 0.0081 (+8%) SD= 0.0015
400 ppm	$\bar{x}$= 0.028 (+22%) SD= 0.008	$\bar{x}$= 0.0096 (+28%) SD= 0.003

Values calculated by reviewer using data from pages 345 to 704, current study.

2. Gross pathology - A very slight increase in enlarged pituitaries and thickened mammary glands in the high dose group were the only findings at gross necropsy which are attributable to compound exposure.

Table 13: Gross Necropsy Findings. n=89 for all groups

	Control	25 ppm	50 ppm	400 ppm
Enlarged Pituitary	0	0	0	3
Thickened Mammary Gland	0	1	0	6

Data from pages 79 and 80, current study

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 concluded that atrazine exposure at 400 ppm (29.4 mg/kg/day) resulted in attenuation of the LH surge and disruption of the estrous cycle. The study author did not believe that the doses of 25 ppm (1.8 mg/kg/day) or 50 ppm (3.65 mg/kg/day) resulted in LH surge attenuation and estrous cycle disruption. The specific estrous cycle disruptions were: increased days in estrus; increases in estrus blocks; and, increases in diestrus blocks. The study author did not believe that the prolactin surge was affected at any dose level. Pituitary weights were not commented on by the study author.

Reviewer comments and conclusions

The reviewer agrees that the LH surge was attenuated at 29.44 mg/kg/day and that the prolactin surge was not altered at any dose. The reviewer also agrees that atrazine exposure clearly altered estrous cycles at the high dose. The reviewer also agrees that atrazine exposure at 25 ppm (1.8 mg/kg/day) did not alter the LH surge or estrous cycles.

There was a 22% and 28% increase in absolute and relative pituitary weights at 29.44 mg/kg/day. These increases indicate that atrazine exposure at the high dose resulted in increased pituitary weights compared to controls. Pituitary weights at the other two doses did not appear to be greatly altered compared to controls.

The reviewer disagrees with the study author in regards to the lack of effect on LH surge and estrous cycles at the MDT of 50 ppm (3.65 mg/kg/day). An effect on both LH surge

and estrous cycles can be seen at this dose. The reviewer also disagrees with the study author on the increase in diestrus blocks at the high dose.

The LH surge is attenuated at 3.65 mg/kg/day -

The attenuation of the LH surge at this dose is evident when one examines Figure 1 of this DER. The attenuation is most evident at the 1800 time point. At this time point the percent increase over baseline (1100 time point) in the untreated animals was +267%. For the 3.65 mg/kg/day animals the percent increase is 157%. The attenuation at 3.65 mg/kg/day is not evident when one examines the data from the non-repeat bleed animals. The data from the two sets of animals - repeat bleed and non-repeat bleed - are contradictory. One set of data shows what appears to be an attenuation at 3.65 mg/kg/day, while the other does not show an attenuation. The estrous cycle data can be used to support the contention that an LH surge attenuation is occurring at this dose. Were the LH surge to be attenuated below the threshold critical for ovulation, the increases in percent days in estrus and estrus blocks would be expected at this dose. Both these events are found to occur at 3.65 mg/kg/day. Percent days in estrus were found to be significantly increased during the 21-22 week measurement interval at 3.65 mg/kg/day as shown in Table 6 above. Values at this time point were 32% in controls and 45% at 3.65 mg/kg/day. Percent days in estrus during the final, 25-26 week interval, were also increased (but not significantly) at 3.65 mg/kg/day compared to control. Values at this time point were 47% in controls and 54% at 3.65 mg/kg/day. There was also a consistent increase in animals with estrus cycle blocks from week 13 onward in the 3.65 mg/kg/day group compared to controls. This data is displayed in Table 7 of this DER. These increases were mild at the 13-14 and 17-18 week time points: 12.2% and 20% of the control animals displayed estrus blocks, respectively, at these time points; compared to 16.7% and 25.6% of the 3.65 mg/kg/day animals. The increases were more severe at the last two time points: 23.3% and 46.7% of the controls at weeks 21-22 and 25-26, respectively, displayed estrus blocks; compared to 42.2% and 54.4% of the 3.65 mg/kg/day animals.

The decreased LH values seen at the 1800, 2000 and 2300

(especially at 1800) time points, combined with the increases in days in estrus, indicate that the LH surge is attenuated at 3.65 mg/kg/day.

