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

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

JUL 28 1997

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cancer Assessment Review Committee Meeting on Atrazine

FROM: Jess Rowland, M.S. Jess Rowland 7/28/97
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addresses

Attached for your review is a package on Atrazine prepared by Dr. Melba Morrow.

A meeting to review the carcinogenicity classification of this chemical is scheduled for **Tuesday August 12, 1997**, at **10:00 am in Room 817, CM2.**

Attachments 6 and 8 will be distributed at a later date.

Addressees

K. Baetcke
L. Brennecke
W. Burnam
M. Copley
T. Crisp
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
P. Hurley
Y. Ioannou
N. McCarroll
H. Pettigrew
E. Rinde
J. Rowland
J. Stewart
L. Taylor
Y. Woo

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

JUL 24 1997

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Fifth Carcinogenicity Peer Review for Atrazine

FROM: Melba S. Morrow, D.V.M.
Registration Action Branch I
Health Effects Division

TO: Jess Rowland, Ph.D.
Executive Secretary, Cancer SARC
Science Analysis Branch
Health Effects Division

Attached is a copy of the Weight of the Evidence Document for the fifth carcinogenicity peer review for Atrazine.

The purpose of this document is to present new data and supporting documentation provided by Ciba Geigy that address the mechanism of carcinogenesis.

The Cancer SARC is asked to evaluate the new information and to classify the chemical accordingly.

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Table I
Serum LH (pg/mL) Concentrations Following Atrazine Administration

Dose (mg/kg)	Sample Time (Hrs)					
	1100	1400	1600	1800	2000	2200
0						
mean	998	1122	3315	5138	2242	761
range	260/2116	497/2593	461/9717	744/13910	716/7027	455/1306
S.D.	614	564	693	4403	1850	288
2.5						
mean	943	1171	2951	4489	1118	486
range	260/1847	262/3032	992/5739	1366/18381	463/1959	260/1925
S.D.	614	802	1315	4345	412	138
5.0						
mean	1140	882	3099	2804	1554	508
range	260/2682	260/3701	420/10408	158/5068	370/5097	164/4731
S.D.	715	925	2521	1345	??	317
40.0						
mean	1219	1125	3518	3246	1740	689
range	385/1681	232/3514	583/17932	292/7181	403/4240	98/1185
S.D.	467	795	4514	1981	1157	373
200.0						
mean	873	1099	1685	2752	1853	1126
range	260/2003	168/3476	250/11844	413/10173	220/4389	374/2888
S.D.	655	863	2962	3137	1139	816

Discussion of the Data: Several of the assumptions made by the registrant in support of the LH mechanism have not been fully investigated investigated in this study and the results are inconclusive. Specific flaws in the LH study include the lack of comparison of the LH surge observed in atrazine treated rats to that observed in normal, untreated middle aged (10 -15 months) rats; failure to demonstrate unovulated or abnormal ovarian follicles either grossly or histologically; failure to provide data that demonstrated a stimulation of prolactin; large standard deviations that in some instances exceed the mean values and indicate a high degree of variability in the data; and the failure to demonstrate or correlate the LH findings to promotion of mammary tumor growth due to the short duration of the study. A critique of the study is provided in the attached May 30, 1996 memo from T.M. Crisp to E. Francis, K. Hammerstrom, K. Baetcke and M. Morrow. (The memo is Attachment 1 to this weight of the evidence document).

In addition to the flaws in the LH study, it is understood that LH is under the control of the gonadotropin releasing hormone (GnRH) that is released by the anterior pituitary. No links or assessments have been conducted to determine the role of the pituitary hypothalamic tract with regard to the hormonal perturbations that are supposedly occurring in these aging rats. Furthermore, if LH is controlled at this site (anterior pituitary) in the body, the changes in this hormone could be the result of an effect at the site of hormonal control.

ii. Evaluation of the LH Surge in Atrazine Exposed Female Sprague Dawley Rats - 6 Month Report
MRID 44152102
October 1996

Atrazine was administered in the diet to female Sprague Dawley rats (90/dose level) at levels of 0, 25, 50 or 400 ppm (0, 1.8, 3.65 or 29.4 mg/kg/day) for 6 months. At 400 ppm (29.4 mg/kg) there was a statistically significant difference in body weight, body weight gain and food consumption when compared to controls. Body weight at 400 ppm was 5 to 9% lower than that reported for controls; body weight gain was 14% lower than that reported for controls and food consumption was 4% lower than controls.

Based on the results obtained from vaginal cytology, rats receiving 400 ppm of atrazine had a greater incidence of abnormal estrous cycles beginning at week 13. Only 53% (48/90) of the animals were experiencing normal cycles and 39% (35/90) remained in estrus for a period in excess of 2 days. By week 17, 18% (16/90) of the animals in the 400 ppm dose group were reported to be in a state of constant estrus and at week 25, treatment- related effects on cyclicity were more apparent and were compounded by the increasing age of the animals. In high dose animals, only 18% of the rats

were cycling normally and 67% had estrus phases greater than 2 days in duration. Of the high dose animals with prolonged estrus, 38/61 or 62% were reported as being in a state of constant estrus.

LH levels in rats scheduled for repeat and non-repeat bleeding, appeared to be affected by the dose of atrazine. The mean LH levels in control, low and mid dose groups peaked at around 1800 hours (biological time, with 1100 being the baseline biological time); however, in rats receiving 400 ppm, there was no surge in LH and blood levels remained close to baseline values.

Atrazine had no apparent effect on prolactin levels.

The following Tables depict the effect of the compound on the estrous cycle and on blood LH levels.

