Image A 5

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

Office of Prevention, Pesticides
and
Toxic Substances

MEMORANDUM

October 30, 2002

TXR#: 0051031

SUBJECT: **ATRAZINE** - Submission by Syngenta Crop Protection, Inc.

TO: Catherine Eiden
Reregistration Branch III, HED (7509C)
and
Kimberly Nesci
Reregistration Branch, SRRD (7508W)

FROM: Linda L. Taylor, Ph.D.
Reregistration Branch I
Health Effects Division (7509C)

THRU: Whang Phang, Ph.D.
Branch Senior Scientist, Reregistration Branch I
Health Effects Division (7509C)

Submitter:	Syngenta Crop Protection, Inc.
Chemical:	2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine
Synonym:	atrazine, ATZ
Caswell No.:	063
CAS No.	1912-24-9
PC Code:	080803
DP Barcode:	D248708
Action Requested:	Review.

INTRODUCTION: The Registrant has submitted two special toxicology studies on atrazine [MRID 45711303 and MRID 45722401] and an overview document [MRID 45711302], which is a summary and interpretation of information and data on atrazine. Both toxicology studies have been evaluated, and the Data Evaluation Records [DERs] are attached. The EXECUTIVE SUMMARY for each DER is provided under (1) and (2) of this memorandum. An assessment of the toxicological aspects of the overview document is provided in this

memorandum under (3).

(1) MRID 45711303 - Eldridge, J. C. (2002). **Effects of Atrazine on the First Spontaneous Ovulation of Female SD Rats Administered Pregnant Mare's Serum Gonadotropin [PMSG] on PND 30.**

EXECUTIVE SUMMARY: In a special study [MRID 45711303] undertaken to determine the doses of atrazine necessary to disrupt parameters of ovulation and reproductive function in peripubertal Sprague-Dawley female rats, groups [ranging from 24-42 rats] of Sprague-Dawley (SD) female rats were dosed once daily [PND 30 to PND 32] *via* gavage [10 mL/kg] with atrazine [98.2%; 1, 5, 10, 50, 100, 300, 500 mg/kg/day] or vehicle [67 rats; carboxymethylcellulose (CMC)] following a subcutaneous injection of an extract of pregnant mare's serum gonadotropin [PMSG] on PND 30.

Body weight was not adversely affected at any dose level. Decreased body-weight gains [PND 30-32] were observed at dose levels of 50 mg/kg/day [80% of control] and above [67%-72% of control]. There was a decrease in the % of females with ova [% ovulation] at the 100 mg/kg/day [60%] and 500 mg/kg/day [54%] dose levels but not at 300 mg/kg/day or at the 1-50 mg/kg/day dose levels. Of the females that did ovulate, there were fewer ova in those females at 500 mg/kg/day than in the control and other dose groups. Since the study was performed over a 16-week period, there were limited time points that were common between control and treated groups. Data are available for comparison of the % ovulating and number of ova for weeks 14 and 15 for the control and the three highest dose groups. This comparison shows a dose-related decrease in the number of ova in those females that ovulated, but the % of females that ovulated was lowest in the control group. Due to the question of whether atrazine was dosed during the critical period to demonstrate an effect on ovulation, no conclusion regarding the apparent lack of an effect at lower dose levels can be made.

The study lacks performance criteria. The control group showed the lowest ovulation rate for 7 of the 14 weeks in which a control group was run. Additionally, the percent of control female groups displaying greater than or equal to 50% ovulation is 57% compared to 100% in all but the 100 mg/kg/day dose group [71%]. Based on this, the failure to demonstrate the capability to consistently induce ovulation in the immature female control rat with the model utilized, and the questionable timing of treatment, no definitive conclusion regarding the effect of atrazine on ovulation in the immature female rat is possible.

For body-weight effects, the NOAEL is 10 mg/kg/day, based on decreased body-weight gain at the LOAEL of 50 mg/kg/day. Although inhibition of ovulation and a decreased number of ova in those females that ovulated were observed at 500 mg/kg/day compared to the control, *no definitive LOAEL/NOAEL can be determined.* This is due to the fact that there are no data to support the selected time [10 am to 12 noon] when the dose of atrazine was administered, and the lack of an effect at lower dose levels may have resulted because exposure to atrazine did not occur during the critical period. Lack of a positive control and a failure to demonstrate the capability to consistently induce the immature female control rat to ovulate with the model used added difficulty to the interpretation of the data.

This nonguideline special study on female rat sexual maturation is classified Unacceptable/non-guideline. The study is unacceptable based on the questionable timing of the atrazine dose and the failure to demonstrate the capability to consistently induce ovulation in the immature female control rat with the model utilized.

(2) MRID 45722401 - Ashby, J. and Tinwell, H. (2002). **The Effects of Atrazine on the Sexual Maturation of Female Alderley Park-Wistar and Sprague-Dawley Rats.**

EXECUTIVE SUMMARY: In a special study [MRID 45722401] that involved four separate experiments, 8-10 Alpk:ApfSD (Wistar-derived; AP) female rats/dose group and 8-10 Sprague-Dawley (SD) female rats/dose group [20-21 day old] were dosed once daily *via* gavage [10 mL/kg] with atrazine [98.2%], vehicle [carboxymethylcellulose (CMC)], or Antarelix™ [a centrally-acting GnRH antagonist] for up to 25 days. The dose levels of atrazine were 10, 30, and 100 mg/kg. Antarelix was dosed at 0.3 mg/kg. The first two experiments involved the determination of uterine weight on either postnatal day 30 [start of puberty] or on postnatal day 33, when uterine growth was assumed to have been completed.

Alpk:ApfSD (Wistar-derived; AP) female rats: Atrazine: ***Uterine growth***: There was a dose-related decrease in uterine weight following exposure to atrazine from postnatal day [PND] 22 to PNDs 29 [8 doses], 32 [11 doses], and 42 [21 doses]. At the high-dose level, the largest decrease in uterine weight was observed in the group receiving the 11 doses [PND 22-32], and the smallest decrease was observed following the longest exposure [PND 22-42]. The magnitude of the decrease in body weight [93% of control]/body-weight gain [87% of control] observed at the high-dose level following the 11-dose regimen does not account for the magnitude of the decrease in uterine weight [blotted 55%/dry 50% lower than control]. At the mid-dose level, the decrease in uterine weight [blotted 22%/dry 16% lower than control (PND 22-29); blotted 18%/dry 19% lower than control (PND 22-42)] was not statistically significant but is considered treatment-related. Body weight and body-weight gains of the mid-dose females were comparable to the control values. ***Vaginal opening [VO]***: At the high-dose level, there was a statistically-significant delay in VO [PND 41] compared to the control [PND 38] following the PND 22-42 dosing regimen. Antarelix: ***Uterine growth***: In comparison, the rats exposed to ANT showed a lack of uterine growth, and in contrast to the atrazine findings, the magnitude of the decrease in uterine weight increased with the increase in the duration of ANT exposure. There was no effect on body weight/body-weight gain. ***Vaginal opening***: None of the ANT females had an open vagina at study termination [PND 43].

Sprague-Dawley (SD) female rats: Atrazine: ***Uterine growth***: Decreased uterine weight was observed at the high-dose level following exposure to atrazine from PND 22-PND 45 [24 doses]. However, statistical significance was not attained, and the magnitude of the effect [blotted 13%/dry 11% lower than control] was slight, as was the body-weight deficit [4% lower than control]. ***Vaginal opening***: There was a statistically-significant delay in VO at the mid- [PND 41] and high-dose [PND 42] levels compared to the control [PND 39] following the PND 22-45 dosing regimen. In contrast to the AP females, a delay in vaginal opening was observed in the SD females at a dose level where uterine weight was not affected by treatment.

The NOAEL is 10 mg/kg/day, based on delayed vaginal opening [SD rats] and reduced uterine growth [AP rats] at the LOAEL of 30 mg/kg/day.

This nonguideline special study on female rat sexual maturation is classified Acceptable/non-guideline.

(3) MRID 45711302 - Breckenridge, C.; Stevens, J.; and Pastoor, T. (2002). **The Selection of Endpoints, Application of FQPA Uncertainty Factors and Risk Extrapolation at the 99.9th Percentile.**

Syngenta believes that the use of the LH endpoint from a 6-month study in adult rats is not appropriate for assessing risk to the young. Additionally, Syngenta concludes that their analyses should alleviate any concerns held by EPA and remove the need for additional [10X] FQPA uncertainty factors.

ISSUE 1. **Sensitivity of Young**. Syngenta states that all evidence indicates that young rats are less sensitive to the neuroendocrine effects caused by atrazine than adult rats. The basis for this conclusion stems from their comparison of the NOAEL/LOAEL from studies on the young animal [those where the young were not directly dosed, two pubertal assays and two recent studies in which young rats were dosed directly] with findings in the available database on the adult animal.

HED Response: Although the NOAELs in some of the adult studies are lower than those in the young, this apparent difference between the age groups may be attributed to dose spacing and/or to a difference in dosing duration. For example, comparison of the 28-day LH surge study in the female adult rat [NOAEL of 5 mg/kg/day; LOAEL of 40 mg/kg/day] with the published pubertal study in female young rat [delayed VO NOAEL of 25 mg/kg/day ; LOAEL of 50 mg/kg/day] shows rather similar LOAELs [40 *vs* 50] for similar durations of dosing [young female 20 days]. If the dose-spacing in the adult study were similar to that in the pubertal study [2X], the NOAELs might have been similar also [20 *vs* 25]. In comparisons made by Syngenta, the 6-month study **duration** far exceeds any study performed in the young animal, and it is well known that doses required to produce an effect following long-duration exposure are lower than for a short-duration exposure. A comparison of the **adult** NOAELs/LOAELs obtained in the 6-month [1.8/3.65 mg/kg/day] and 28-day [5/40 mg/kg/day] studies illustrates this also.

Based on one of the recent studies [described above] in which immature female rats were dosed directly **[21-24 days]**, the lowest NOAEL was 10 mg/kg/day, based on effects [delayed vaginal opening and reduced uterine weight] at 30 mg/kg/day. Comparison of this study with the NOAEL observed in the adult female **28-day** LH surge study [NOAEL = 5/mg/kg/day; LOAEL = 40 mg/kg/day] also **does not support the conclusion** that the young female rat is less sensitive than the adult female rat.