Days in estrus are increased. Days in diestrus are not -

Atrazine clearly increased percent days in estrus and estrus blocks in the HDT of 29.44 mg/kg/day. As described above, atrazine exposure at 3.65 mg/kg/day also increased percent days in estrus and number of animals with estrus blocks.

Percent days in diestrus was decreased at 3.65 and 29.4 mg/kg/day during weeks 21-22 and 25-26. Such a decrease would be expected given the concurrent increases in days in estrus that are occurring during these two time periods at these two doses. The study author states on page 30 of the study report that there were significant increases in diestrus blocks earlier in the 29.44 mg/kg/day animals. This statement is difficult to support. As shown in Table 8, there is an increase in the number of animals with diestrus blocks at the high dose during the early time point of 1-2 weeks. The percentage of control animals displaying at least one diestrus block was 24.44% while the percentage of 29.4 mg/kg/day animals displaying at least one diestrus block was 36.7%. However, at the very next time point, the situation is reversed with 17.8% of the control animals displaying a diestrus block and only 10% of the 29.4 mg/kg/day animals displaying at least one diestrus block. These dramatic variations between time points so close together implies that the differences are non-dose-related variations.

The study author also states, on page 30, that the increases in diestrus blocks corroborate findings described in: Cooper, *et al.*, 1995. *Effects of atrazine on the hormonal control of the ovary*. The Toxicologist 15:24. This reference is only an abstract and does not, in any case, describe an increase in days in diestrus. Rather, this abstract states only that atrazine exposure at high doses, "eliminated ovarian cycles within 4 days". A full journal publication using this data was later published: Cooper, *et al.* 1996. *Effect of atrazine on ovarian function in the rat.* Reproductive Toxicology, 10:4, pp. 257-264. The studies described in this publication exposed both SD and Long Evans (LE) females to 0, 75, 150, or 300 mg/kg/day of atrazine by

gavage for 21 consecutive days. Atrazine significantly increased days in diestrus in both strains, *but only at the 150 and 300 mg/kg/day doses*. The 75 mg/kg/day dose did not increase days in diestrus in either strain. The exposure in the Cooper study was for 21 days only compared to the six month exposure used in the current study. Had Cooper used exposures of longer duration then perhaps ≤75 mg/kg/day would have resulted in increases in days spent in diestrus.

The study author also cites, on page 30, a registrant-submitted one month gavage study with atrazine. It is stated on page 30 that this study also provides collaborative evidence that supports the contention that 29.44 mg/kg/day in the current study resulted in increases in days in diestrus. This study, MRID 43934406, is discussed in detail in a separate DER. In this study SD females were exposed to 0, 2.5, 5, 40 and 200 mg/kg/day of atrazine by gavage for 28 days. There was a statistically significant increase in the number of animals with diestrus blocks at both the 40 and 200 mg/kg/day doses.

The results from these three studies do not provide strong evidence that atrazine exposure at doses ≤ 29.44 mg/kg/day result in increases in days in diestrus or diestrus blocks. The Cooper study did not see increases in percent days in diestrus after 21 days of exposure to 75 mg/kg/day. The current study saw increases in animals with diestrus blocks only at the 29.44 mg/kg/day dose at the first and last two sampling time points. The percentage of days spent in diestrus during the last two time points was actually significantly decreased at the last two time points, despite the increase in animals displaying diestrus blocks. The increases in high dose animals with diestrus blocks at the first time point are compromised by the dramatic decrease in high dose animals displaying a diestrus block at the next time point. The only study which found a clear increase in days in diestrus at a dose close to 29.44 mg/kg/day was the one-month gavage study (MRID 43934406). In this study a statistically significant increase in animals with diestrus blocks was seen at 40 mg/kg/day.

