DATA EVALUATION RECORD

ATRAZINE/080803

STUDY TYPE: NON-GUIDELINE - EFFECT ON LUTEINIZING HORMONE SURGE - RAT
MRID 45622309

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Task Order No. 02-52

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DATA EVALUATION RECORD
TXR#: 0050773

STUDY TYPE: Luteinizing Hormone Surge - Rat
Non-Guideline

PC CODE: 080803 **DP BARCODE:**D281938
SUBMISSION NO.: S612886

TEST MATERIAL (PURITY): Atrazine (97.1%),
Simazine (98.3%)
DACT (96.8%)

SYNONYMS: Atrazine - Atrex; 6-chloro-N-ethyl-N'-isopropyl-1,3,5-triazine-2,4-diamine
Simazine - CDT; Simadex; 2-chloro-4,6-bis(ethylamino-1,3,5-triazine
DACT; Diaminochlorotriazine; 6-chloro-1,3,5-triazine-2,4-diamine

CITATION: Minnema, D.J. (2002). 52-Week toxicity study of simazine, atrazine, and DACT administered in the diet to female rats. Covance Laboratories Inc., 9200 Leesburg Pike, Vienna, VA. Laboratory Identification Number 6117-399. February 21, 2002. MRID 45622309. Unpublished

Sielken, R.L. (2002). Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine, or DACT for Six Months: Statistical Analysis of the LH Surge: Supplemental Analysis. Sielken and Associates Consulting, Inc., Bryan, TX. Laboratory Identification Number 6117-399. March 4, 2002. MRID 45629402. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: A study was done to evaluate the effects of atrazine (Lot No. SG8029BA10, purity 97.1%), simazine (Lot No. SG202028GB10, purity 98.3%), or diamino-chlorotriazine (DACT) (Lot No. GP-720301, purity 96.8%) treatment on the estrous cycle and luteinizing hormone (LH) surge in response to exogenously administered estrogen to female rats after ~ 6 months of treatment and on the female reproductive organs after 52 weeks of treatment (MRID 45622309). For six months, female Sprague-Dawley Crl:CD®(SD)IGS BR rats received atrazine at 1.8, 3.4, 4.9, and 29.1 mg/kg/day (25, 50, 70, and 400 ppm), simazine at 1.6, 3.2, 4.6, 26.8 mg/kg/day (23, 47, 66, or 374 ppm), and DACT at 1.2, 2.4, 3.4, and 19.7 mg/kg/day (17, 34, 48, or 270 ppm). During the overall study, high-dose animals received 28.2, 25.9, and 18.8 mg/kg/day of atrazine, simazine, and DACT, respectively.

High doses of both atrazine and simazine decreased total body weight by the second week of the study and remained ~85% of control through the remainder of the study. Total body weight gain of female rats in the high-dose treatment groups of atrazine and simazine were ~70% of control at weeks 29 and 52. Treatment with high doses of DACT and with lower doses of atrazine and simazine had relatively little impact of total body weight and body weight gain. In contrast, food consumption of high-dose atrazine and simazine treated rats was essentially unaffected by treatment, suggesting food efficiency was decreased in these two high-dose groups. Food consumption was also not affected by treatment for female rats in lower dose atrazine and simazine groups, or in any dose groups treated with DACT.

No significant effect of treatment with high doses of atrazine, simazine, or DACT were apparent in vaginal smears assessing percent days in diestrus or estrus, or percent days in diestrous or estrous blocks. Six months after treatment of female rats with equal molar doses of atrazine or simazine, no effect on LH_{max} , LH AUC and $Time_{max}$ was found. However, LH_{max} and LH AUC were decreased in female rats treated for 6 months with high doses of DACT. Time to LH_{max} was unaffected by treatment with DACT. Similarly, when the concentration of plasma LH was analyzed by observation time, it was found that high-dose DACT decreased LH surge.

No macroscopic or microscopic treatment-related effects were found in tissues of the reproductive tract of female rats and no increase in brain weight was found. The incidence of pituitary adenomas was slightly increased in all three high-dose test material groups relative to the controls. In addition, the incidence of mammary carcinoma was increased in the high-dose DACT treatment group rats. The significance of these increases is uncertain since they were generally within the background percent incidence found in female rats of this strain.