The second study submitted recently in support of their argument about sensitivity is unacceptable, based mainly on the questionable timing of atrazine treatment. Additionally, the authors did not demonstrate proficiency with the model used, and there is a lack of performance criteria. The control group showed the lowest ovulation rate for 7 of the 14 weeks in which a control group was run. Additionally, only 57% of the control groups displayed greater than or equal to 50% ovulation compared to 100% in all but the 100 mg/kg/day atrazine group [71%]. Based on this, the lack of a positive control, and the questionable timing of atrazine treatment, no definitive conclusion regarding differences in response between the control and treated animals or between the adult and young animals is possible.

ISSUE 2. **LH Surge Inappropriate Endpoint for Young.** The registrant argues that the GnRH axis is "not operative in prepubertal animals", and the role of LH remains quiescent until onset of puberty, when the brain "wakes up" and LH begins to exert its actions on sexual development and, later in life, on maintenance of reproductive function. According to Syngenta, until puberty, LH plays no role and therefore cannot be affected by atrazine. Syngenta further argues that an endpoint that is based on LH or physiological functions that depend on LH are appropriate only for the peripubertal developmental stage and adults. Prepubertal endocrine-related effects that have been reported, such as prostatitis, are considered by Syngenta to be the result of effects in the mother, not in the young animal. Syngenta states that studies with other, life-stage

specific, endpoints are available and are scientifically more appropriate for assessing risk to the young. Syngenta disagrees with the Agency's use of the endpoint [LH surge attenuation and estrous cycle disruption] from the 6-month adult study for risk assessment of the young.

The registrant also states that the prepubescent surge of LH in immature female rats initiates ovarian estradiol synthesis, the growth of the uterus, acquisition of vaginal patency [VO], and the institution of regular ovarian cyclicity. The recent study submitted was conducted to "test the equivalence in dosimetry for the effects of atrazine" in peripubertal and adult female rats. The time of dosing was designed to assess atrazine's effects on sexual development at the critical moments of GnRH/LH "awakening". Syngenta states that the results support the conclusion that the pituitary/hypothalamic axis in peripubertal female SD rats is less sensitive than in adult female SD rats, as evidenced by the lower no-effect level in the adult [1.8 mg/kg/day] than in the peripubertal [10 mg/kg/day] rat.

HED Response: As noted previously, the endpoint [LH surge attenuation and estrous cycle alterations] serves as a **surrogate** for the effect of atrazine on the hypothalamic-pituitary axis/function. The hypothalamic-pituitary axis is involved in the development of the reproductive system and its maintenance and functioning in adulthood. Additionally, the reproductive hormones **modulate the function of numerous other metabolic processes, including bone formation, and immune, CNS, and cardiovascular functions**. A potential exists for reproductive as well as developmental disruption to occur as a consequence of hypothalamic-pituitary-gonadal disturbance. As discussed in the SAP report, neurogenesis is not limited to the intrauterine period and may continue throughout the lifespan. Brain development goes at an explosive pace during the first few years of life. During that time, neurons and glia are migrating and dendrites are sprouting and are being pruned back. A three year old has fewer synapses than a two year old because of the pruning process. This pruning is tightly orchestrated and under the influence of the genes and the experiences of the child. A synaptic connection that is reinforced by experience at this time is more likely to persist. Any perturbation of CNS metabolism at this time may decrease the specificity and increase the randomness of these connections. The effect of atrazine on LH and prolactin are the result of altered GnRH output, and this is mediated by neurotransmitters, NE and DA. Prolactin is regulated by DA. Because of the rapid developmental brain changes noted above, the influence of atrazine on neurotransmitters in the hypothalamus and on GnRH may well have a differential, permanent effect on children. Therefore, altered hypothalamic-pituitary function, which can potentially broadly affect an individual's functional status, is considered relevant to humans of **all population subgroups**. With respect to the comparison of NOAEL/LOAEL in the adult 6-month study with new immature rat study, both the duration of exposure [6 months *vs* 25 days] and the endpoints monitored [LH surge attenuation and estrous cyclicity disruption *vs* delayed VO and decreased uterine weight] differ.

With regard to the use of life-stage specific endpoints for assessing risk to the young, the acute dietary [females 13-50 years of age; delay or lack of ossification of several sites in the rat developmental study; NOAEL 10/LOAEL 70] and short-term incidental oral, dermal, and inhalation [delay in preputial separation in the pubertal screening study; NOAEL 6.25/LOAEL 12.5] exposure assessments are based on effects observed in the young animal. It is recognized, however, that the developmental toxicity study involved indirect dosing of the young animal.

It is to be noted that the 6-month study is being used for **the intermediate-term** and **long-term** risk assessments only. There are no studies in the young animal of sufficient duration with which to assess intermediate-term and long-term exposure.

ISSUE 3. **Inappropriate Application of the FQPA 10X Uncertainty Factor.** The registrant argues that the two, recently-submitted, studies in the prepubertal female SD rat demonstrate that the pituitary/hypothalamic axis in the peripubertal/immature rat is less sensitive than in the adult female SD rat. Syngenta also provides rationale "to remove the additional FQPA uncertainty factors". This includes (1) their argument that LH plays no role in the young animal until puberty and, therefore, cannot be affected by atrazine; (2) their determination that "a great deal of data are available that describe atrazine's NOELs during the pubertal period of development." Syngenta cites the results of the recently submitted studies on sexually immature rats, which they state support the conclusion that the hypothalamic-pituitary axis in the immature female rat is less sensitive than in the adult female. The registrant points out that the study on ovulation was "unable to demonstrate significant disruption of the ovulatory process at acute doses of 300-500 mg/kg atrazine in animals 30 days old, whereas LH attenuation can be easily demonstrated by one-tenth of this atrazine dose, when administered to animals that are 6-8 months age." Based on the above, Syngenta believes EPA should remove the additional 10-fold uncertainty factor.

HED Response: With respect to the lack of a significant response in the 30-day old female SD [peripubertal] rat following a 3-day exposure to atrazine doses of 300-500 mg/kg/day, it is to be noted that there was no attenuation of the LH surge in adult female SD [ovariectomized] rats following 3 days of exposure to 300 mg/kg/day [Toxicol. Sci. 53,297-307 (2000)]. As pointed out elsewhere in this memorandum, the recently-submitted studies do **not** demonstrate that the young animal is less sensitive to the effects of atrazine than the adult animal. The FQPA Safety Factor was **retained due to residual concerns** identified by the HIARC for the effects of the neurocrine mode of action described for atrazine on the development of the young. The concern results from a lack of an assessment of exposure of the young animal to atrazine **throughout development**. Additionally, the residual concerns for the young animal are not just for disruption of the ovulatory process and reproductive effects, but for possible effects on the function of numerous other metabolic processes, including bone formation, and immune, CNS, and cardiovascular functions. Further, the 10X FQPA safety factor determination for atrazine was not only based on uncertainties in the hazard data for the immature animal but on the limitations found in the exposure monitoring data for drinking water.

CONCLUSION: The two recently-submitted special studies on young female SD rats do not demonstrate that the young animal is less sensitive to the effects of atrazine than the adult animal. The endpoint used for risk assessment for all population subgroups [LH surge attenuation and estrous cycle alterations] serves as a surrogate for the effects of atrazine on the hypothalamus-pituitary axis/function. It is considered appropriate for all populations and is being used for the intermediate-term and long-term risk assessments. Additionally, there are no studies in the young animal of sufficient duration for use in these latter two assessment. The FQPA Safety Factor was retained due to residual concerns for the effects of the neuroendocrine mode of action on the development of the young. The concern results from a lack of an assessment of exposure of the young animal to atrazine throughout development. Finally, the 10X FQPA safety factor determination for atrazine was not only based on uncertainties in the hazard database for the immature animal but on the limitations found in the exposure monitoring data for drinking water.

B

[ATRAZINE] **Special Study [First Spontaneous Ovulation in SD Females] (§ none)**

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 10/29/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. Whang Phang 10/31/02
Branch Senior Scientist, Reregistration Branch I, Health Effects Division (7509C)

TXR #: 0051031

DATA EVALUATION RECORD

STUDY TYPE: Special Study - [female rat] OPPTS none [§ none]
DP BARCODE: D284705
P.C. CODE: **080803**
CAS Number: 1912-24-9
TEST MATERIAL (PURITY): Atrazine [98.2% a.i.]
SYNONYMS: ATR
CHEMICAL: 2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine
CITATION: Eldridge, J. C. (2002). Effects of Atrazine on the First Spontaneous Ovulation of Female SD Rats Administered Pregnant Mare's Serum Gonadotropin [PMSG] on PND 30. Wake Forest University School of Medicine, Department of Physiology & Pharmacology, Winston-Salem, NC. Syngenta Number 1755-02; July 3, 2002. MRID 45711303. Unpublished.
SPONSOR: Syngenta Crop Protection, Inc.

EXECUTIVE SUMMARY: In a special study [MRID 45711303] undertaken to determine the doses of atrazine necessary to disrupt parameters of ovulation and reproductive function in peripubertal Sprague-Dawley female rats, groups [ranging from 24-42 rats] of Sprague-Dawley (SD) female rats were dosed once daily [PND 30 to PND 32] *via* gavage [10 mL/kg] with atrazine [98.2%; 1, 5, 10, 50, 100, 300, 500 mg/kg/day] or vehicle [67 rats; carboxymethylcellulose (CMC)] following a subcutaneous injection of an extract of pregnant mare's serum gonadotropin [PMSG] on PND 30.

Body weight was not adversely affected at any dose level. Decreased body-weight gains [PND 30-32] were observed at dose levels of 50 mg/kg/day [80% of control] and above [67%-72% of control]. There was a decrease in the % of females with ova [% ovulation] at the 100 mg/kg/day [60%] and 500 mg/kg/day [54%] dose levels but not at 300 mg/kg/day or at the 1-50 mg/kg/day dose levels. Of the females that did ovulate, there were fewer ova in those females at 500 mg/kg/day than in the control and other dose groups. Since the study was performed over a 16-week period, there were limited time points that were common between control and treated groups. Data are available for comparison of the % ovulating and number of ova for weeks 14 and 15 for the control and the three highest dose groups. This comparison shows a dose-related decrease in the number of ova in those females that ovulated, but the % of females that ovulated was lowest in the control group. Due to the question of whether atrazine was dosed during the critical period to demonstrate an effect on ovulation, no conclusion regarding the apparent lack of an effect at lower dose levels can be made.