Table II
Vaginal Cytology Results

	Dose	Weeks on Atrazine						
Normal cycles	(ppm)	1	5	9	13	17	21	25
n= 90	0	64	72	81	76	64	56	41
	25	71	80	80	73	64	50	38
	50	69	75	82	69	60	49	32
	400	50	78	74	48	45	29	16
Diestrus								
> 4 days	0	22	16	5	3	8	13	11
	25	17	7	5	6	10	9	14
	50	20	9	3	7	8	6	11
	400	33	9	7	9	7	21	16
Estrus								
> 2 days	0	4	3	4	11	18	21	42
	25	2	3	5	12	18	31	40
	50	1	6	5	15	23	38	49
	400	9	4	9	35	39	50	61
Constant estrus								
	0	0	0	1	6	8	8	17
	25	0	0	0	3	7	17	15
	50	0	1	1	6	9	19	25
	400	0	0	0	8	16	20	38

Taken from Table 5 of report

were cycling normally and 67% had estrus phases greater than 2 days in duration. Of the high dose animals with prolonged estrus, 42/61 or 68% were reported as being in a state of constant estrus.

LH levels in rats scheduled for repeat and non-repeat bleeding, appeared to be affected by the dose of atrazine. The mean LH levels in control, low and mid dose groups peaked at around 1800 hours (biological time, with 1100 being the baseline biological time); however, in rats receiving 400 ppm, there was no surge in LH and blood levels remained close to baseline values.

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Table II
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Normal cycles	Dose (ppm)	Weeks on Atrazine 1	5	9	13	17	21	25
n= 90	0	64	72	81	76	64	56	41
	25	71	80	80	73	64	50	38
	50	69	75	82	69	60	49	32
	400	50	78	74	48	45	29	16
Estrus > 4 days	0	22	16	5	3	8	13	11
	25	17	7	5	6	10	9	14
	50	20	9	3	7	8	6	11
	400	33	9	7	9	7	21	16
Constant estrus	0	0	0	1	6	8	8	17
	25	0	0	0	3	7	17	15
	50	0	1	1	6	9	19	25
	400	0	0	0	8	16	20	38

Taken from Table 5 of report

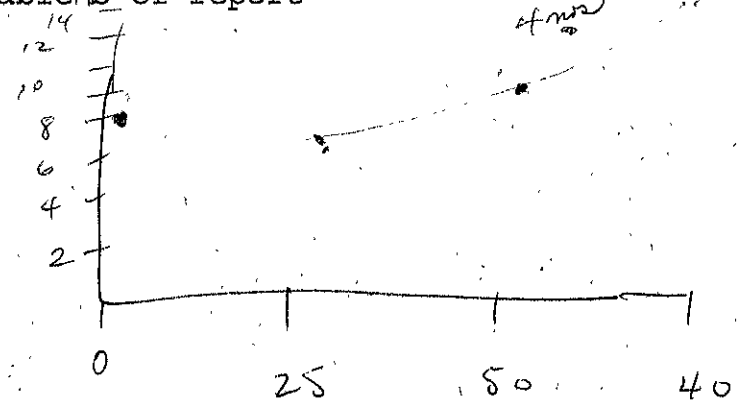

Table III
Serum LH (pg/mL) Concentrations Following Atrazine Administration
Repeat Bleed

Dose (ppm)	Sample Time (Hrs)					
	1100	**1400**	**1600**	**1800**	**2000**	**2300**
0						
mean	909	1136	2213	3336	3388	1672
S.D.	410	554	2562	3138	3344	426
25						
mean	1075	1468	1603	3631	2510	1229
S.D.	621	977	682	2732	1138	492
50						
mean	972	984	2277	2500	2409	1271
S.D.	353	466	1470	1897	1525	559
400						
mean	1005	1155	850	858	1042	953
S.D.	482	620	352	416	627	549

Taken from Table 6B of report.

Discussion of the data: Based on the results reported in this 6 month study, a possible correlation can be made between the dose of atrazine, the disruption of the normal estrus cycle and a possible attenuation of LH. There is a wide variation in the standard deviations reported for blood levels of LH following both repeat (shown in Table III) and non-repeat bleeding. This variation in the data may be due to the small sampling window that is available for LH and could adversely compromise the study with regard to the reliability of the data.

The registrants earlier hypothesis pertaining to the effects of atrazine on prolactin are not supported by the results from this study. Prolactin levels were unaffected by the compound.

Based on the data presented, the NOEL for LH activity should be established at 25 ppm. This is based on the fact that at 50 ppm the surge is somewhat attenuated when depicted graphically. This is not as apparent from the mean numbers provided for animals receiving 50 ppm.

Several of the deficiencies cited in the 28 day study would also apply to this study, specifically, failure to determine whether the observed effects on LH are occuring at the pituitary hypothalamic tract, failure to demonstrate gross or microscopic effects in ovarian tissue and the existence of large standard deviations in the data. A memo from T. Crisp to M. Morrow dated June 5, 1997 is attached.

b. Chronic Studies

i. One Year Combined Feeding/Oncogenicity Study in Female Sprague Dawley Rats
Authors: Pettersen and Turnier
December 8, 1995
(MRID 43934402)

This special toxicity study was designed to determine the effects of 12 months of dietary exposure of atrazine to female CD rats on mammary glands, pituitary glands, estrous cycle, and plasma hormones. Estrous cycle evaluation (by vaginal smear and histology) and plasma hormone (estradiol, progesterone, prolactin, and luteinizing hormone) concentrations are to be reported later.