To summarize:

- **Increases in days in estrus and estrus blocks were seen at 3.65 and 29.44 mg/kg/day;**

- **Attenuation of the LH surge was seen at 3.65 and 29.44 mg/kg/day;**

- **The prolactin surge was not altered at any dose;**

- **Days in diestrus or animals with diestrus blocks were not *increased* by compound exposure, though a dose-related *decrease* in percent days in diestrus was seen at 3.65 and 29.44 mg/kg/day in the last two time points (such a decrease is expected given the increase in days in estrus);**

- **Pituitary weights were increased at 29.44 mg/kg/day.**

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 = 3.65/1.80 mg/kg/day;

Prolactin Surge LOAEL/NOAEL = not established/> 29.44 mg/kg/day

Estrous cycle alterations = LOAEL/NOAEL = 3.65/1.80 mg/kg/day

B. Study deficiencies

This study contained several deficiencies. The Morseth, 1996 study, cited on page 30, is not included in the references. Measurements of pituitary tissue hormones levels have yet to be provided. Histopathology of the tissues prepared have yet to be provided. Estradiol values, neither group mean nor individual values, are not given. Figures 1 and 2 on pages 35 and 36 show asterisks indicating statistical significance for the repeat bleed LH measurements, but specific p values are not given here or in the text.