The study lacks performance criteria. The control group showed the lowest ovulation rate for 7 of the 14 weeks in which a control group was run. Additionally, the percent of control female groups displaying greater than or equal to 50% ovulation is 57% compared to 100% in all but the 100 mg/kg/day dose group [71%]. Based on this, the failure to demonstrate the capability to consistently induce ovulation in the immature female control rat with the model utilized, and the questionable timing of treatment, no definitive conclusion regarding the effect of

atrazine on ovulation in the immature female rat is possible.

For body-weight effects, the NOAEL is 10 mg/kg/day, based on decreased body-weight gain at the LOAEL of 50 mg/kg/day. Although inhibition of ovulation and a decreased number of ova in those females that ovulated were observed at 500 mg/kg/day compared to the control, *no definitive LOAEL/NOAEL can be determined.* This is due to the fact that there are no data to support the selected time [10 am to 12 noon] when the dose of atrazine was administered, and the lack of an effect at lower dose levels may have resulted because exposure to atrazine did not occur during the critical period. Lack of a positive control and a failure to demonstrate the capability to consistently induce the immature female control rat to ovulate with the model used added difficulty to the interpretation of the data.

This nonguideline special study on female rat sexual maturation is classified Unacceptable/non-guideline. The study is unacceptable based on the questionable timing of the atrazine dose and the failure to demonstrate the capability to consistently induce ovulation in the immature female control rat with the model utilized.

COMPLIANCE: Unsigned and undated GLP and Data Confidentiality statements were provided. The study was not performed according to GLP; however, it is stated that it was conducted in accordance with good and acceptable scientific practices. There is no Quality Assurance statement, but there is a Report Approval signature page, which is not signed or dated.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: atrazine
Description: not provided
Batch #: SG-302011 BA, # FL-931480
Purity: 98.2% a.i.
Stability of Compound: not provided
Source: Syngenta Crop Protection, Inc. Greensboro, NC
CAS #: 1912-24-9

2. Vehicle: 0.5% carboxymethylcellulose [CMC, Sigma C-4888]
Batch #: not provided
Source: Sigma Chemical Company

5. Test animals: Species: rat
Strain: female Sprague-Dawley (SD)
Age at start of dosing: 21 days [acquired immediately after weaning from supplier]
Weight at start of dosing: 63-96 grams
Source: Harlan Sprague-Dawley [Charleston, WV]
Housing: 3 per cage
Diet: not specified (lab chow), ad libitum
Water: ad libitum
Acclimation period: not stated.

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: not provided.

2. Animal assignment -There were 16 sets, each containing 21 rats, which were shipped [**one set per week**] from supplier. The rats were randomly divided among 7 hanging wire cages (3 rats/cage). Each set of 21 rats was divided into four dose groups [1 vehicle control group and 3 atrazine dose groups]. After being weighed, the rats were redistributed slightly to achieve balanced distribution of weights in each dose. Two cages [6 rats] were assigned to one of the 3 dose groups, and one cage [3 rats] was assigned to the 4th dose group. There were 8 atrazine dose groups [Table 1]. *Although the number of animals that were stated to be included in the study was **336**, the number of rats for which data were presented was **282**.* On PND 30, each rat was administered 4-8 IU Pregnant Mares Serum Gonadotropin [PMSG, Sigma G-4877], in 0.1 mL saline, subcutaneously [at 10:00 am]. On PND 30, 31, and 32 [between 10:00 am-12:00 noon], each rat was administered atrazine or vehicle *via* gavage [1 mL/100 grams body weight]. NOTE: There was no discussion as to the timeframe over which each group was dosed relative to each other. Since there were 16 weekly sets of 21 animals, and these were divided among 4 groups/week, a maximum of four groups could be dosed per week [Table 2].

Table 1. Study Design

Group	# rats/group	dose [mg/kg/day]	concentration [mg/mL]
1	67	0	0
2	24	1	0.1
3	30	5	0.5
4	30	10	1.0
5	30	50	5
6	42	100	10
7	35	300	30
8	24	500	50

Table 2. Weeks on Study

Week on study	Dose (mg/kg/day)							
	0	1	5	10	50	100	300	500
1	X							
2	X			X	X			
3	X	X		X		X		
4								
5	X		X		X		X	
6								
7	X	X		X		X		
8	X		X		X		X	
9	X	X				X		
10	X			X			X	
11	X		X		X		X	
12	X		X	X		X		
13	X	X				X		X
14	X					X	X	X
15	X					X	X	X
16	X		X		X			X

X: week of treatment

3. Dose selection rationale: No rationale was provided for the dose levels of atrazine.

4. Dose preparation and analysis - Atrazine was suspended in 0.5% carboxymethylcellulose in tap water [concentrations shown in Table 1]. Storage conditions were not provided, and there was no discussion of homogeneity, stability, or concentrations attained.

5. Statistical analyses: In the results section of the report, various analyses were mentioned in the discussion of the findings. A one-way analysis of variance and a paired t comparison were conducted on the body-weight findings. Linear regression analysis, Cochran-Armitage trend test, and the Chi square test were performed on the % of rats ovulating. One-way analysis of variance was used on the number of ova/ovulation. Regression analysis was performed for ovulation *vs* body-weight change.

C. METHODS

1. Observations - There was no information provided.

2. Body weight/body-weight gain - Body weight was recorded for each rat for 6 days prior to dosing [PND 24-PND 30] and for 2 days during treatment [PND 30 to PND 32]. It is stated that body-weight gain per 2 days was calculated for each rat by subtracting the PND 24 weight from the PND 30 weight, then dividing the total by 3. This pre-treatment value [g/2 days] served as a contrast to the weight difference during treatment [PND 32-PND 30].

3. Food consumption and compound intake: Food consumption was not monitored [gavage study].

4. Ophthalmoscopic examination - Ophthalmological examinations were not performed.

5. Blood - No blood parameters, such as hormone levels, were provided.

6. Termination of study: The rats were terminated by carbon dioxide gas on PND 33. At necropsy, the reproductive organs were dissected and the oviduct of each ovary was isolated. Both oviducts were squeezed between two glass microscopeslides and examined with a microscope. Ova, when present, were easily visible as single, very large, round cells surrounded by numerous small cumulus oopherus cells, most often found within a distinctive clear area of each oviduct. The number of visible ova was recorded.

II. RESULTS

A. BODY WEIGHT/BODY-WEIGHT GAIN

1. The overall mean body weight of the control was slightly lower than all of the atrazine groups on PND 24 [3%-7%] and PND 30 [2%-7%]. Although body weights were comparable among the groups on PND 32, the control body weight remained slightly lower [2%-5%] than the 1-100 mg/kg/day atrazine dose groups [Table 3]. Body-weight gains over the pre-dosing period [PNDs 24-30] were comparable among the groups. The four highest dose levels displayed decreases in body-weight gain compared to the control and to their own 2-day average gain prior to dosing.

2. OVULATION DATA/NUMBER OF OVA

None of the groups had a 100% ovulation rate following a single injection of gonadotrophin on PND 30. The highest ovulation rates were observed in the atrazine groups [1 (83%), 5 (70%), 10 (77%), 50 (77%), and 300 (77%) mg/kg/day; Table 4]. Among the controls, 67% ovulated, and the ovulation rates of the atrazine groups were 70% or greater, with the exception of the 100 mg/kg/day [59.5%] and 500 mg/kg/day [54.2%] dose groups. At the highest dose level [500 mg/kg/day], there was a decrease in the mean number of ova [8.9±2.9] compared to the control [14.9±11.2] and other dose groups [14.1±13.8-16.6±16.0]. However, due to the large standard deviations observed, this decrease was not statistically significant.