Technical atrazine (97.1% a.i.) was administered in the diet to 55 female Crl:CD(SD)BR rats per dose group at dose levels of 0, 15, 30, 50, 70 or 400 ppm, corresponding to 0.8, 1.7, 2.8, 4.1, or 23.9 mg/kg/day. Ten females per dose group had trunk blood collection and estrous cycle evaluation made at interim sacrifices of 3, 6, 9, or 12 months. Fifteen females per dose group had interim orbital blood samples and estrous cycle evaluations made at 3, 6, 9, and 12 months followed by sacrifice at 12 months. No effects upon clinical signs or mortality were noted.

The 400 ppm group had statistically significant decreases in mean body weight compared to controls beginning at week 2 (96% of controls), decreasing to 88-92% of controls for the last 10 months of the study. The 400 ppm group had statistically significantly decreased mean body weight gain from weeks 2-46 with mean values ranging from 82-89% of controls. Food consumption for the 400 ppm group ranged from 88-101% of controls, statistically significant at 6 weekly intervals in the first 11 weeks. The 400 ppm group had food consumption close to that of controls for the last 9 months of the study. Food efficiency for the 400 ppm group was decreased in comparison to controls during the first 12 weeks of the study. After 12 weeks, the 400 ppm group had similar, or occasionally increased food efficiency in comparison to controls. Mammary gland hypertrophy (11/55 vs 6/55) and skin masses (9/55 vs 4/55, apparently associated with mammary tumors) were increased in high-dose females compared to controls. Mammary gland adenocarcinomas were increased in high-dose females compared to controls (6/55 vs 1/55), mammary gland adenomas were similar in incidence in high-dose animals compared to controls (1/5 vs 0/55), and mammary gland fibroadenomas were only slightly increased in high-dose females in comparison to controls (4/55 vs 2/55). The increase in mammary gland adenocarcinomas in high-dose females was not statistically significant, however the increase of combined mammary gland adenomas, adenocarcinomas, and fibroadenomas (11/55 vs 3/55 combined) was significant ($p \leq 0.05$). There were no significant differences for onset time among treatment groups when mammary gland adenomas, adenocarcinomas, and fibroadenomas were considered

separately, however, there was a significant positive trend ($p \leq 0.05$) when fibroadenomas were combined with either adenomas or adenomas and adenocarcinomas.

The NOEL is 70 ppm (4.1 mg/kg/day) and the LOEL is 400 ppm (23.9 mg/kg/day) in females based on decreased body weight, body weight gain, and increased mammary gland adenocarcinomas. A statistical Analysis by L. Brunsman of SAB is attached (Attachment 2) along with the DER from K. Farwell (Attachment 3).

Discussion of the Data: The special one year combined chronic/oncogenicity study in female Sprague Dawley rats was classified **supplementary**, did **not** satisfy the guideline requirement for a chronic oral study in the rat, and could not be upgraded. The following guideline deficiencies were noted: only females were tested; only 25 animals per dose group were planned for terminal sacrifice; no clinical chemistry, hematology, urinalysis, or ophthalmology testing; liver and kidneys were not weighed; microscopic exam only included the pituitary, ovaries, uterus, vagina, inguinal skin with mammaries, and masses; and not all results were reported (hormone analysis and estrous cycle evaluations).

ii. Chronic 12/24 Month Study in Rats with Atrazine Technical, Supplement to EPA Guideline 83-1.
Author: S.L. Morseth, Ph.D.
MRID44152103,
Study # CHV2386-108
October 24, 1996

Technical Atrazine (97.1%) was administered at dietary levels of 0, 25, 50, 70 or 400 ppm (equal to 0, 1.5, 3.1, 4.3 or 24.5 mg/kg average) to ovariectomized and intact female Sprague Dawley rats (80/group and a total of 160 animals for each dietary level). Animals received atrazine for 52 weeks. Ovariectomies were performed on designated rats when the animals were 7 weeks of age.

There were no compound-related effects on survival in any of the groups. At 400 ppm, body weight and body weight gain were significantly lower ($p < 0.05$) than corresponding controls in both intact and ovariectomized rats. Body weight was 5 to 7% lower in intact high dose rats and 7 to 9% lower in ovariectomized high dose rats when compared to controls. Body weight gain at the end of 51 weeks was 13% lower than controls in intact females and 11% lower than in ovariectomized females.

Palpable masses were present in all groups of intact animals and in the ovariectomized control group. In intact females, the incidence of palpable masses was 2/80, 4/80, 4/80, 4/80 and 8/80* (* $p < 0.05$) for ascending doses levels. At 400 ppm, the first palpable mass was reported at week 14 and at weeks 29, 28, 38 and

32 for control, 25, 50 and 70 ppm dietary levels, respectively. The masses were palpated at various anatomical locations and were not confined to regions where mammary tissue was located. although most followed glandular distribution

There were no reported compound- related effects on organ to body weight ratios. Gross findings were similar between groups and were not associated with the administration of atrazine. In ovariectomized rats, there were no mammary neoplasms reported. However in intact females, mammary neoplasms were reported at a higher frequency (6/25) in animals receiving 400 ppm. This was not statistically significant when compared to control animals.

The following table shows the incidence and classification of mammary tumors in intact animals.

Table IV
Incidence of Neoplastic Lesions in Intact Female SD Rats

Tumor type	Atrazine Dose Level (ppm)				
	0	25	50	70	400
Mammary Gland fibroadenoma (%)	0/22 (0)	2/22 (9)	2/23 (9)	2/22 (9)	1/25 (4)
carcinoma	2/22 (9)	2/22 (9)	0/23 (0)	2/22 (9)	6/25 (24)
✓ combined	2/22 (9)	3/22 (14)	2/23 (9)	4/22 (18)	6/25 (24)

Total numbers include the 20 animals scheduled for interim sacrifice and any animals dying or sacrificed during the study.
in ~~group~~ 25ppm and 400 ppm group one animal had both tumor types

Discussion of the Data: While absolute numbers and percentages of animals with mammary tumors increased there was no statistical significance associated with the increase. It is possible that since no tumors were found in ovariectomized animals, the ovaries may play a hormonally mediated role in the development of mammary tumors in intact animals.