In summary, atrazine and simazine behaved similarly in this study (i.e., no effect on LH surge), while DACT suppressed the exogenously stimulated LH surge. However, confidence in this study is low. Based on historical control data, the induction of LH surge in control animals appears to be suboptimal. Rats exhibited an infection that may have impacted LH induction. In addition, estradiol data were not submitted to evaluate LH induction response. Also, in a separate study (MRID# 44152102), atrazine had a significant effect on LH surge (LOAEL = 3.65 mg/kg/day; NOAEL = 1.8 mg/kg/day). A definitive effect on LH surge could not be determined, given the deficiencies of the study.

Based on decreased body weight and body weight gain, the systemic LOAELs for female rats treated with atrazine or simazine are ~29 mg/kg bw/day for atrazine and ~26.4 mg/kg bw/day for simazine. The corresponding NOAEL is 4.9 mg/kg bw/day for atrazine and 4.6 mg/kg bw/day for simazine. The systemic LOAEL for DACT is > 19.3 mg/kg bw/day and the systematic NOAEL for DACT is ~19.3 mg/kg bw/day.

A definitive effect on LH surge could not be determined, given the deficiencies of the study.

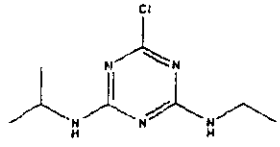
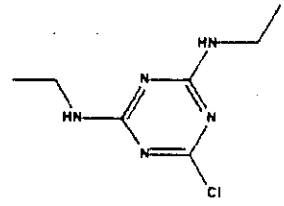
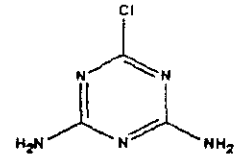
This LH surge study in the rat is **Unacceptable/Non-guideline** and does not satisfy the intent of investigating the effect of atrazine, simazine, and DACT on the LH surge of the female SD rats. A definitive effect on LH surge could not be determined, given the deficiencies of the study.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

	Atrazine	Simazine	DACT
Description:	white powder	white powder	white powder
Lot/Batch #:	SG8029BA10	SG202028GB10	GP-720301
Purity:	97.1%	98.3%	96.8%
Compound Stability:	1 year	6 years	1 year
CAS # if TGAI:	1912-24-9	122-34-9	3397-62-4
Structure:			

2. Vehicle and/or positive control: Diet for study; sesame oil for replacement hormone

3. Replacement Hormone: Estradiol, purity 100%, Lot No. 77H37751

4. Test animals:

Species:	rat
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR
Sex:	female
Age/weight at study initiation:	young adult, 167-216 g
Source:	Charles River Laboratories, Raleigh, NC
Housing:	individually in hanging stainless steel cages
Diet:	Harlan Teklad Certified Rodent Diet, *ad libitum*
Water:	tap, *ad libitum*
Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air Changes:	10/hour
Light Cycle	14/10 light/dark
Acclimation period:	2 weeks

B. STUDY DESIGN:

1. In life dates: Begin - 08/24/1999; End - 09/06-07/2000

2. Animal assignment: Rats were assigned to the groups shown in Table 1 by using a computerized block randomization procedure based on body weight.

TABLE 1: Study design

Group	Test Material	Dietary Conc. (ppm)	Dose (mmoles/ kg feed)	# Females	
				Interim sacrifice (29 weeks)	Terminal sacrifice (52 weeks)
1	Control	-	-	32	50
2	Atrazine	25	0.116	16	-
3		50	0.232	16	-
4		70	0.325	16	-
5		400	1.854	16	20
6	Simazine	23	0.116	16	-
7		47	0.232	16	-
8		66	0.325	16	-
9		374	1.854	16	20
10	DACT	17	0.116	16	-
11		34	0.232	16	-
12		48	0.325	16	-
13		270	1.854	16	20

Data from p. 17, MRID 45622309

3. **Dose selection rationale:** Atrazine was used as the standard to set doses for simazine and DACT. The maximum tolerated dose for atrazine in the diet was 400 ppm based on the reduction of the luteinizing hormone (LH) surge occurring during normal cycling in the young female SD rat. The other dietary concentrations (25, 50, and 70 ppm) were chosen to demonstrate a no-observed effect level for LH surge, estrus cycle, and possibly tumorigenic effects. The dietary concentrations for simazine and DACT were chosen as molar equivalents.