Table 3. Body Weight✖/Body-Weight Gain✖ [grams] and % females with ova✖							
Dose/unit (n)	PND 24	PND 30	PND 32	ΔPND 24-30 [÷3]	ΔPND 24-32	ΔPND 30-32	% w/ ova
0 mg/kg/day							
10	-	70.3±4.3	84.7±5.8	-	-	14.4±1.9	90
9	39.9±2.5	73.0±4.2	83.3±5.1	33.1±2.4 [11.0]	43.4±3.0	10.3±1.5	78
3	53.0±5.2	77.0±7.2	87.0±7.9	24.0±2.6 [8.0]	34.0±3.0	10.0±1.0	100
3	55.3±3.5	86.0±7.9	97.3±11.0	30.7±4.6 [10.2]	42.0±7.8	11.3±3.2	33
3	55.3±5.8	87.0±7.9	98.3±8.5	31.0±3.0 [10.3]	42.3±4.6	11.3±2.5	0
3	55.3±1.2	85.7±1.5	97.0±2.0	30.3±0.6 [10.1]	41.7±1.2	11.3±0.6	0
9	53.4±3.4	82.1±5.5	90.4±6.3	28.7±0.7 [9.6]	37.0±3.6	8.3±1.2	89
9	52.8±3.0	80.4±5.8	90.2±5.2	27.7±5.9 [9.2]	37.4±5.5	9.8±1.4	56
3	50.3±2.5	82.3±5.1	90.7±8.1	32.0±2.6 [10.7]	40.3±5.6	8.3±3.2	33
3	49.7±2.1	80.0±2.0	89.3±4.0	30.3±0.6 [10.1]	39.7±2.3	9.3±2.1	100
3	48.7±0.6	76.6±2.5	84.7±3.1	28.0±2.6 [9.3]	36.0±3.0	8.0±1.0	100
3	47.7±2.1	69.7±2.1	80.3±5.1	22.0±0.0 [7.3]	32.7±3.1	10.7±3.1	67
3	52.0±1.7	80.0±2.6	89.7±2.1	28.0±1.0 [9.3]	37.7±0.6	9.7±0.6	33
3	49.3±1.5	76.0±1.0	84.7±1.5	26.7±1.5 [8.9]	35.3±1.5	8.7±0.6	67
mean [*mean*]√	[*50.3±5.7*]	[*78.0±7.0*]	[*88.4±7.1*]	[*9.7±1.3*]		[*10.4±2.5*]	**60.4±35.5**
1 mg/kg/day							
6	57.2±4.0	82.0±5.6	92.3±6.2	25.0±1.4 [8.3]	35.2±2.5	10.3±1.0	67
6	53.7±1.6	82.2±3.5	92.5±4.8	28.5±2.1 [9.5]	38.8±3.4	10.3±1.8	100
6	53.7±2.7	86.2±5.0	95.3±6.7	32.5±2.7 [10.8]	41.7±4.2	9.2±1.7	67
6	51.3±2.1	79.3±3.1	87.3±2.7	28.0±1.4 [9.3]	36.0±1.7	8.0±1.3	100
mean [*mean*]√	[*54.0±3.3*]	[*82.4±4.8*]	[*91.9±5.8*]	[*9.5±1.1*]		[*9.5±1.7*]	**83.5±19.1**
5 mg/kg/day							
6	57.2±2.5	86.3±3.5	96.8±4.3	39.7±3.1 [13.2]	39.7±3.1	10.5±1.4	100
6	55.0±3.1	84.8±4.0	94.8±4.5	39.8±2.9 [13.3]	39.8±2.9	10.0±1.8	67
6	54.2±1.7	87.2±3.8	97.0±4.2	42.8±2.7 [14.3]	42.8±2.7	9.8±1.0	67
6	54.5±1.5	84.0±2.4	93.0±2.5	38.5±2.3 [12.8]	38.5±2.3	9.0±0.9	67
6	50.0±2.5	78.5±2.6	87.2±2.2	37.2±1.3 [12.4]	37.2±1.3	8.7±0.5	50
mean [*mean*]√	[*53.7±3.0*]	[*83.9±4.3*]	[*93.3±4.9*]	[*10.0±0.8*]		[*9.4±1.3*]	**70.2±18.2**
10 mg/kg/day							
6	40.3±3.2	73.3±4.6	84.2±6.0	33.0±3.0 [11.0]	45.2±4.0	10.8±1.6	50
6	58.5±3.1	82.7±4.6	95.8±4.8	24.2±2.0 [8.0]	37.3±2.4	13.2±0.8	83
6	56.7±2.9	88.2±2.0	98.5±3.3	31.5±2.3 [10.5]	41.8±1.7	10.3±2.4	67
6	58.2±3.1	78.0±4.7	85.2±5.3	19.8±7.1 [6.6]	27.0±7.3	7.2±1.2	100
6	49.0±4.0	77.2±5.1	85.7±6.2	28.2±3.0 [9.4]	36.7±4.1	8.5±2.0	83
mean [*mean*]√	[*52.5±7.8*]	[*79.9±6.9*]	[*89.9±7.7*]	[*9.1±2.1*]		[*10.0±2.6*]	**76.6±18.9**
50 mg/kg/day							
6	39.0±3.0	73.0±3.2	80.8±4.0	34.0±2.1 [11.3]	41..8±2.9	7.8±1.6	67
6	57.7±3.7	88.0±5.1	98.0±5.9	30.3±1.6 [10.1]	40.3±2.3	10.0±1.7	67
6	54.2±2.7	84.3±4.2	93.5±5.9	30.2±1.9 [10.1]	39.3±3.6	9.2±1.8	83
6	54.2±4.3	85.8±5.8	90.8±6.0	31.7±2.0 [10.6]	36.7±2.3	5.0±0.9	83
6	52.8±3.9	83.2±5.8	92.5±5.0	30.3±3.4 [10.1]	39.7±3.1	9.3±1.6	83
mean [*mean*]√	[*51.6±7.4*]	[*82.9±7.0*]	[*91.1±7.7*]	[*10.4±0.9*]		[*8.3±2.3*]	**76.6±8.8**

Table 3. Body Weight✖/Body-Weight Gain✖ [grams] and % females with ova✖

Dose/unit (n)	PND 24	PND 30	PND 32	▵PND 24-30 [÷3]	▵PND 24-32	▵PND 30-32	% w/ ova
100 mg/kg/day							
6	57.2±4.2	83.0±4.4	94.8±6.9	25.8±1.9 [8.6]	37.7±4.2	11.8±3.0	33
6	55.7±4.7	88.2±5.2	96.2±7.0	32.5±5.2 [10.8]	40.5±3.2	8.0±5.5	50
6	54.5±2.7	82.7±3.0	88.2±2.4	28.2±1.7 [9.4]	33.7±1.8	5.5±1.0	83
6	53.8±2.9	85.5±2.6	92.8±3.9	31.7±2.9 [10.6]	39.0±4.5	7.3±1.6	17
6	50.2±3.0	78.2±2.6	83.7±4.3	28.0±2.0 [9.3]	33.3±3.4	5.5±1.9	83
6	53.2±3.7	78.3±4.7	84.5±4.5	25.2±2.0 [8.4]	31.3±2.2	6.2±1.9	83
6	52.2±2.5	79.7±4.1	88.2±5.8	27.5±2.3 [9.2]	36.0±3.6	8.5±2.5	67
mean [*mean*]√	[*53.8±3.9*]	[*82.2±5.1*]	[*89.8±6.7*]	[*9.7±1.2*]		[*7.5±3.4*]	**59.4±26.8**
300 mg/kg/day							
6	57.7±3.1	88.2±5.5	96.3±5.3	30.5±2.6 [10.2]	38.7±2.9	8.2±2.1	50
5	56.2±2.0	85.8±5.0	93.6±4.8	29.6±3.1 [9.9]	37.4±2.9	7.8±1.8	60
6	55.0±3.5	76.8±5.8	80.2±6.8	21.8±2.9 [7.3]	25.2±4.1	3.3±2.3	100
6	50.2±3.5	81.5±4.4	90.5±5.2	31.3±1.6 [10.4]	40.3±2.5	9.0±1.3	100
6	50.7±2.5	73.5±4.3	81.0±4.9	22.8±2.5 [7.6]	30.3±3.0	7.5±1.2	100
6	53.7±2.9	82.2±4.3	88.5±4.8	28.5±2.1 [9.5]	34.8±2.9	6.3±1.4	50
mean [*mean*]√	[53.8±3.9]	[*81.2±6.8*]	[*88.2±7.9*]	[*9.4±1.3*]		[*7.0±2.5*]	**76.7±26.8**
500 mg/kg/day							
6	52.7±3.3	82.0±3.3	88.8±3.7	29.3±1.9 [9.8]	36.2±2.1	6.8±1.3	50
6	51.3±1.8	74.5±2.9	81.0±5.0	23.2±1.6 [7.7]	29.7±3.7	6.5±3.3	67
6	52.5±1.4	81.7±2.9	89.7±3.1	29.2±1.7 [9.7]	37.2±2.0	8.0±1.1	50
6	54.0±2.3	81.8±4.3	88.5±5.5	27.8±2.2 [9.3]	34.5±3.4	6.7±1.6	67
mean [*mean*]√	[*52.6±2.4*]	[*80.0±4.5*]	[*87.0±5.5*]	[*9.5±0.7*]		[*7.0±2.0*]	**58.5±9.8**

✖ calculated by reviewer using data from Tables 3-10 (pages 22-31) of the report; ▵ gain during interval; [*mean*]√ provided in report

Table 4. Ovulation/Ova Data

Group mg/kg/day	# w/ ova [% ovulating]	mean # ova ✖
0	45/67 [67]	14.9±11.2
1	20/24 [83]	16.3±11.7
5	21/30 [70]	14.3±12.3
10	23/30 [77]	14.6±13.3
50	23/30 [77]	16.6±16.0
100	25/42 [60]	14.4±12.0
300	27/35 [77]	14.1±13.8
500	13/24 [54]	8.9±2.9

✖ calculated by reviewer using data from Tables 3-10 (pages 22-31) of the report;
Data from Table 2 [page 21] of the report

DISCUSSION

This study was undertaken to determine the doses of atrazine necessary to disrupt parameters of ovulation and reproductive function in peripubertal Sprague-Dawley female rats. The model selected was one in which immature female rats can be induced to ovulate just prior to the time of their own spontaneously-generated initial ovulation. As discussed in the report, one subcutaneous [s. c.] injection of an extract of pregnant mare's serum gonadotropin [PMSG] on PND 30 causes the appearance of ova on the morning of PND 33 [an initial spontaneous ovulation would normally be expected around PND 36-38]. The single injection of PMSG recruits ovarian follicles to begin maturation, as the adult rat's own FSH and LH do during each cycle. Estrogen secreted from the follicles initiates an LH surge on the afternoon of PND 32. The objective of the study was to determine whether orally-administered atrazine could inhibit ovulation that was stimulated by a single injection of PMSG to immature female rats. The endpoints monitored were body weight, body-weight gain, occurrence of ovulation, and the number of ova.

Body weight was not adversely affected at any dose level. Decreased body-weight gains [PND 30-32] were observed at dose levels of 50 mg/kg/day [80% of control] and above [67%-72% of control].

There was a decrease in the % of females with ova [% ovulation] at the 100 mg/kg/day [60%] and 500 mg/kg/day [54%] dose levels but not at the 300 mg/kg/day or 1-50 mg/kg/day dose levels. Of the females that did ovulate, there were fewer ova in those females at 500 mg/kg/day than in the control and other dose groups.

This study was performed over a 16-week period, and the highest dose level was run during the last four weeks of the study [weeks 13-16]. Data are available for comparison of % ovulating and number of ova for weeks 14 and 15 for the control and the three highest dose groups [Table 5]. This comparison shows a dose-related decrease in the [mean over 2 weeks] number of ova in those females that ovulated, but the **% that ovulated was lowest in the control group during these two weeks**. The overall % ovulating in the control group [there was a control group in 14 of the 16 weeks] was 67%. Table 6 demonstrates a **lack of effect** on the % of females ovulating **at the 500 mg/kg/day** dose level when the control data for weeks 13-16 are compared to the 500 mg/kg/day dose groups that were run only during these four weeks.