Based on the results reported in the study, the NOEL is 70 ppm and the LOEL is 400 ppm based on statistically significant decreases in body weight gain. Data from vaginal cytology were not provided in this report. The study is supplementary and will be classified based on satisfaction of Guideline criteria upon its completion.

c. **Journal Articles Provided by Ciba Geigy to Address Mechanisms of Carcinogenicity**

1. Theory of Estrogenicity

In their attempt to address the mechanism of action of atrazine in Sprague Dawley rats, Ciba Geigy proposed several mechanisms, most of which were not supported by ancillary and specialized studies. The previously proposed mechanisms entertained the possibility that atrazine possessed estrogenic properties that in turn resulted in a hormonal imbalance in Sprague Dawley rats which led to premature senescence and finally resulted in a decreased latency and an increased incidence of mammary tumors. The journal articles provided information from studies conducted in vivo and in vitro, involving different types of assays to determine whether atrazine had estrogenic properties when compared to estradiol.

Discussion of the Data:

The results from seven studies are summarized in the following Table V. Once these studies were completed, the theory of exogenous estrogenic activity was no longer pursued. Additionally, a consensus panel, convened by Ciba Geigy to evaluate the hormonal mechanism of mammary carcinogenesis concluded that 7 out of 9 in vivo studies conducted to determine the estrogenicity of atrazine, were negative. One of the positive tests had not been validated and the other positive test was inconsistent with other published reports.

4. Previously Reviewed Information
Studies Conducted in Sprague Dawley Rats:

a. 2-Year Combined Feeding/Oncogenicity in Sprague Dawley Rats
Author: Mayhew, et.al.
Date: April 29, 1986

(This study was the subject of the first HED Cancer Peer Review held on September 10, 1987 and was the basis of the cancer classification).

Atrazine was administered in the diet of Sprague Dawley rats at dose levels of 0, 10, 70, 500 or 1000 ppm for 24 months. This is equivalent to 0, 0.5, 3.5, 25 or 50 mg/kg/day. In males there was an increase in the incidence of testicular tumors at the highest dose tested. This finding was stated in the peer review report to have occurred at a level which exceeded the MTD and was not considered in the carcinogenicity classification. In males, the systemic NOEL was 3.5 mg/kg/day and the LOEL was 25 mg/kg/day based on decreased body weight gain (14%). At the highest dose tested of 50 mg/kg, there was decreased body weight gain (14-16%) and an increase in the incidence of degeneration of the rectus femoris muscle. Histopathological lesions were also present in males and included prostatic hyperplasia and renal calculi.

In females, there was a statistically significant increase in the incidence of mammary carcinomas at doses of 3.5 mg/kg/day and higher. A significant increase in fibroadenomas/adenomas was reported at 50 mg/kg/day and a significant increase in all mammary tumors was reported at 25 and 50 mg/kg/day. In spite of the carcinogenic response at 3.5 mg/kg/day, this dose level was determined to be the NOEL for systemic effects of atrazine during the chronic portion of the study. The LOEL for systemic effects was 25 mg/kg/day based on decreased body weight gain (18 to 23%). Females receiving 50 mg/kg/day had decreased body weight gain (26 to 34%), decreased survival, decreased red blood cell count, hematocrit and hemoglobin, and an increased incidence of retinal degeneration, liver necrosis and degeneration of the rectus femoris muscle. This study also served as the basis for the RfD. The study was classified as core minimum and satisfied the requirements for a chronic/oncogenicity study in rodents. (See Table VII for incidence of mammary tumors).

Table VI
Incidence of Neoplastic Lesions in Male and Female SD Rats

Tumor type	Atrazine Dose Level (ppm)				
	0	10	70	500	1000
Mammary Gland					
fibroadenoma	29/89	29/65	36/70	39/68	45/88**
(%)	(33)	(45)	(51)	(57)	(51)
adenoma	1/57	0/51	1/57	1/54	2/50
	(2)	(0)	(2)	(2)	(4)
adenocarcinoma	15/90	16/68	27/70*	27/68*	45/90**
	(17)	(23)	(39)	(40)	(50)
combined	36/90	40/68	47/70	48/68	65/90*
	(40)	(59)	(67)	(71)	(72)
Testes					
interstitial	1/58	3/59	2/59	2/60	7/64*
cell tumor	(2)	(5)	(3)	(3)	(11)

*p< 0.05, **p <0.01. An analysis of this data was conducted by SAB for the first HED Cancer Peer Review for atrazine.

b. 2-Year Oncogenicity Study in Female Sprague Dawley Rats
Author: A.K. Thakur
January 27, 1992

Atrazine was administered to 60 female Sprague Dawley rats per dose group for 24 months at dietary levels of 0, 70 or 400 ppm (0, 3.8 or 23 mg/kg/day). The NOEL for systemic effects was 3.8 mg/kg/day and the LOEL was 23 mg/kg/day based on decreases in body weight gain. At this dose level, the combined incidence of fibroadenomas and carcinomas were statistically increased when the numbers were adjusted for survival. A decreased latency in the onset of mammary tumors was also reported at the highest dose level, with 6 carcinomas being reported at the high dose vs 0 in the controls at the 12 month interim sacrifice. At the 12 month interim sacrifice, the total number of mammary tumors was 2, 3 and 9 for control, 70 ppm and 400 ppm dose levels, respectively. (See Table VII below for non-adjusted incidence). At 24 months, there was no significant difference in the total tumor incidence between control and treated groups.