4. **Diet preparation and analysis:** Diets were prepared weekly and adjusted to 100% chemical purity. The diets were prepared by adding the appropriate amount of test material to ~200 g diet and blending in a Waring blender until homogenous. The diets were mixed for ~1 minute/kg/10 kg (or ~10 minutes for batches <10 kg). Two samples/test material were taken from each mixed batch and stored at -20°C until analysis by HPLC or gas chromatography. Duplicate homogeneity analyses were done on the low- and high-dose diets for each test material. Diet stability analyses from the low- and high-dose levels of each test material were done to determine 10-day stability.

Results:

Homogeneity: The concentration of the three test materials were 97-103% of nominal in the top, middle, and bottom of all prepared diets.

Stability: All diets containing the test materials were stable for at least 10 days from preparation.

Concentration Analyses: All test material concentrations were within ± 10%.

5. **Statistics:** ANOVA was used for body weight, average body weight change, and mean food consumption. Statistical analyses for all treatment groups were compared against control, and in addition against the various test material groups. If data were heterogenous, it was rank transformed. If this failed to achieve homogeneity, ANOVA was still done.

   LH surge was assessed by: 1. Maximum increase in LH over baseline (LH_{max}); 2. time to peak LH surge (T_{max}); and area-under–curve for LH vs time (AUC).

   Multiple linear regression for each of the treatment materials was done on the scored daily vaginal smears for: 1. percentage of days in diestrus (% days D); 2. percentage of days in estrus (% days E); 3. percentage of days in diestrous blocks (% Days D-block [4 consecutive days in diestrous]); and 4. percentage of days in estrous blocks (% days E-blocks [2 consecutive days]). The control group was used as the 0 ppm group. Dose and week were used as the independent variables.

C. METHODS

1. **Cageside Observations:** The rats were observed twice daily for morbidity and moribundity. Body weights and food consumption were recorded weekly

2. **Estradiol Implantation:** The implants were prepared by slicing medical grade silastic silicon tubing into 20 mm lengths. Teflon beading (5 mm) was inserted into the tubing, made flush, and glued into place. Estradiol benzoate (4 mg/mL sesame oil) was inserted into the open end of the tubing followed by small cut sections of untrimmed Teflon beading that were cut flush with the end and sealed with adhesive.

3. **Determination of Cycling:** Daily vaginal smears following lavage with 0.9% saline were taken for 14 consecutive days every 4 weeks beginning on Day 1 through the end of the study. The lavage material was spotted on a glass slide, stained with toluidine blue and allowed to air dry. The smears were examined and scored as proestrus, estrus, or diestrus. The percent number of days spent in estrus was determined for each rat/group/treatment/time interval and the group mean and standard error of the mean determined. In addition, the percent of rats in each dose group that spent 7 or more days in estrous during each interval was calculated and the proportion of rats with normal estrous cycles determined. For the purposes of the study the estrous cycle was considered abnormal if 2 or more consecutive days were spent in either proestrous or estrus or 4 or more consecutive days were spent in diestrous.

4. **LH Surge:** Beginning on week 29, interim-sacrifice rats were ovariectomized. Because of the large number of rats involved, the ovariectomies were staggered over a period of 8 days. Six days after the ovariectomy, each rat was given an estradiol implant and 3 days later blood was collected to determine plasma LH. Blood was collected at 6 intervals during the day: 8, 11, and 13 hours after the lights were turned on, and 1, 3, and 5 hours after the lights were turned off. The plasma samples were stored at -70°C until time of analysis.

5. Necropsy: All rats found dead, killed *in extremis*, or sacrificed on schedule were subjected to gross pathological examination. After collection of the final blood sample, interim sacrifice

rats were weighed and sacrificed. After 52-weeks of treatment, the remaining surviving rats were weighed, sacrificed, and exsanguinated. The following tissues and organs were collected from all rats: mammary masses, mammary tissue, uterus, ovaries, vagina, brain (including hypothalamus), salivary glands, a section of skin with fur, and miscellaneous lesions.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed.

2. **Mortality:** No treatment-related effects on survival were observed.