Table 5. Comparison of Weeks 14 and 15 [mean±std # ova (% ovulating)]

week on study	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	500 mg/kg/day
14	9.5±0.7 (67)	9.2±5.3 (83)	6.8±3.4 (100)	7.5±2.4 (67)
15	11.0±0 (33)	8.9±3.7 (67)	11.7±4.9 (50)	9.3±2.3 (50)
mean	10.0±1.0 (50)	8.7±4.4 (75)	8.4±4.4 (75)	8.3±2.4 (58)

weekly N=3 control; 6 atrazine; calculated by reviewer using data from Tables 3, 8-10 (pages 23, 28, 30, 31) of the report

Table 6. Comparison of % Females Ovulating During Weeks 13-16 (% ovulating)]

Week on study	Dose (mg/kg/day)	
	0	500
13	100	50
14	33	67
15	33	50
16	67	50
mean	**51**	**54**

Table 7 displays comparisons of the three highest dose levels with the control groups run concurrently during the weeks in which these treated groups were run. From these comparisons, it can be seen that for the latter weeks [13-16] during which most of the high-dose groups were run, all three treated groups displayed fewer ova than the concurrent control. The only other comparisons possible for these groups are weeks 5, 8, 10, ans 11 for the 300 mg/kg/day group and weeks 3, 7, 9, and 12 for the 100 mg/kg/day group. At 300 mg/kg/day during weeks 5-11, a larger number of ova were observed than in the control. However, the control mean ovulation rate over this time period was 39% compared to 78% at 300 mg/kg/day, and the mean number of ova in the control was lower than the mean number observed in the control groups during other time periods. A lack of data for the 500 mg/kg/day dose group for weeks prior to week 13 also raises a question as to whether the apparent effect [fewer ova] may be due to factors other than treatment.

Table 7. Comparison of 3 Highest Dose Groups to Control on a Weeks' Tested Basis [% Ovulating and # Mean Ova]

common weeks tested	0 mg/kg/day	100 mg/kg/day	300 mg/kg/day	500 mg/kg/day
weeks 13, 14, 15, 16 # ovulating/n (%) mean # ova range # ova	8/12 (67) 19.1 6-41			13/24 (54) 8.9 5/16
weeks 13, 14, 15 # ovulating/n (%) mean # ova range # ova	6/9 (67) 17.3 9-41	14/18 (78) 12.2 3-31		10/18 (56) 9.0 6-16
weeks 14, 15 # ovulating/n (%) mean # ova range # ova	3/6 (50) 10.0 9-11	9/12 (75) 8.7 3-18	9/12 (75) 8.4 2-15	7/12 (58) 8.3 5-12
weeks 5, 8, 10, 11 # ovulating/n (%) mean # ova range # ova	7/18 (39) 14.5 7-52		18/23 (78) 17.0 2-59	
weeks 10, 11 # ovulating/n (%) mean # ova range # ova	9/15 (60) 22.9 8-52		18/18 (100) 21.4 2-59	
weeks 3, 7, 9, 12 # ovulating/n (%) mean # ova range # ova	14/18 (78) 19.4 7-40	11/24 (46) 17.1 7-61		

There was no discussion in the report regarding the time selected for administering atrazine [10 am to 12 noon]. It is to be noted that the time of the day when atrazine is administered can affect the results. In discussions with Dr. Ralph Cooper at the Reproductive Toxicology Division of EPA's National Health and Environmental Effects Research Laboratories [NHEERL] at Research Triangle Park, N. C., he indicated that traditionally, reproductive studies examining the neuroendocrine basis of ovulation employ a 14-hour light-10-hour dark lighting schedule [lights on at 5 am and off at 7 pm]. It has been shown that with this light schedule, the activation of the neural [hypothalamic] mechanisms that trigger the ovulatory surge of LH and ovulation occur on the afternoon [somewhere between 2 pm and 3 pm] of vaginal proestrus [or in the current study on PND 32]. Thus, investigators time the dose of a test material so that the maximum concentration of the material to the brain occurs at the so-called critical period [between 2 pm and 4 pm]. In general, this would be 1-1:30 pm or one hour before the critical period. If a test material were injected too early, the dose required to block the LH surge and ovulation was higher; if injected too late [4 pm], the dose may be totally ineffective. In a study on thiram

[Stoker, *et al.*, (1993). Reprod. Toxicol. 7(3): 211-218], ovulation was blocked in all rats given dose levels of 25 mg/kg and 50 mg/kg at 1300 hours [1 pm], but when injected at 1100 hours [11 am] or 1800 hours [6 pm], only the 50 mg/kg dose was effective [ovulation blocked in 60% and 17%, respectively]. Based on this, dosing in the current study may have been performed at the wrong time. Dr Cooper indicated that in his experience, the maximum effect of atrazine on catecholamines occurs around one hour after dosing.

The author concludes that these data indicate that the immature female rat is less sensitive than the adult with respect to reproductive function, in light of the fact that LH surge suppression and estrous cycle disruption occur at lower doses in the adult than were effective [according to the authors] in the present study. However, the current study does not provide LH data for comparison with the LH data in the adult rat, and there are no similar data on ova number and % ovulating in the adult female for comparison. Additionally, the current study was of limited duration [3 doses], and the studies where the low doses were found to affect LH surge and produce estrous cycle disruption in the adult were of longer durations [28 days and 6 months]. In a study in the adult female rat in which dosing was for a 3-day period [comparable to the current study], the LOAEL was 50 mg/kg/day [lowest dose tested], based on suppression of the estrogen-induced LH and prolactin surges. It is noted that this latter study was performed in ovariectomized SD rats.

The current study lacks performance criteria. The authors did not demonstrate a capability to induce ovulation in the immature female rat with the model utilized, as evidenced by the fact that the rate of ovulation in the control varied greatly throughout the study. The control group showed the lowest ovulation rate for 7 of the 14 weeks in which a control group was run. Additionally, the percent of control female groups displaying greater than or equal to 50% ovulation is only 57% compared to 100% in all but the 100 mg/kg/day dose group [71%]. Based on this, the lack of a positive control, and the questionable timing of treatment, no definitive conclusion regarding differences in response between the control and treated animals is possible.

For body-weight effects, the NOAEL is 10 mg/kg/day, based on decreased body-weight gain at the LOAEL of 50 mg/kg/day. Although inhibition of ovulation and a decreased number of ova in those females that ovulated were observed at 500 mg/kg/day compared to control, *no definitive LOAEL/NOAEL can be determined for these endpoints*. This is due to the fact that there are no data to support the selected time [10 am to 12 non] when the dose of atrazine was administered, and the apparent lack of an effect at lower dose levels may have resulted because exposure to atrazine did not occur during the critical period. The lack of a positive control and a failure to demonstrate the capability to consistently induce the immature female control rat to ovulate with the model used added difficulty to the interpretation of the data.

Discrepancies: There are several aspects of this study that diminish its usefulness. As noted above, there are no data to support the time [between 10 am and 12 noon] during which the atrazine dose was administered. The design of the study was not ideal, and the methods' section of the study report was difficult to follow with respect to procedure. Logistically, the use of so many animals requires that the study be conducted over an extended period of time. However, the study could have been more useful had fewer doses been run. For example, sets of 28 rats/week could have been divided equally [7 rats/group] among the control and three dose groups, and several weeks of data for these same groups could have been generated. As shown below [Tables A-D] and Table 5 above, there are only three instances in the current study in which data for more than one week are available for the same 3 atrazine dose groups. Additionally, the number of rats per group varied among the groups, as did the number of weeks each group was treated. The atrazine groups each consisted of 6 rats/week, while for 10 of the 14 weeks in which the control was included, only 3 control rats/week were used. The total number of rats used per group varied from 24, 30, 35, 42 in the atrazine groups to 67 in the control. It is noted that of the 14 weeks in which there was a control group, there were two weeks in which none of the control rats

ovulated, and three weeks in which only 33% of the control rats ovulated. In contrast, the high-dose group was dosed during only 4 weeks, but at least 50% of the rats ovulated in each of the 4 weeks.

The Individual Data Tables in the report for the 10 mg/kg/day [Table 6] and 50 mg/kg/day [Table 7] dose levels show the same mean and standard deviation for all of the body weight and body-weight gain data. Table 6 of the report contains the wrong means and standard deviations. The data listed in Summary Table 1 for 10 mg/kg/day should be the data in Table 6. In Table 2 of the report, the number of ova for each group is identical to the standard error of the mean (SEMs) for each group; e.g., the control is 14.9±14.9. In the Individual Data Tables [3-10], the mean number of ova for each group is the same as those listed in Table 2, but standard deviations (stds) [not SEM] are listed [all of which are different from the mean]. Table 9 of the report is paginated as Page 30 of 29 and Table 10 is paginated as Page 31 of 29.