Table VII
Mammary Tumor Incidence

Tumors	Dose Level (ppm)		
	0	70	400
Fibroadenoma	39/60	30/60	41/60
Adenocarcinoma	17/60	13/60	22/60
Combined	46/60	34/60	49/60

p= 0.05 using life Table tests.
(From review by H. Spencer 2/09/94, no SAB review of data appended).

c. 2-Year Oncogenicity Study in Female Sprague Dawley Rats
Author: A.K. Thakur
October 1991

Atrazine was administered at dietary dose levels of 0, 70 or 400 ppm for 24 months to female Sprague Dawley rats. At 1, 3, 9, 12, 15, 18 and 24 months, 10 animals per group were sacrificed for hormonal determinations. The systemic NOEL in this study was 70 ppm (3.5 mg/kg) based on a 13% decrease in body weight gain. The ovaries, uterus, vagina, mammary glands and pituitaries were evaluated histologically and an assessment was made using vaginal cytology to determine the phase of estrous that the animals were in at the time of sacrifice.

The mammary tumor incidence is summarized in Table VIII. In this study, there was a decreased time to tumor at 12 months in the 400 ppm group. The number of palpable masses at 12 months that were later confirmed as mammary tumors (either adenomas or carcinomas) was also increased in the highest dose level. The incidence at 12 months was 4/50, 3/48 and 14/50 for control, 70 ppm and 400 ppm dose groups, respectively. The incidence at the end of the study was not significantly different between control and high dose groups.

Table VIII
Mammary Tumor Incidence

Tumors	Dose Level (ppm)		
	0	70	400
Fibroadenoma	8/50	12/48	13/50
Adenocarcinoma	9/50	4/48	11/50
Combined	16/50	16/48	22/50

Discussion of the Data: The vaginal smears used in the assessment of stage of the estrus did not provide substantial proof that animals at higher doses were more inclined to remain in proestrus. Additionally, hormonal determinations were questionable with regard to the significance of the variability in the ranges that were reported for estrogen and progesterone. Reviews conducted by T.M. Crisp under memo of March 31, 1994, outlined discrepancies in hormonal data and cited that there was a concern that the experimental design of this study may be flawed. Among the discrepancies addressed in the Crisp memo were whether the blood samples collected for hormonal assay were collected at the appropriate time; whether the vaginal smear cytology data were reliable, the confidence of reported increases in specific hormones at designated sampling intervals and the reliability of group means. These items were addressed in the response to the PD 1; however, Ciba's response did not provide evidence that the data were reliable or the study design was unflawed.

5. Additional Information Provided by the Registrant

a. Comparative Studies in Fisher 344 and Sprague Dawley Rats

Carcinogenicity studies were conducted in Fisher 344 rats to demonstrate the strain differences in the carcinogenic response. Studies were also conducted in females, only to compare hormonal activity and perturbations in cyclicity to findings in Sprague Dawley rats.

1. Two-Year Oncogenicity in Fisher 344 Rats
   Author: A.K. Thakur
   February 18, 1992

Atrazine was administered for 24 months to male and female Fisher 344 rats at dietary levels of 0, 10, 70, 200 or 400 ppm. The NOEL in both sexes was 70 ppm (3.4 mg/kg/day in males and 4.4 mg/kg/day in females). At 400 ppm there was an 11% decrease in body weight gain in both males and females. At this dose level, there was also a significant increase in leukemia associated hepatopathy in females. The leukemia associated hepatopathy was not believed to be associated with the administration of atrazine. At 200 ppm, there was also a significant decrease in body weight gain (8%) in both sexes from weeks 0 through 76. The NOEL for this study was 70 ppm and the LOEL was 200 ppm.

Atrazine was not associated with carcinogenicity in this strain of rats. Incidences of mammary and pituitary neoplasia were not statistically different from controls.

The study was classified as core guideline and satisfied the data requirements for an oncogenicity study.

2. Two Year Oncogenicity Study in Female Fisher-344 Rats
   Author: A.K.Thakur
   November 8, 1991

Atrazine was administered to female Fisher 344 rats at dietary dose levels of 0, 10, 70, 200 or 400 ppm (0, 0.5, 3.5, 10 or 20 mg/kg/day). Animals were sacrificed at 1, 3, 9, 15 and 18 months for estrous cycle staging and hormonal evaluation. This strain of rats was negative for carcinogenicity. The NOEL was 3.5 mg/kg/day and the LOEL was 10 mg/kg/day based on decreased body weight gain.

The results from the two studies in Fisher rats were compared to the results obtained in Sprague Dawley with regard to hormonal data and cycle staging to evaluate the patterns of reproductive aging that may be responsible for the differences observed in the development of mammary tumors in both strains.

The registrant concluded that in Sprague Dawley rats, there was an increase in serum estrogen and prolactin as the animals aged and in Fisher 344 rats there was a decrease in estrogen and an increase in progesterone and prolactin. Sprague Dawley rats were reported to spend more time in estrus as they age, whereas, Fisher 344 rats were reported to spend more time in proestrus. The purpose of the strain comparison was to determine, using vaginal cytology and hormonal aging patterns, which strain would be more closely compared to humans.

b. **Articles and Papers Addressing Strain Differences**

(The articles below provides additional comparison of results following the administration of atrazine to both Fisher and Sprague Dawley strains).