B. BODY WEIGHT AND WEIGHT GAIN: Body weights for the three treatment regimens are shown in Table 2 and body weight gains are shown in Table 3. Mean body weight in the high dose group for all three test chemicals was lower than control throughout the study, with an apparent order of atrazine ≈ simazine >> DACT. High-dose atrazine and simazine consistently significantly decreased body weight throughout the study, while DACT significantly decreased body weight only up to week 44. These differences in body weight were present from the second week. Female rats receiving the mid high-dose of atrazine and simazine also had statistically significant decreases in body weight; however, the decreases were less than 6%. As would be expected, the body weight gain of female rats receiving the high dose of atrazine and simazine was also significantly decreased at 29 and 52 weeks. DACT significantly decreased body weight gain at week 29, but not week 52.

TABLE 2. Mean gram body weight (± SD) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]

Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1	193±8.8	192±10	193±10	191±8	192±8	190±9	191±11	193±10	192±8	193±8	192±7	192±9	192±9
2	214±11	212±10	212±12	210±14	204±8* (-5%)[b]	208±12* (-3%)	208±15	209±12	204±11* (-5%)	214±10	212±9	213±9	206±11* (-4%)
10	294±22	281±21* (-4%)	289±22	281±18* (-4%)	262±17* (-11%)	284±27	280±28* (-5%)	275±21* (-6%)	259±22* (-12%)	286±17	282±22* (-5%)	294±17	273±17* (-7%)
20	331±30	308±27* (-7%)	316±29	310±24* (-6%)	285±21* (-14%)	316±29	310±42* (-6%)	300±26* (-9%)	286±32* (-14%)	324±30	316±29	328±23	309±25* (-7%)
29	341±32	320±33* (-6%)	328±27	321±24* (-6%)	298±24* (-13%)	328±31	321±42* (-6%)	313±31* (-8%)	296±31* (-13%)	334±31	326±32	336±20	319±26* (-6%)
40	364±34	–	–	–	311±33* (-15%)	–	–	–	313±42* (-14%)	–	–	–	342±34* (-6%)
53	391±45	–	–	–	321±34* (-18%)	–	–	–	334±51* (-15%)	–	–	–	376±43 (-4%)

[a]Data from Table 4, pages 80-91 of MRID 45622309
[b]Results in parentheses are percent change relative to control
*$p \leq 0.05$

TABLE 3. Average body weight gain (g) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]													
Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1-29	148±27	126±23* (-15%)[b]	135±27	130±18	106±24*** (-28%)	136±24	125±30	114±29**	104±28*** (-30%)	137±37	124±36*	141±21	124±19*** (-16%)
1-52	199±43	–	–	–	129±32*** (-35%)	–	–	–	142±48*** (-29%)	–	–	–	185±40 (-7%)

[a]Data from Table 5, pages 92-105, MRID 45622309
[b]Results in parentheses are percent change relative control
*p≤0.05, **p≤0.01, ***p≤0.001 calculated by reviewer

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption**: In contrast to the significantly decreased body weights of rats treated with high doses of atrazine or simazine, food consumption of the high dose-group was only minimally decreased (≤12%) in these groups (Table 4). The decrease in food consumption of rats treated with high-doses of DACT was less (<7%). No significant treatment-related effects were found for the mid-high, mid, and low doses of atrazine, simazine, or DACT.

TABLE 4. Average food consumption (g) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]													
Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1	135±12	133±12	134±11	132±12	124±11* (-8%)	133±13	125±11* (-7%)	130±12*	122±9* (-10%)	134±14	134±12	136±12	127±11* (-6%)
2	145±15	141±12	137±13* (-6%)	136±14* (-6%)	133±15* (-8%)	138±11	132±13* (-9%)	136±11* (-6%)	128±9 (-12%)	140±13	140±15	140±15	135±10* (-7%)
10	136±14	131±13	131±17	134±13	131±16	130±13	127±13* (-7%)	129±10	124±14* (-9%)	128±15* (-6%)	127±13* (-7%)	139±15	136±10
20	144±17	137±19	142±17	137±12	133±15* (-8%)	136±15	132±18* (-8%)	134±12* (-7%)	132±18* (-8%)	145±18	138±20	147±16	145±14
29	148±21	144±24	139±26	142±17	137±17* (-7%)	139±17	135±18* (-9%)	137±18* (-7%)	134±17* (-9%)	140±25	138±21	149±23	149±16
40	146±17	–	–	–	137±18	–	–	–	138±20	–	–	–	147±14
52	153±16	–	–	–	144±25	–	–	–	144±16	–	–	–	152±14
1-52	7283	–	–	–	6928 (-5%)	–	–	–	6776 (-7%)	–	–	–	7181 (-1%)