Table A. Comparison of Ova Data by Week on Study [mean±std # ova (% ovulating)]

Week on study	Dose (mg/kg/day)			
	0	1	10	100
3	9.0±2.0 (100)	8.8±2.2 (67)	5.6±2.6 (83)	8.0±0.0 (33)
7	0 (0)	8.2±3.7 (100)	12.3±4.3 (67)	11.3±2.9 (50)
mean	9.0±2.0 (50)	8.4±3.1 (83)	8.6±4.8 (75)	10.0±2.7 (42)

weekly N=3 (control); 6 (atrazine)

Table B. Comparison of Ova Data by Week on Study [mean±std # ova (% ovulating)]

Week on study	Dose (mg/kg/day)			
	0	5	50	300
5	9.0±0.0 (33)	9.3±2.2 (100)	6.8±4.6 (67)	6.0±4.4 (50)
8	0 (0)	5.8±2.5 (67)	10.2±5.4 (83)	10.3±2.1 (60)♪
11	52±0 (33)	25.5±17.5 (67)	45.2±3.9 (83)	29.0±22.3 (100)
mean	15.9±16.1 (22)	12.4±11.8 (83)	23.6±19.5 (83)	18.6±18.7 (71)

weekly N=3 (control); 6 (atrazine) except ♪n=5

Table C. Comparison of Ova Data by Week on Study [mean±std # ova (% ovulating)]

Week on study	Dose (mg/kg/day)			
	0	5	50	500
16	8.5±3.5 (67)	7.3±2.5 (50)	10.0±3.0 (83)	8.7±2.5 (50)

weekly N=3 control; 6 atrazine

Table D. Comparison of Ova Data by Week on Study [mean±std # ova (% ovulating)]

Week on study	Dose (mg/kg/day)			
	0	1	100	500
13	35.3±4.9 (100)	26.8±10.6 (100)	18.6±7.9 (83)	10.7±4.6 (50)

weekly N=3 (control); 6 (atrazine)

Table E. Comparison of % Females with Ova by Week on Study

Week on study	Dose (mg/kg/day)							
	0	1	5	10	50	100	300	500
1	90	-	-	-	-	-	-	-
2	78	-	-	50	67	-	-	-
3	100	100	-	83	-	33	-	-
4	-	-	-	-	-	-	-	-
5	33	-	100	-	67	-	50	-
6	-	-	-	-	-	-	-	-
7	0	100	-	67	-	50	-	-
8	0	-	67	-	83	-	60	-
9	89	67	-	-	-	83	-	-
10	56	-	-	100	-	-	100	-
11	33	-	67	-	83	-	100	-
12	100	-	67	83	-	17	-	-
13	100	100	-	-	-	83	-	50
14	33	-	-	-	-	83	100	67
15	33	-	-	-	-	67	50	50
16	67	-	50	-	83	-	-	50
# ≥50% (%)	8/14 (57)	4/4 (100)	5/5 (100)	5/5 (100)	5/5 (100)	5/7 (71)	6/6 (100)	4/4 (100)
# <70% (%)	8/14 (57)	1/4 (25)	4/5 (80)	2/5 (40)	2/5 (40)	4/7 (57)	3/6 (50)	4/4 (100)
# ≤50% (%)	6/14 (43)	0	0	1/5 (20)	0	3/7 (44)	2/6 (33)	3/4 (75)

- group not included

DATA FOR ENTRY INTO ISIS

Special Ovulation Study in Immature Female - rats (non-guideline)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
080803	45711303	special/ ovulation	rats	3 days	oral	gavage	1-300	1, 5, 10, 50, 100, 300, 500	10	50		decreased body weight
080803	45711303	special/ ovulation	rats	3 days	oral	gavage	1-300	1, 5, 10, 50, 100, 300, 500	not determined	not determined	ovary	due to questionable timing of the dose [among other problems], the study is unacceptable; inhibition of ovulation/ decreased # ova

[ATRAZINE] T

Special Study [Female Sexual Maturation] (§ none)

EPA Reviewer: Linda L. Taylor, Ph.D. _____
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. _____ 10/16/02
Branch Senior Scientist, Reregistration Branch I, Health Effects Division (7509C)

TXR #: 0051031

DATA EVALUATION RECORD

STUDY TYPE: Special Study - [female rat]
DP BARCODE: D284705
P.C. CODE: **080803**
CAS Number: 1912-24-9
TEST MATERIAL (PURITY): Atrazine [98.2% a.i.]
SYNONYMS: ATZ
CHEMICAL: 2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine
SPONSOR: Syngenta Crop Protection, Inc.

OPPTS none [§ none]
SUBMISSION NO.: S

CITATION: Ashby, J. and Tinwell, H. (2002). The Effects of Atrazine on the Sexual Maturation of Female Alderley Park-Wistar and Sprague-Dawley Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Syngenta Number 1775-02; July 3, 2002. MRID 45722401. Unpublished.

EXECUTIVE SUMMARY: In a special study [MRID 45722401] that involved four separate experiments, 8-10 Alpk:ApfSD (Wistar-derived; AP) female rats/dose group and 8-10 Sprague-Dawley(SD) female rats/dose group [20-21 day old] were dosed once daily *via* gavage [10 mL/kg] with atrazine [98.2%], vehicle [carboxymethylcellulose (CMC)], or Antarelix™ [a centrally-acting GnRH antagonist] for up to 25 days. The dose levels of atrazine were 10, 30, and 100 mg/kg. Antarelix was dosed at 0.3 mg/kg. The first two experiments involved the determination of uterine weight on either postnatal day 30 [start of puberty] or on postnatal day 33, when uterine growth was assumed to have been completed.

Alpk:ApfSD (Wistar-derived; AP) female rats: Atrazine: ***Uterine growth***: There was a dose-related decrease in uterine weight following exposure to atrazine from postnatal day [PND] 22 to PNDs 29 [8 doses], 32 [11 doses], and 42 [21 doses]. At the high-dose level, the largest decrease in uterine weight was observed in the group receiving the 11 doses [PND 22-32], and the smallest decrease was observed following the longest exposure [PND 22-42]. The magnitude of the decrease in body weight [93% of control]/body-weight gain [87% of control] observed at the high-dose level following the 11-dose regimen does not account for the magnitude of the decrease in uterine weight [blotted 55%/dry 50% lower than control]. At the mid-dose level, the decrease in uterine weight [blotted 22%/dry 16% lower than control (PND 22-29); blotted 18%/dry 19% lower than control (PND 22-42)] was not statistically significant but is considered treatment-related. Body weight and body-weight gains of the mid-dose females were comparable to the control values. ***Vaginal opening [VO]***: At the high-dose level, there was a statistically-significant ,delay in VO [PND 41] compared to the control [PND 38] following the PND 22-42 dosing regimen. Antarelix: ***Uterine growth***: In comparison, the rats exposed to ANT showed a lack of uterine growth, and in contrast to the atrazine findings, the magnitude of the decrease in uterine weight increased with the increase in the duration of ANT exposure. There was no effect on body weight/body-weight gain. ***Vaginal opening***: None of the ANT females had an open vagina at study termination [PND 43].

Sprague-Dawley (SD) female rats: Atrazine: ***Uterine growth***: Decreased uterine weight was observed at the high-dose level following exposure to atrazine from PND 22-PND 45 [24 doses]. However, statistical significance was not attained, and the magnitude of the effect [blotted 13%/dry 11% lower than control] was slight, as was the body-weight deficit [4% lower than control]. ***Vaginal opening***: There was a statistically-significant delay in VO at the mid- [PND 41] and high-dose [PND 42] levels compared to the control [PND 39] following the PND 22-45 dosing regimen. In contrast to the AP females, a delay in vaginal opening was observed in the SD females at a dose level where uterine weight was not affected by treatment.

The NOAEL is 10 mg/kg/day, based on delayed vaginal opening [SD rats] and reduced uterine growth [AP rats] at the LOAEL of 30 mg/kg/day.

This nonguideline special study on female rat sexual maturation is classified Acceptable/non-guideline.

COMPLIANCE: Unsigned and undated GLP and Data Confidentiality statements were provided. The study was not performed according to GLP; however, it is stated that it was conducted in accordance with good and acceptable scientific practices. There is no Quality Assurance statement, but there is a Report Approval signature page, which is not signed or dated.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: atrazine
Description: not provided
Batch #: not provided
Purity: 98.2% a.i.
Stability of Compound: not provided
Source: Syngenta USA
CAS #: 1912-24-9

2. Positive control: Antarelix™
Description: not provided
Batch #: not provided
Purity: >98%
Stability of Compound:
Source: synthesized at Zeneca Central Toxicology Laboratory, UK

3. Vehicle: carboxymethylcellulose (CMC; sodium salt, medium viscosity); dissolved in sterile deionized water to give a final concentration of 0.5% (w/v)
Batch #: not provided
Source: Sigma Chemical Company, Poole, Dorset, UK

4. Test animals: Species: rat
Strain: female Sprague-Dawley (SD) and Alpk:ApfSD (Wistar-derived; AP)
Age at start of dosing: 20-21 days
Weight at start of dosing: 35-45 grams
Source: SD [Charles River UK]; AP [barriered animal breeding unit. (AstraZeneca, Macclesfield, UK]
Housing: housed up to 5 per cage
Diet: Rat and Mouse No. 1 [RM1; Special Diets Services, Ltd., Witham, Essex, UK], ad libitum
Water: ad libitum
Acclimation period: 24 hours

5. In-life dates: not provided.

B. PROCEDURES AND STUDY DESIGN

1. Study schedule and animal assignment: There are four parts to this study [Table 1]. In the first two experiments, uterine and body weights were determined on either postnatal day [PND] 30 (start of puberty) or on PND 33 (uterine growth assumed to have been completed) for the AP rats exposed to vehicle, atrazine, or ANT following either 8 or 11 doses, respectively. The third experiment involved dosing AP females with vehicle, atrazine, or ANT until completion of vaginal opening [VO] in the vehicle group, at which time the rats were terminated and uterine and body weights were determined

[PND 43]. The fourth experiment involved dosing SD rats with either vehicle or atrazine until completion of VO in the vehicle control group, at which time the rats were terminated [PND 46] and uterine and body weights were determined. The uterine weights were recorded 24 hours after 8, 11, or 21 daily treatments for the AP rats and 24 hours after 24 daily doses for the SD rats. In experiments 3 and 4, the females dosed for 21 [AP] or 24 [SD] days were monitored on a daily basis from the start of dosing for vaginal opening. The age at the start of VO and the body weight on that day were recorded for each female.

Table 1. Study Design

Experiment #/ strain/[N]/termination day	# of doses/compound/dose	parameters monitored		
		body weight	uterine weight	age at vaginal opening
Experiment 1 AP [8] PND 30	dosed on PND 22-29 (8 doses) CMC ATZ 10, 30, 100 mg/kg ANT 0.3 mg/kg	X	X	-
Experiment 2 AP [10] PND 33	dosed on PND 22-32 (11 doses) CMC ATZ 10, 30, 100 mg/kg ANT 0.3 mg/kg	X	X	-
Experiment 3 AP [10] PND 43	dosed on PND 22-42 (21 doses) CMC ATZ 10, 30, 100 mg/kg ANT 0.3 mg/kg	X	X	X
Experiment 4 SD [10] PND 46	dosed on PND 22-45 (24 doses) CMC ATZ 10, 30, 100 mg/kg ANT 0.3 mg/kg	X	X	X

PND postnatal day; [N] # of rats per group;

There were 8 female rats/group in the first experiment and 10/group in the other experiments. There was no information regarding randomization. The rats were dosed *via* gavage [10 mL/kg dosing volume] once daily for 8, 11, 21, or 24 days [see Table 1]. The objectives of the study were to test the equivalence in dosimetry reported for the effects of atrazine in peripubertal female to adult rats; and to compare the effects of atrazine treatment on the timing of uterine growth and VO in peripubertal AP rats. It appears that the rats were not individually identified. The individual data shows that rat numbers in both experiment 1 and 4 are 1-40, those in experiment 2 are 1-60, and those in experiment 3 are 1-50.