1. Chronic Effects of Atrazine on Estrus and Mammary Tumor Formation in Female Sprague Dawley and Fisher 344 Rats MRID 43598615

This article provided a comparison of the results obtained in long term studies in which atrazine was administered at similar dietary dose levels to two different strains of rats (0,10, 70, 200 or 400 ppm in Fisher rats and 0, 70 or 400 ppm in Sprague Dawley rats). The authors concluded that the levels of atrazine were at or exceeded the MTD in Sprague Dawley rats, which in turn led to lengthening of the estrous cycle primarily based on the increased number of days in estrus. Earlier onset of galactocele formation was also demonstrated at the high dose level (400 ppm) and an earlier onset of mammary tumors with no increase in the overall incidence at study termination was reported. A hormonal profile revealed that plasma estradiol levels were significantly elevated at three months, only. Other hormonal data were unremarkable and the prolactin data during the first two sampling intervals were hemolyzed.

Based on this article, Sprague Dawley rats develop mammary tumors spontaneously as a part of the aging process and the response at the dose levels administered in this study was expected. The reproductive aging process is different in Fisher rats and much of the endocrine control of ovarian function as observed in Sprague Dawley rats is not clearly evident in the Fisher strain.

Discussion of the Data: The MTD issue and its relevance to mammary tumor formation is discussed in this article and has been addressed elsewhere in this document and in previous Cancer Peer Review documents.

In these studies, body weight gain appears to be the only parameter affected and only at the highest dose level in Sprague Dawley rats (15% decrease at 400 ppm). Other studies conducted with higher

doses (500 ppm) did not demonstrate an exacerbation or increase in the clinical signs; however, tumor incidence, specifically adenocarcinomas, was significantly increased above concurrent and historical controls.

The hormonal data are inconsistent, with no patterns being established throughout the study. Additionally, at terminal sacrifice, there was an inadequate number of animals to make a meaningful assessment of the results.

2. Short Term Effects of Chlorotriazines on Estrus in Female Sprague Dawley and Fisher 344 Rats
MRID 43598614

This paper summarized the results of a 2 week study in which atrazine and simazine were administered daily by gavage for two weeks to groups of Sprague Dawley and Fisher rats. The dose levels were 0, 100 or 300 mg/kg. The study was conducted to examine the effects of the compounds on ovaries, uterus, and adrenals, estrous cycling, vaginal cytology and hormonal levels. The results discussed in this entry will pertain to atrazine only.

Significant effects (reductions) in body weight were present in both strains at 100 (14%, Sprague Dawley) and 300 mg/kg (26%, Sprague Dawley) along with significant reductions in absolute and relative ovarian and uterine weights ($p < 0.05$). Absolute and relative adrenal weights were increased. Plasma hormone levels were assessed in proestrus and revealed a marked decrease in estradiol at both dose levels, significant increases in progesterone at 300 mg/kg, slight and insignificant increases in prolactin at 300 mg/kg and a decrease in mean corticosterone levels at 300 mg/kg in Sprague dawley rats. In Fischer 344 rats, none of the hormones measured, estrogen, progesterone, prolactin or corticosterone, were altered by the administration of atrazine.

When both atrazine groups of Sprague Dawley rats were compared to the control groups, there appeared to be a significant increase in cycle length. This was characterized by an increase in the length of time spent in estrus and a decrease in the amount of time spent in diestrus. The time spent in estrus was confirmed by an increase in the number of cornified and nucleated cells at both 100 and 300 mg/kg. No changes in cycle phases were reported for Fischer 344 rats.

Discussion of the Data: It is noted that this study examined the effects of both atrazine and simazine. Interestingly, when the control groups are compared to each other, there is a degree of variation that suggests that some of the findings and conclusions made in this study did not take into account the variability within the species. For instance, the control value for the number of

days spent in a estrus was 4.2 ± 0.4 days for the concurrent atrazine control and 4.7 ± 1.0 day for the concurrent simazine control. Furthermore, with a study of only 2 weeks duration, conclusions pertaining to an increase in cycle length and hormone levels based on a single proestrus sampling, would be premature. The duration of the study would be insufficient to determine whether the results represent an inconsequential finding in the data or a real pattern associated with the administration of the compound.

3. Rat Tumorigenesis: Relevance of Hormonal Imbalance to Dose Selection (a paper, presented by Jim Stevens, Ciba Geigy) MRID 43598613

Jim Stevens, a toxicologist with Ciba Geigy Corporation, presented a paper in which he discussed the mechanisms of hormonal imbalance and the mechanisms which may be involved in mammary tumor pathogenesis in rats. The paper suggested that imbalances in hormonal levels involve either blockade of hormone synthesis or secretion, interference with enzyme systems or competition with receptor sites. Hormones are under the control of the hypothalamus and the pituitary and may serve as switches that bring about a response. These hormones may also increase tissue susceptibility to compounds which act as initiators. The paper touched on the importance of binding for hormonal responsiveness and mentioned that prolactin levels influence the number of estrogen receptors in mammary tissue and estrogen levels affect the number of progesterone receptors in the liver.

The paper discusses and highlights the differences in the reproductive hormonal make-up of aging Fisher 344 rats as compared to aging Sprague Dawley rats. In Sprague Dawley rats, senescence is marked by a decrease in FSH, estradiol and progesterone and an increase in prolactin. An increase in the length of the estrous cycle followed by acyclicity also occurs with aging in this strain. In Fisher rats, reproductive aging is characterized by an increase in progesterone levels and the existence of a state of pseudopregnancy. Galactocoeles, which are considered as markers of prolactin exposure, are more prevalent in aged Sprague Dawley strain than in the aged Fisher strain.

In his paper, Stevens asserts that the mammary tumors observed with atrazine in Sprague Dawley rats are an over- dose phenomenon and occur only when the MTD is exceeded.