[a]Data from Table 6, pages 106-118 of MRID 45622309
[b]Results in parentheses are percent of control
*p≤0.05

2. **Food efficiency**: Food efficiency was not reported in the study report; however, with the decreased body weights of high-dose atrazine and simazine rats and relatively normal food intakes for these groups, food efficiency would be decreased.

3. **Compound consumption**: Average compound consumption for the three test materials is shown in Table 5.

TABLE 5. Average compound consumption (mg/kg bw/day) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]

Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1 - 29	0	1.8	3.4	4.9	29.1	1.6	3.2	4.6	26.8	1.2	2.4	3.4	19.7
1 - 52	0	–	–	–	28.2	–	–	–	25.9	–	–	–	18.8

[a]Data from page 29 of MRID 45622309

D. ESTROUS CYCLE DETERMINATIONS:

Estrous cycles did not appear to be altered by any of the treatment compounds at any dose, as shown in Table 6 and Appendix 1. There were also no treatment-related effects on percent days in diestrus, percent days in diestrous blocks, or % days in estrous blocks (Appendix 1).

TABLE 6. Percent Days in Estrus [a]

Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1-2	22±5.1	22±3.6	21±5.8	21±6.0	23±4.6	21±4.1	26±4.4	23±4.1	23±5.8	22±4.9	23±5.4	22±4.3	22±4.1
5-6	23±4.3	23±3.2	23±3.2	23±5.3	24±8.7	22±3.4	24±7.3	23±4.1	22±4.8	24±4.4	21±5.5	23±3.9	26±9.4
9-10	24±5.0	24±3.6	33±27	33±25.1	28±17.1	22±4.4	26±4.3	28±11.2	28±12.3	24±4.4	24±3.4	22±4.4	25±14.7
13-14	31±18.2	34±25	34±27	33±26.5	34±26.8	34±26.7	25±4.5	28±14.8	38±28.0	29±17.7	22±1.8	28±17.9	32±23.1
17-18	37±30	40±32	43±34	37±26.6	46±35	41±36.3	40±29.3	44±28.6	42±31.9	40±31.7	27±20.6	36±29.8	41±31.7
21-22	47±34	49±33	48±36	41±30.4	53±34.4	50±36.0	53±33.0	51±31.7	51±35.4	50±35.6	34±27.5	46±36.8	50±32.2

[a]Data from Appendix 12, pages 1142-1147 of MRID 45622309
*$p \leq 0.05$

E. LUTEINIZING HORMONE:

As shown in Table 7, the evaluation of LH_{max}, AUC and T_{max} indicated that atrazine and simazine did not produce a treatment-related effect on LH surge, as compared to control. However, DACT at 270 ppm significantly decreased LH_{max} and AUC, but not T_{max}. Although not treatment-related, DACT at 34 ppm was found to increase LH_{max} and AUC. Similar treatment-related effects of DACT at the high dose (270 ppm) on LH surge are also found when analyzing the concentration of LH (ng/ml) at the observation time (Table 8). DACT significantly decreased the LH surge at 270 ppm, as indicated by the decrease in LH surge at 1400.