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

Figure 1: LH Measurements - Repeat Bleed

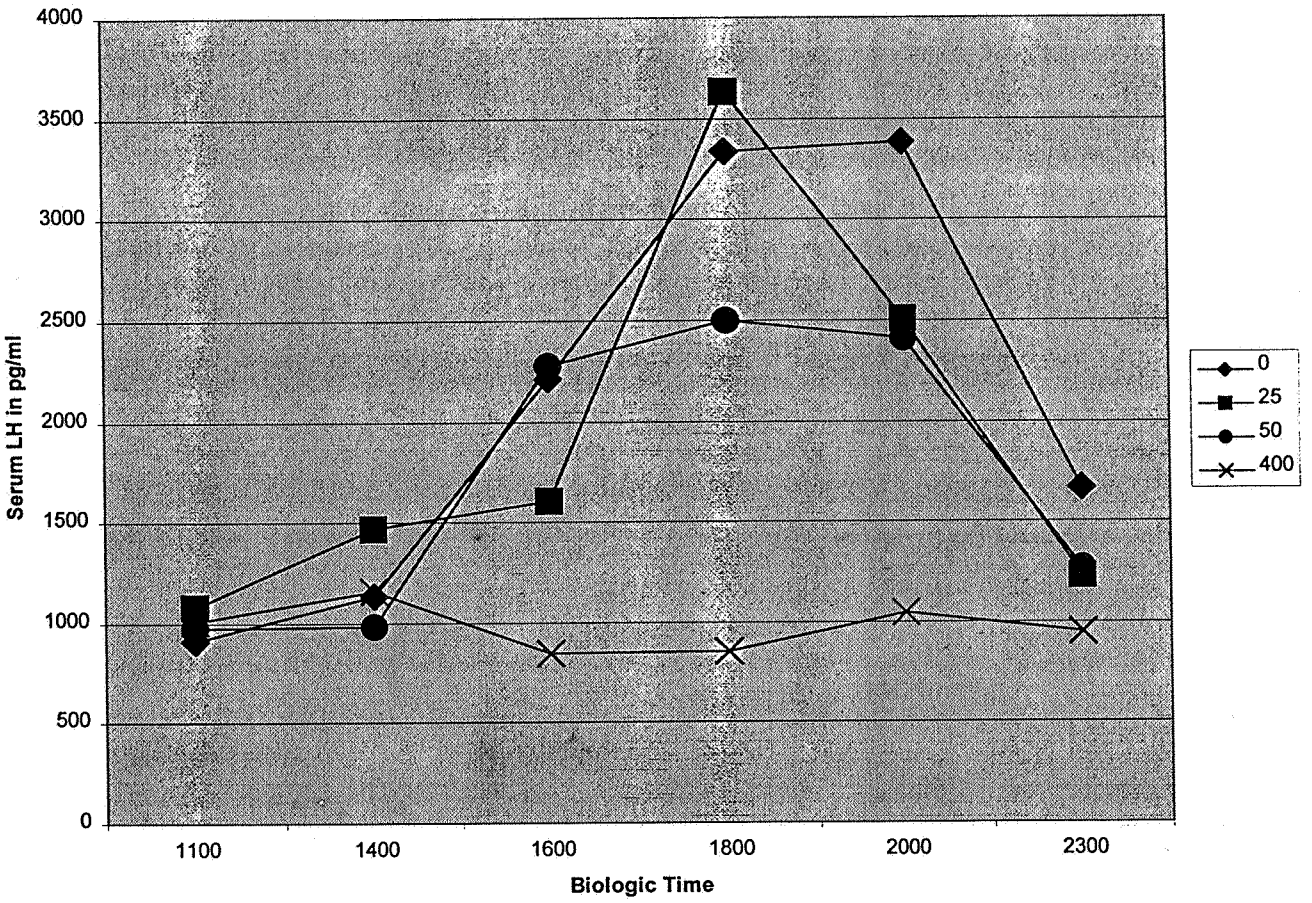

Figure 2: LH Measurements - Nonrepeat Bleed

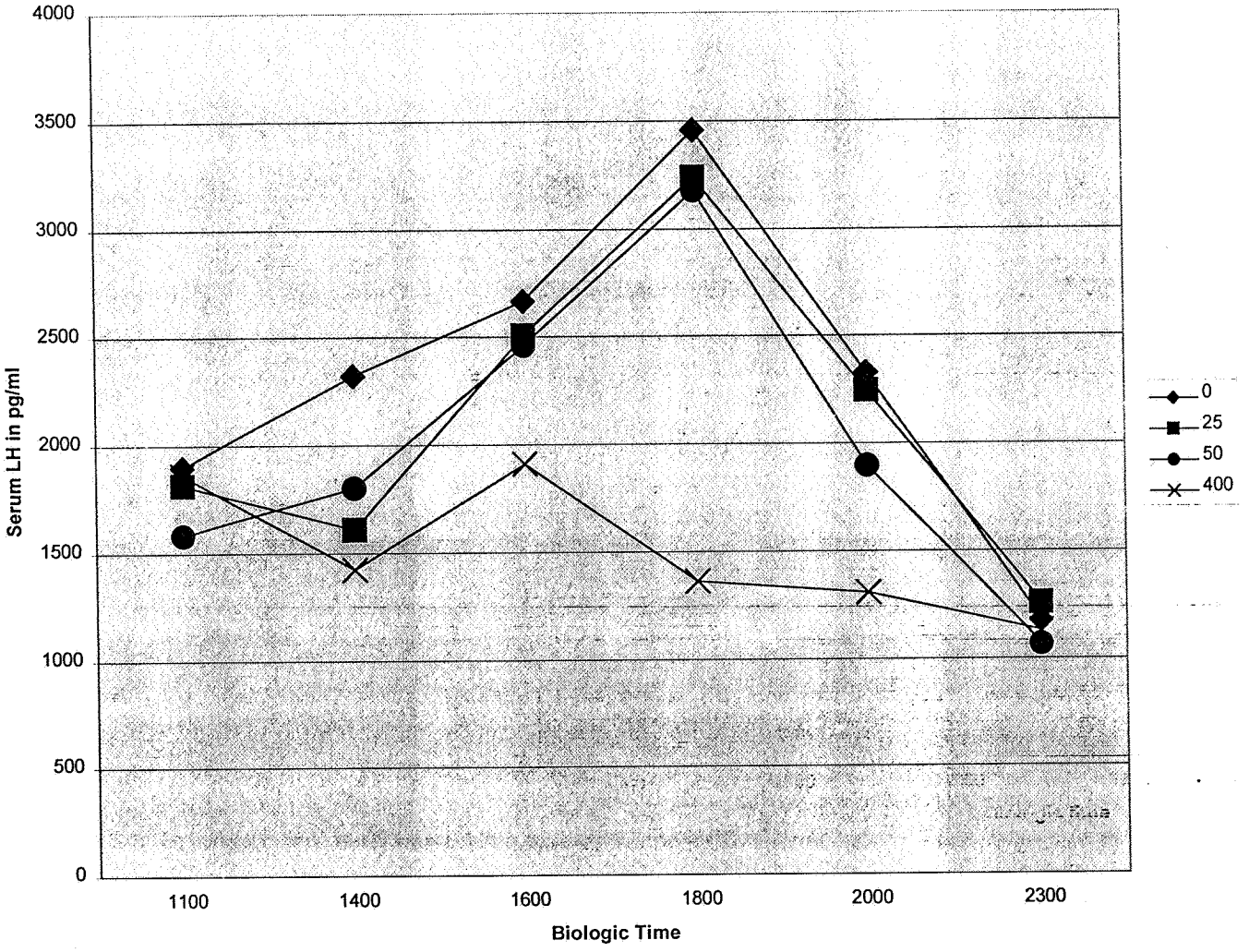

Figure 3: Prolactin Measurements