Discussion of Data: It should be pointed that the only signs of toxicity with atrazine were a reduction in body weight and an increase in the incidence of mammary tumors at the dose levels

examined. Nothing else suggests an overt toxicity that would bring about other physiological changes and result in tumor formation. The paucity of clinical signs of toxicity in Sprague Dawley rats at dietary doses up to and including 500 ppm have been demonstrated in the four long-term studies cited on pages 9 through 12 of this weight of the evidence document.

If hormones are acting as "switches", further examination of effects on hormonal imbalances such as a disruption in the ratios of one hormone to another should be examined. Some of Steven's proposals have already been refuted, specifically, the blockade of the synthesis of estradiol. The effects of other hormones on the carcinogenicity of atrazine have not been fully investigated nor have the effects on, or the role of the hypothalamus been sufficiently examined. Interference with transforming or metabolizing systems alluded to in the Stevens paper as a possible mechanism for the production of mammary tumors, has also not been investigated.

4. Evaluation of a Hormonal Mechanism for Mammary Carcinogenesis: Consensus Panel Report
MRID 43598620

This document provided a basis for conclusions reached by an independent group of scientists, convened by the registrant, with regard to a mechanism for carcinogenesis and the additional areas where studies would be needed to account for the influence of sex, species and strain. The panel addressed the suitability of the Sprague Dawley rat as a model for evaluating mammary carcinogens and concluded that the strain was inappropriate because of the endocrine processes that take place in the strain as it ages.

With regard to an age based mechanism, the panel concluded that in the Sprague Dawley, reproductive senescence is characterized by a persistent elevation in the hormones prolactin and estrogen. In affected animals, a persistent estrus exists and is due to an ovulation failure. The panel also believes that atrazine disrupts the LH secretory mechanism in Sprague Dawley rats resulting in an acceleration of age related endocrine problems and a high background incidence of mammary tumors.

Based on their proposed mechanism(s) of action, the panel suggested additional studies, primarily to determine the effect of LH on tumorigenic response in general and the effect on mammary tissue, specifically. References are made within the document to the high level of exposure, not just to atrazine, but to other chlorotriazines and to the neuroendocrine regulation of reproductive aging.

Discussion of the Data: Many of the assumptions made by the technical panel have now been demonstrated to some degree; others have been refuted (induction of estrogenic responses, initially believed to be the mechanism of carcinogenicity). With regard to neuroendocrine aging, no studies providing a comparison or contrast of events in normal aging animals and in those receiving atrazine have been conducted. Suggestions that a threshold mechanism exists are plausible; however, it is confounded by the fact that in at least one study in Sprague Dawley rats, an increase in mammary tumor incidence was present at 10 ppm and in another study, an increase was apparent at 70 ppm.

As indicated in the report, atrazine does not appear to have a significant stimulatory effect on estrogen secretion; effects on prolactin remain obscure. With regard to the studies that were suggested by the panel, the results are now in and add some clarity to the panel's proposed mechanism with regard to the effects of LH on premature aging. The correlation between these effects and mammary tumorigenesis still needs to be demonstrated.

c. Other Proposed Mechanisms

1. Hypothesized Pituitary- Hypothalamic Effects

Ultrastructural Changes in the Rat Hypothalamus Arcuate Nucleus following DACT Feeding
MRID 43598616

Sprague Dawley rats, 20 weeks of age were fed DACT, a metabolite of atrazine, at dose levels of 0 or 1000 ppm. The study was conducted to test the theory that estrogens enhance hypothalamic structural gliosis. In cases of neuronal degeneration (etiology undefined), it has been proposed that the astrocytes and microglial cells enlarge and accumulate dense bodies. The degree of enlargement and granular content are used as indices of hypothalamic damage. Glial activity has been observed in the arcuate nucleus of the hypothalamus, the region that plays a role in the secretion of gonadotropins. It is also speculated in this report that the ultrastructural pathology reported in the hypothalamus may possibly be related to stimulation of endogenous peroxidase activity which has been reported to occur during periods of prolonged estrus.

In this study the granular content in astrocytes was increased by 4% at week 32 and by 36% at week 48. The microglial cells were increased in number at both weeks 32 and 48; however, the increases were not significant and the microglial cells were not classified as reactive (meaning they were not enlarged and did not contain dense bodies).

Discussion of the Data: An evaluation of the results showed that the granular content of the astrocytes was not homogeneous and that the microglial cells, although increased in number, were not considered reactive cells analogous to those which have been reported in cases of neuronal degeneration. The results of this study as they pertain to the effects of the atrazine metabolite, DACT, are ambiguous and the contribution of the changes observed in the hypothalamus to the increased incidence of mammary tumors in Sprague Dawley rats would need to be more thoroughly probed and correlated.

2. Additional Information on the Carcinogenic Potential of Atrazine

Weight of the Evidence on the Oncogenic Potential of Atrazine
Consensus Panel Report
43598624

The consensus panel report from a group of independent scientists who were convened by Ciba Geigy concluded the following with regard to the carcinogenic potential of Atrazine:

Atrazine induces adenocarcinoma in Sprague Dawley rats and accelerates the onset at doses greater than or equal to the MTD, with the exception of one study where increases in mammary tumors were observed below the MTD. This could not be reproduced in additional studies conducted at the same dietary level.

Atrazine is negative for carcinogenicity in male Sprague Dawley rats, in both sexes of Fisher rats and in both sexes of CD-1 mice.

Atrazine accelerates age related reproductive changes in Sprague Dawley rats causing a state of constant estrus (prolonged estrogen exposure) albeit atrazine has not been demonstrated to be estrogenic by in vivo and in vitro tests. The female Sprague Dawley rat is not considered to be a good model for mammary tumor induction in humans because of the differences in reproductive cyclicity between the SD rat and human females.