TABLE 7. Average LH_{max}, AUC, and T_{max} of female rats that received atrazine, simazine, or DACT for 29 weeks[a]

Dose (ppm)	No. Rats	LH_{max} (ng/mL)	AUC (ng-hr/mL)	T_{max} $(hr)^{-1}$
Control	26	1.872	5.370	14.385
Atrazine				
25	11	2.187	7.725	14.273
50	15	2.959	7.213	14.000
70	15	2.443	7.049	13.933
400	15	2.371	6.941	13.600
Simazine				
23	16	1.701	4.468	13.688
47	16	2.870	8.051	14.625
66	14	2.054	5.866	13.357
374	15	1.611	3.332	13.333
DACT				
17	13	3.361	8.866	13.846
34	12	3.618*	12.764*	13.083
48	14	2.496	6.355	14.643
270	16	0.750*	0.807*	13.375

Data from Table 10, p. 134, MRID 45622309
*$p \leq 0.05$

Table 8. Group Mean (±SD) Plasma LH (ng/ml)*

Dose group	Time				
	900	1200	1400	1600	1800
Control					
0	0.52±.46	0.93±.94	1.76±1.8	1.29±.95	0.84±1.16
Atrazine					
25	0.53±0.40	1.27±1.5	2.1±2.1	1.87±2.09	0.54±.34
50	0.63±.61	1.20±1.38	2.46±2.5	1.5±2.1	1.05±1.6
70	0.51±.53	1.14±.67	2.41±2.8	1.3±1.17	0.61±.53
400	0.56±.53	0.94±1.25	2.61±4.7	1.45±2.3	0.69±.67
Simazine					
23	0.73±.89	1.69±1.5	1.07±.88	1.30±1.3	0.95±.97
47	0.43±.51	0.85±.78	1.97±2.2	1.99±2.1	1.22±1.4
66	0.58±.48	1.24±.96	2.26±2.6	0.10±.91	0.58±.59
373	0.70±.80	1.62±2.6	0.94±1.5	1.07±1.2	0.50±.56
DACT					
17	0.41±.40	1.01±1.3	2.22±3.7	2.27±2.8	0.40±.26
34	0.77±.78	3.04±2.4	2.87±2.9	2.39±2.4	0.38±.28
48	0.71±.67	0.85±.79	2.08±1.3	2.37±2.2	0.65±.75
270	0.60±.65	0.79±.73	0.74±.56*	0.67±.52*	0.52±.51

* Data obtained from pages24-28, MRID 45269402???? (check this MRID)

F. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** No treatment-related effects on brain weight, the only organ weighed at interim sacrifice, were found for female rats of the three treatment groups relative to control rats.

2. **Gross pathology:** No treatment-related effects were found in female rats of the three treatment groups at interim or final sacrifice, or in rats of unscheduled deaths.

3. **Microscopic pathology:** None of the three test materials induced treatment-related lesions in the reproductive organs examined. The incidence of pituitary adenomas in rats receiving the high-dose of the three test materials was increased relative to control rats (Table 9) and was significantly increased (as calculated by reviewer) for female rats in the DACT treatment group. However, the percent incidence was less than the spontaneous incidence of ~23% for rats of this species at 12-13 months of age. No increase in pituitary adenomas was found at lower doses of the three test materials. The incidence of mammary carcinoma was also

increased in female rats treated with a high dose of DACT, an incidence slightly greater than the maximum spontaneous increase of ~10% found in female rats of this species and age. No increase in mammary lesions were found in the lower-dose groups.

TABLE 9. Total incidence of selected neoplastic lesions found in female rats treated up to 52 weeks with high doses of atrazine, simazine, or DACT

Treatment (ppm)	No. Rats examined	Pituitary adenoma	Mammary Carcinoma	Mammary Fibroadenoma
Control (0)	82	7 (9%)*	4 (5%)	2 (2%)
Atrazine (400)	36	7 (19%)	0	0
Simazine (373)	36	5 (14%)	3 (8%)	0
DACT (270)	36	8 (22%)*	6 (17%)*	2 (6%)

Data from Table 19, p. 180-185, MRID 45622309
*Percent incidence
*p≤0.05 by Fishers exact test calculated by reviewer

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that female SD rats exposed to 270 ppm DACT in their feed for 29 weeks had decreases in LH surge (LH_{max} and AUC). A similar effect was not found in female rats treated with equal molar doses of atrazine or simazine. In addition, female rats treated with 270 ppm DACT had an increased incidence of pituitary adenomas, mammary carcinomas and mammary fibroadenomas. A slight increase in the incidence of pituitary adenomas were also found in female rats treated with high-doses of atrazine or simazine.