2. Dose selection rationale: No rationale was provided for the dose levels of atrazine, although the EXECUTIVE SUMMARY states that the studies were designed to compare the doses that interfere with GnRH signaling seen in previous studies [not identified] in adult Sprague-Dawley rats [LH surge suppression] with doses that impair GnRH signaling in peripubertal rats. The dose levels used in the previous 14-day (gavage), 28-day (diet), and 6-month (diet) studies were 100, 200, and 400/300 mg/kg/day; 2.5, 5, 40, and 200 mg/kg/day; and 1.8, 3.65, and 29.44 mg/kg/day, respectively.

3. Dosage preparation and analysis

Atrazine was homogenized in 0.5% CMC to give suspensions with the final concentrations of 10, 30, and 100 mg/10 mL [based on Laws, *et al.*, 2000], which were stored at room temperature. ANT was homogenized in 0.5% CMC to give a 0.3 mg/2.5 mL solution [based on Ashby, *et al.*, 2002] and stored at +4°C. All preparations were stirred continuously during dosing. There is no discussion of homogeneity, stability, or concentrations attained.

4. Termination of Study

The rats were terminated by an overdose of halothane followed by cervical dislocation [described in Ashby and Tinwell, 1998]. The uterus was removed, trimmed free of fat, gently blotted and weighed. It was then placed in a pre-weighed vial, dried for 24 hours at 70°C and re-weighed. In the second AP uterine weight experiment, a group of naive rats were terminated at the start of dosing in order to provide a baseline for uterine growth or its inhibition.

C. ANALYSES

D. DATA ANALYSIS

Statistical analyses: Body weights were considered by analysis of covariance on day 22 body weights. Uterine weights and developmental landmarks were considered by analysis of variance. Analyses were carried out separately for atrazine and Antarelix groups. All analyses carried out using SAS version 8.2.

II. RESULTS

A. UTERINE WEIGHTS AND BODY WEIGHTS

1. Experiment 1: **Alpk:ApfSD (Wistar-derived; AP) female rats**: There was a dose-related decrease in uterine weight [both blotted and dry] following atrazine exposure on PND 22-29 [termination PND 30], but statistical significance was not attained at either the low- [84%/89% of control (blotted/dry)] or mid-dose [78%/84% of control (blotted/dry)] level [Table 2]. Significantly decreased uterine weight was observed following Antarelix exposure also, and the magnitude of the effect [39%/53% of control (blotted/dry)] was greater than that observed at the high-dose atrazine level [59%/66% of control (blotted/dry)]. A comparison of the uterine weight [blotted 20.2 mg/dry 4.0 mg] of the naive controls [age PND 22 in Experiment 2] with the ANT uterine weights [blotted 23.1 mg/dry 5.2 mg] on PND 30 shows a lack of uterine growth. Terminal body weights were significantly decreased compared to the control [93% of control] at the high-dose level of atrazine, and body-weight gain was decreased also [86% of control]. Body weight and body-weight gain of the ANT rats were comparable to the control.

Table 2. Uterine Weights and Body Weights - AP Rats Dosed on PND 22-29 [terminated PND 30]					
Compound/Dose	mean uterine weight [mg]		mean body weight [gm]		body-weight gain √ PND 22-30
	blotted	dry	initial	terminal	
atrazine					
0	43.1±20.2	7.6±2.8	45.3±4.5	80.8±8.1	35.5
10	36.3±4.9 [84]♪	6.8±0.8 [89]	44.0±3.9	78.7±7.0	34.7
30	33.6±5.6 [78]	6.4±1.0 [84]	45.1±3.0	79.7±5.4	34.6
100	25.5±3.7** [59]	5.0±0.8** [66]	44.5±3.9	74.9±5.5** [93]	30.4 [86]
ANT					
0.3	16.9±2.8** [39]	4.0±0.7** [53]	44.8±3.0	81.7±6.5	36.1

Data from Table 1, page 17 of the report; (N=8); ♪ [% of control];* p<0.05; ** p<0.01
√ calculated by reviewer using data from Appendix Table 2, page 22 (no statistics performed)

2. Experiment 2: **Alpk:ApfSD (Wistar-derived; AP) female rats**: Decreased uterine weights were observed at the mid-[blotted 89%/dry 92% of control] and high-dose [blotted 45%/dry 50% of control] levels following atrazine exposure during PND 22-32 and at the dose level used for Antarelix [blotted 21%/dry 29% of control], but the mid-dose atrazine value did not attain statistical significance [Table 3]. A comparison of the uterine weight [blotted 20.2 mg/dry 4.0 mg] of the naive controls [age PND 22] with the ANT uterine weights [blotted 23.1 mg/dry 5.2 mg] on PND 33 shows a lack of uterine growth. Terminal body weights were significantly decreased compared to the control [93% of control] at the high-dose level of atrazine, and body-weight gain was decreased also [87% of control]. Body weight and body-weight gain were comparable [greater than] to the control in the ANT rats.

Table 3. Uterine Weights and Body Weights - AP Rats Dosed on PND 22-32 [terminated PND 33]					
Compound/Dose	mean uterine weight [mg]		mean body weight [gm]		body-weight gain √ PND 22-33
	blotted	dry	initial	terminal	
atrazine					
0	111.6±50.4	18.1±8.1	44.7±2.8	96.1±5.9	51.4
10	122.7±46.0	19.9±8.1	43.9±2.7	98.2±6.9	54.3
30	99.3±44.5 [89] ♪	16.6±7.1 [92]	44±3.3	96.3±8.2	52.3
100	50.3±8.7** [45]	9.0±1.7** [50]	44.6±2.7	89.4±3.9** [93]	44.8 [87]
ANT					
0.3	23.1±5** [21]	5.2±0.9** [29]	43.1±2.9	98.5±7.1*	55.4
Naive [PND 22]	20.2±2.7	4.0±0.4	45.4±3.3	-	-

Data from Table 1, page 17 of the report; (N=10); ♪ [% of control];* p<0.05; ** p<0.01
√ calculated by reviewer using data from Appendix Table 4, page 22 (no statistics performed)

3. Experiment 3: **Alpk:ApfSD (Wistar-derived; AP) female rats**: There was a dose-related decrease in uterine weights [both blotted and dry] following atrazine exposure to AP female rats during PND 22-42, but statistical significance was not attained for the blotted weights at any dose level or for the dry weights at either the low- or mid-dose level [Table 4]. Decreased uterine weight [blotted 13%/dry 15% of control] was observed following Antarelix exposure also, and the magnitude of the effect was greater than that observed at the high-dose atrazine level [blotted 79%/dry 77% of control]. Comparison of the naive control uterine weights on PND 22 to the uterine weights of the ANT females shows a lack of uterine growth. Terminal body weights were significantly decreased compared to the control [89% of control]

at the high-dose level, and body-weight gain was decreased also [85% of control]. Body weights and body-weight gains were comparable to the control for the ANT rats following all exposure durations.

Table 4. Uterine Weights and Body Weights - AP Rats Dosed on PND 22-42 [terminated PND 43]

Compound/Dose	mean uterine weight [mg]		mean body weight [gm]		body-weight gain √ PND 22-43
	blotted	dry	initial	terminal	
atrazine					
0	182.7±57.8	34.2±10.5	45.4±2.2	155.4±7.5	110
10	177.4±49.2 [97]	31.9±6.9 [93]	44.2±4.0	149.2±11.4	105
30	150.5±37.1 [82]	27.7±6.6 [81]	44.9±2.2	151.2±5.0	106.3
100	144.6±44.8 [79]	26.4±7.3* [77]	45.2±2.8	138.4±6.8** [89]	93.2 [85]
ANT					
0.3	22.9±2.6** [13]	5.1±1.0** [15]	44.8±1.9	160.1±11.2*	115.3

Data from Table 1, page 17 of the report; (N=10); ♪ [% of control];* p<0.05; ** p<0.01
√ calculated by reviewer using data from Appendix Table 6, page 30 (no statistics performed)

4. Experiment 4: **Sprague-Dawley (SD) female rats**: Decreased uterine weights [blotted 87%/dry 89% of control] were observed only at the high-dose level following atrazine exposure during PND 22-45, although statistical significance was not attained [Table 5]. ANT was not administered to the SD female rats for comparison, and no explanation was provided. There was a slight decrease in terminal body weight at the mid- and high-dose levels [96% of control] compared to the control and a slight decrease in body-weight gain [93%-94% of control].

Table 5. Uterine Weights and Body Weights - SD Rats Dosed on PND 22-45 [terminated PND 46]

Compound/Dose	mean uterine weight [mg]		mean body weight [gm]		body-weight gain √ PND 22-45
	blotted	dry	initial	terminal	
atrazine					
0	203.8±56.6	36.3±8.3	43.4±3.2	165.5±12.3	122.1
10	247.9±64.1	42.3±8.6	43.5±4.2	165.4±11.3	121.9
30	213.4±59.1	37.7±8.7	43.6±3.1	158.9±5.2* [96]	115.3 [94]
100	178.3±71.2 [87]♪	32.3±11.5 [89]	44.1±3.1	158.2±9.7* [96]	114.1 [93]

Data from Table 1, page 17 of the report; (N=10); ♪ [% of control];* p<0.05; ** p<0.01
√ calculated by reviewer using data from Appendix Table 8, page 33 (no statistics performed)

B. VAGINAL OPENING

1. **Alpk:ApfSD (Wistar-derived; AP) female rats**: At the high-dose level, there was a statistically-significant delay in the age at vaginal opening [VO] in AP females dosed from PND 22 to PND 42 [Table 6]. Although not significantly different than the control, it is noted that the standard deviation of the age at VO at the mid-dose level is large compared to the other dose levels and the number of mid-dose females greater than 40 days old at VO [3] was increased compared to the low and control groups [0].There was a statistically-significant decrease in terminal body weight [11%] at this dose level. However, body weight on the day of vaginal opening was slightly greater than the control body weight at VO at both the mid- and high-dose levels [Table 8], which one might expect since the atrazine females were older. Body-weight gains [Table 7] from the start of dosing [PND 22] until the day of vaginal opening were increased at the mid- and high-dose levels also. None of the ANT-dosed rats had an open vagina at study termination [PND 43]. Body weight was not affected in the ANT rats.