Atrazine was not considered to be genotoxic in 31/37 mutagenicity studies. The remaining 6/37 studies were considered either positive or equivocal.

Atrazine is completely absorbed and rapidly eliminated via the urine. Dealkylation is the major route of metabolism and the primary metabolite is DACT.

Atrazine is structurally related to simazine in that it contains a 2-chloro-4,6-bis-(alkylamino)-s-triazine ring and both have been associated with the formation of mammary tumors (increased incidence and early onset). The panel report states that atrazine at doses of 400 ppm or greater accelerates senescence in female Sprague Dawley rats and is characterized by an increase in serum estradiol levels, early onset of constant estrus and ovulatory failure. Changes in mammary histomorphology appear to be associated with an imbalance of endogenous estrogen and not caused by an exogenous source of estrogen.

Discussion of the Data: The consensus panel suggested a mechanism for the development of mammary tumors in Sprague Dawley rats; however, the possible influences of the hypothalamus are not investigated thoroughly or followed in this report. The effect of atrazine on the ability of LHRF (GnRF) to evoke a neuronal response has not been addressed. Furthermore, it has been stated that there is an age related decline in estrogen receptors; however, this phenomenon has not been correlated with the decline in neuroendocrine control of the estrous cycle. The panel report has alluded to the possibility that a neuroendocrine component exists in the development of mammary tumors.

In the neuroendocrine scheme of aging the following series of events are believed to take place:

Under neuroendocrine control female Sprague Dawley rats lose their ability to ovulate. This leads to an increase in the secretion on estradiol. New follicles are formed but, because ovulation is impaired, there is a decrease in the number of corpora lutea formed and a decrease in the progesterone level. The balance between the progesterone/estrogen activity is shifted, with estrogen becoming the more persistent hormone. This estrogenic persistence leads to overstimulation of estrogen responsive tissues such as the uterus, mammary gland and anterior pituitary. Estrogenic effects on the anterior pituitary would lead to prolactin secretion that would in turn stimulate the rate of growth of existing mammary tumors.

While portions of the different mechanisms of carcinogenic activity have been examined, they have not been proven, correlated or reproduced with a level of confidence that would rule out all other feasible mechanisms. The LH studies, while providing possible explanation as to the affect of atrazine on LH, still have not been tied in with the development of mammary tumors in one complete study. It is possible that the carcinogenic effect of atrazine may lie in its ability to **promote** phenotypic expression of mammary cancer in a sensitive strain or species. It is also possible that the mechanism is not a singular, triggering incident, but a series of events.

Within the consensus panel report there was a comparison of reproductive senescence in humans and rats. The emphasis should not be on events related to aging across species, but rather the potential for this compound to cause or be associated with the development of cancer over the lifetime of a human. A factor for consideration in the risk equation, when extrapolating from rats to humans with regard to breast cancer, is the paucity of information on hormonal disruption and the impact of any such disruption during various stages of development in humans. Additionally, the etiology of human breast cancer is for the most part unknown. Mammary cancer is not a concern solely in aging humans, but a concern over the lifetime of an individual and specifically during periods when hormonal equilibrium is disturbed (i.e. menarche, pregnancy, lactation and menopause).

The studies conducted in Sprague Dawley rats at dose levels up to and including 400 ppm have not demonstrated that atrazine has overt systemic toxicity. Furthermore, doses between 70 ppm and 400 ppm were not investigated in Sprague Dawley rats as they were in Fisher 344 rats so it is not clearly established that the signs of toxicity (weight loss, decreased tumor latency) observed at 400 ppm would not have occurred at dose levels between 70 and 400 ppm. A table providing dose response information from long-term and intermediate term studies is attached.

6. Metabolism

When C14 labelled atrazine was administered to rats, the distribution was found to be dose dependent and to follow first order kinetics. Red blood cells stored the highest level of atrazine, followed in decreasing order by the liver, kidneys, ovaries, pituitary, brain, and the pectoral region of the mammaries. The half life of atrazine in the body was 1.61 days, with 95% of the administered dose being excreted within 7 days. Atrazine is metabolized primarily by N- dealkylation. The compound is eliminated by both the urinary and fecal routes.

7. Structure Activity Relationship (SAR)

Atrazine is an s-triazine pesticide and is related to simazine, cyanazine, propazine, terbuthylazine and terbutryn, all of which have been associated with the induction of mammary gland adenomas and/or adenocarcinomas in Sprague Dawley rats. Terbutryn has also been associated with carcinogenicity involving the thyroid and the liver. The structures for the chemicals are attached (Attachment 4, Figure 1).

In a report dated January 17, 1991 which addressed the SAR analysis of s-triazine pesticides and related compounds, it was stated that the carcinogenic activity of any given s-triazine was dependent on

to simazine, cyanazine, propazine and terbutryn, all of which have been associated with the induction of mammary gland adenomas and /or adenocarcinomas in Sprague Dawley rats.

Based on the information presented, the HED Cancer SARC is asked to address the following:

1. Has a hormonal mechanism for mammary carcinogenesis been ~~adequately described~~ reasonably supported for atrazine in Sprague Dawley rats? – yes

2. In the studies in which a positive carcinogenic response has been demonstrated has the MTD been exceeded? – dosing adequate, not excessive

3. Is it likely that carcinogenic effects observed in Sprague Dawley rats would ~~not~~ be expected in humans? No reason to believe that it would not be true, given similarities. We do not know the full etiology of mammary cancer in rodents

~~4. With the theories and data that have been presented on the possible hormonal mechanism of carcinogenesis can other mechanisms/modes of action be ruled out?~~

~~5. What alternatives for classifying the carcinogenic potential of atrazine are applicable/appropriate if there is a threshhold and a possible mechanism?~~

Risk Assessment :