B. REVIEWER COMMENTS: In this study, female rats were treated with equal molar doses of atrazine, simazine, or DACT for a period of ~29 weeks and 52 weeks. High doses of both atrazine and simazine decreased total body weight by the second week of the study. The body weights of these two high-dose groups continued to decrease through week 10, where they remained ~85% of control through the remainder of the study. Total body weight gain of female rats in the high-dose treatment groups of atrazine and simazine were ~70% of control at weeks 29 and 52. Treatment with high doses of DACT and with lower doses of atrazine and simazine had relatively little impact on total body weight and body weight gain. In contrast, food consumption of high-dose atrazine and simazine treated rats was essentially unaffected by treatment, indicating food efficiency was decreased in these two high-dose groups. Food consumption was also not affected by treatment for female rats in lower dose atrazine and simazine groups, as well as all dose groups treated with DACT.

No significant effect of treatment with high doses of atrazine, simazine, or DACT were apparent in vaginal smears assessing percent days in diestrus or estrus, or percent days in diestrous or estrous blocks. A slight marginally significant effect noted for percent days in diestrous blocks of female rats treated with DACT was consistent with variability in response over time, particularly by rats treated with 34 ppm DACT over time. This response can be ruled out as treatment-related since the effect was not of the magnitude expected and showed no dose-related trend. Therefore, the reviewer considers treatment

with atrazine, simazine, and DACT as having no biologically significant effect on the estrous cycle.

Six months after treatment of female rats with equal molar doses of atrazine or simazine, no effect on the LH surge was found. However, LH_{max} and LH AUC were decreased in female rats treated for 6 months with the high dose of DACT. Time to LH_{max}, was not affected by treatment. However, confidence in this study is low. Based on historical control data, the induction of LH surge in control animals appears to be suboptimal. Rats exhibited an infection that may have impacted LH induction. In addition, estradiol data were not submitted to evaluate LH induction response. Also, in a separate study (MRID# 44152102), atrazine had a significant effect on LH surge (LOAEL = 3.65 mg/kg/day; NOAEL = 1.8 mg/kg/day). A definitive effect on LH surge could not be determined, given the deficiencies of the study.

No macroscopic or microscopic treatment-related effects were found in tissues of the reproductive tract of female rat and no increase in brain weights was found. However, the incidence of pituitary adenomas was increased in all three test material high-dose groups relative to the control group. In addition, the incidence of mammary carcinoma was increased in the high-dose DACT treatment group.

Based on decreased body weight and body weight gain, the systemic LOAELs for female rats treated with atrazine or simazine are ~29 mg/kg bw/day for atrazine and ~26.4 mg/kg bw/day for simazine. The corresponding NOAEL is 4.9 mg/kg bw/day for atrazine and 4.6 mg/kg bw/day for simazine. The systemic LOAEL for DACT is > 19.3 mg/kg bw/day and the systematic NOAEL for DACT is ~19.3 mg/kg bw/day.

A definitive effect on LH surge could not be determined, given the deficiencies of the study.

C. **STUDY DEFICIENCIES:** During weeks 21-23, many rats in all groups had an SDAV (Sialodacryoadenitis virus) infection. The effects of the infection included reduced food consumption, body weight loss, and ocular discharge. Because of the infection, the interim sacrifice was delayed from week 26 to week 29 to allow recovery from the infection. The study authors state that the impact of the infection on the LH surge is unknown. Infection may have impacted LH induction Based on historical control data, the induction of LH surge in control animals appears to be suboptimal. In addition, estradiol data were not submitted to evaluate LH induction response. Also, in a separate study (MRID# 44152102), atrazine had a significant effect on LH surge (LOAEL = 3.65 mg/kg/day; NOAEL = 1.8 mg/kg/day).

DATA FOR ENTRY INTO ISIS

Non-guideline - Rodent

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	45622309	Non-guideline	rat	up to 52 weeks	oral	feed	0 - ~30	0, 1.8, 3.4, 4.9, ~29 atrazine 0, 1.6, 3.2, 4.6, ~26.4 simazine 1.2, 2.4, 3.4, ~19.3 DACT	4.9 atrazine 4.6 simazine 3.4 DACT	~29 atrazine ~26.4 simazine 19.7 DACT	Bdy wt Atra-zine & simazine Pituitary - DACT	None

APPENDIX 1

DATA FROM PAGES 46-51, MRID 45622309

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