2. **Sprague-Dawley (SD) female rats**: There was a statistically-significant delay in the age at vaginal

opening in SD females dosed at the mid- and high-dose levels of atrazine from PND 22 to PND 45 compared to the control [Table 6]. Although there was a statistically-significant decrease in body weight at these dose levels, the magnitude of the decrease was slight [4%]. However, body weight on the day of vaginal opening was slightly greater than the control body weight at VO at both the mid- and high-dose levels [Table 8], which one might expect since the atrazine females were older. Body-weight gains [Table 7] from the start of dosing [PND 22] until the day of vaginal opening were increased at the mid- and high-dose levels also. ANT was not administered to SD female rats.

Table 6. Age at Vaginal Opening - PND

Strain/Dose [mg/kg/day]	AP Rats Dosed on PND 22-42 [terminated PND 43]			SD Rats Dosed on PND 22-45 [terminated PND 46]		
	mean	range	#>40	mean	range	#≥40
atrazine						
0	38±1.5	36-40	0	39±2.9	34-44	3
10	37±1.5	35-40	0	39±2.0	38-44	4
30	38±3.4	31-42	3	41.5±3.1*	35-45	8
100	41±1.5**	38-43	6	42±2.2**	38-45	8✖
ANT						
0.3	-√	-	-			

PND postnatal day; √ no ANT female had an open vagina at termination [PND 43];
ANT not administered to SD female rats; (N=10; except ✖ (9)): * p<0.05; ** p<0.01;
data from Tables 5-8, pages 28-33 of the report;

Table 7. Body-Weight Gain [PND 22-day of VO]

Dose [mg/kg/day]/Strain	AP	SD
atrazine		
0	80.7±9.2	86.9±12.7
10	74.1±10.0	88.1±13.9
30	82.5±21.4	95.7±14.3
100	82.9±5.0	96.6±14.4

Calculated by reviewer using data from Tables 5 and 7, pages 26-29 and 31-32 of the report
No statistics performed

Table 8. Age at VO and Body Weight on Day of VO				
Strain/Dose/rat #	AP rats [dosed PND 22-42]		SD rats [dosed PND 22-45]	
	body weight [g] on day of VO	day of VO	body weight [g] on day of VO	day of VO
atrazine				
0 mg/kg/day				
1	121	36	142	41
2	126	37	110	34
3	131	38	132	37
4	129	38	128	37
5	139	40	144	41
6	115	36	135	39
7	144	40	146	39
8	116	36	115	36
9	117	38	115	37
10	123	39	136	44
average BW at VO√	**126.1±9.8**		**130.3±13.0**	
10 mg/kg/day				
11	123	36	155	41
12	121	37	132	38
13	110	35	137	40
14	115	37	145	40
15	140	40	157	44
16	123	38	123	38
17	105	36	129	38
18	122	39	121	38
19	99.5	38	105	38
20	124	37	112	38
average BW at VO	**118.3±11.4**		**131.6±17.3**	
30 mg/kg/day				
21	121	37	160	45
22	127	39	157	43
23	140	41	139	41
24	130	38	134	40
25	121	37	141	43
26	118	37	152	45
27	133	39	117	35
28	145	42	114	38
29	79.4	31	140	42
30	159	43	139	43
average BW at VO	**127.3±21.0**		**139.3±15.2**	
100 mg/kg/day 1				
31	134	40	151	41
32	124	41	119	38
33	130	41	147	42
34	133	39	158	45
35	133	41	-♪	-
36	121	38	140	41
37	120	39	157	44
38	129	42	128	44
39	127	41	146	43
40	130	43	120	40
average BW at VO	**128.1±5.0**		**140.7±15.0**	

√ calculated by reviewer using data from Appendix Tables 5 and 7 [pages 28 and 32];
♪ female did not displayed VO on the last observation day [PND 46]

DISCUSSION

Alpk:ApfSD (Wistar-derived; AP) female rats: Atrazine: ***Uterine growth***: There was a dose-related decrease in uterine weight following exposure to atrazine from postnatal day [PND] 22 to PNDs 29 [8 doses], 32 [11 doses], and 42 [21 doses]. At the high-dose level, the largest decrease in uterine weight was observed in the group receiving the 11 doses [PND 22-32], and the smallest decrease was observed following the longest exposure [PND 22-42]. The magnitude of the decrease in body weight [93% of control]/body-weight gain [87% of control] observed at the high-dose level following the 11-dose regimen does not account for the magnitude of the decrease in uterine weight [blotted 55%/dry 50% lower than control]. ***Vaginal opening***: At the high-dose level, there was a statistically-significant delay in VO [PND 41] compared to the control [PND 38] following the PND 22-42 dosing regimen. Thirty percent of the mid-dose females and 60 percent of the high-dose females were greater than 40 days old at VO compared to zero percent in the control and low-dose groups. Antarelix: ***Uterine growth***: In comparison, the rats exposed to ANT showed a lack of uterine growth, and in contrast to the atrazine findings, the magnitude of the decrease in uterine weight increased with the increase in the duration of ANT exposure. There was no effect on body weight/body-weight gain. ***Vaginal opening***: None of the ANT females had an open vagina at study termination [PND 43].

Sprague-Dawley (SD) female rats: Atrazine: ***Uterine growth***: Decreased uterine weight was observed at the high-dose level following exposure to atrazine from PND 22-PND 45 [24 doses]. However, statistical significance was not attained, and the magnitude of the effect [blotted 13%/dry 11% lower than control] was slight, as was the body-weight deficit [4% lower than control]. ***Vaginal opening***: There was a statistically-significant delay in VO at the mid- [PND 41] and high-dose [PND 42] levels compared to the control [PND 39] following the PND 22-45 dosing regimen. In contrast to the AP females, a delay in vaginal opening was observed in the SD females at a dose level where uterine weight was not affected by treatment.

For the endpoint of vaginal opening, no delay was observed at the 10 mg/kg/day dose level in SD females following exposure to atrazine from PND 22 to PND 45. For the endpoint of uterine growth, no decrease in uterine weight was observed at 10 mg/kg/day dose level in AP females following exposure to atrazine from PNDs 22-29, PNDs 22-32, and PNDs 22-42.

Although there is a dose at which no effect was observed on body weight, uterine weight, or day of vaginal opening [10 mg/kg/day], the study is of limited scope. For example, there was only one exposure period [PND 22-45] examined in the SD females, and the exposure duration examined was similar to the one in the AP females where the least effect on uterine growth was observed. From the available data, it is not known whether an effect would have been observed on VO of the AP females dosed from PND 22-32, the duration of dosing that produced the greatest effect on uterine growth. Additionally, there are no cyclicity data provided on the females following vaginal opening, and there are no data on hormone/estrogen levels.

The results of the current study are consistent with those obtained in a previous study. In the previous study [Laws, *et al.*, 2000], delayed vaginal opening [Wistar] was observed at 50 mg/kg/day and above [NOAEL 25 mg/kg/day] compared to the control, although it is noted that the age at which VO occurred in the previous study was earlier [PND <35] than in the current study [PND≥35].

The authors conclude that the results of the current study demonstrate that the NOAEL for effects of atrazine on GnRH signaling in sexually immature rats is nearly an order of magnitude greater than the no-observed effect level in mature female rats and, therefore, the pituitary-hypothalamic axis in peripubertal female SD rats is less sensitive than in adult female SD rats. HED points out that the duration of the studies being compared by the authors differ; i.e., the immature female was dosed for 24 days and the adult female was dosed for 6 months. A more appropriate comparison would be the 28-day LH surge study in which adult female rats were dosed for 28

days [exposure duration more appropriate]. The NOAEL for LH surge suppression was 5 mg/kg/day and the LOAEL was 40 mg/kg/day. This comparison indicates comparable sensitivity between the adult and young SD female rat with respect to these endpoints.

Discrepancies: (1) Number of doses: In the EXECUTIVE SUMMARY of the report, it states that atrazine was administered daily from postnatal day 21 up to postnatal day 46, which is **26** exposures]. In the MATERIALS AND METHODS section it states that the rats received a daily dose for up to **25** days. In the Observation of Vaginal Opening section, it states that the females were exposed for **25** (SD rats) days, and in the Uterine Weight Determination section it states that uterine weights were recorded for SD rats following **24** daily exposures. (2) Dosing regimens: There was only one dosing duration [PNDs 22-45] for the SD females, whereas there were 3 durations [PNDs 22-29, PNDs 22-32, and PNDs 22-42] for the AP females. It is to be noted that the PND 22-45 interval is similar to the longest duration of exposure to the AP females [PND 22-42] where the least effect on uterine weight was observed. No explanation was provided for why shorter-duration exposures were not performed with the SD rats. Given the fact that VO was delayed in the SD female at the mid dose as well as the high dose [AP affected only at high dose], a shorter duration of dosing of the SD females might produce an effect on uterine growth at a lower dose level than was observed. Vaginal opening assessment: VO was monitored only following the longest exposure duration for both strains, but since this is the duration of exposure where the smallest effect was observed on uterine weight, it is not known whether VO would be delayed following a shorter dosing period.

DATA FOR ENTRY INTO ISIS

Special Sexual Maturation Study in Immature Female - rats (non-guideline)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
080803	45722401	special/sexual maturation	rats	up to 25 days	oral	gavage	10-100	10, 30, 100	10	30	uterus	delayed vaginal opening; reduced uterine growth

13544

056652

Chemical:	**Atrazine**
PC Code:	**080803**
HED File Code	**13000 Tox Reviews**
Memo Date:	**10/30/2002**
File ID:	**TX051031**
Accession Number:	**412-03-0019**

HED Records Reference Center
01/17/2003