UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C. 20460

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JUN 24 1988

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MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANC

SUBJECT: Atrazine - Company Response to Toxicology Branch Reviews of the Rat and Rabbit Teratology Studies. Submitted March 25, 1988 by Ciba-Geigy Corporation.
Tox. Branch Project No.: 8-0744 Tox. Chem. No.: 63

TO: Robert Taylor
Product Manager #25
Registration Division (TS-767C)

FROM: Judith W. Hauswirth, Ph.D. Judith W. Hauswirth 6/24/88
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

THRU: Theodore M. Farber, Ph.D., Chief
Toxicology Branch/HED (TS-769C)

Action Requested: Determine whether the submitted data justify upgrading the Core classification of the rat and rabbit teratology studies on Atrazine from Core Supplementary to Core Minimum.

Discussion:

1. Rabbit Teratology Study (MRID 405663-01)

This study was classified as Core Supplementary pending submission of the purity of the technical product. The registrant has submitted information indicating that the purity of the technical product was approximately 96.3%.

2. Rat Teratology Study (MRID 405663-02)

This study was classified as Core Supplementary pending submission of the purity of the technical product and since a NOEL for runting was not demonstrated. The registrant has submitted information indicating that the purity of the technical product was approximately 96.7% and historical control data to address the absence of a NOEL for runting in the study. The historical control data along with the incidence of runting in the study are summarized in the following table.

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Incidence of Runting

	Dosage Group (mg/kg) 0	10	70	700	Hist. Control[1]
No. of fetuses	292	350	351	67	8712
No. of runts	1(0.3)[2]	5(1.4)	7(2.0)	5(7%)	90(1.0)[3]
No. of litters	23	23	25	5	663
No. of litters with runts	1(4.3)	3(13)	4(16)	5(100)	65(9.8)[4]

1 Data from 25 studies were submitted. Data from one study, conducted in 1981, was not included by this reviewer. Only data from studies conducted from 1982 to 1986 are summarized. The study under consideration was dated 1984.
2 Numbers in parentheses are percentage incidence.
3 Range = 0 - 4.0%
4 Range = 0-26.3%

The incidence of both runts and litters with runts was within the historical control range for the low and mid dose groups; however, for both of these parameters and dosage groups the incidence was slightly higher than the mean historical control value. The concurrent control values, on the other hand were low when compared to the historical control mean values. This reviewer feels that the incidence of runts in the low and mid dose group of this study is not treatment related, based upon the submitted historical control data and the comparatively low concurrent control values seen in the Atrazine study. In addition, the increase over control values in both the number of runts and the number of litters with runts was not statistically significant at the low or mid dose by Fischer's Exact test at the 0.05 level (Statistics done by this reviewer).

At the mid dose, there were statistically significant increases by both fetal and litter incidence in skeletal variations indicating delayed ossification. These included: skull not completely ossified, presphenoid not ossified, teeth not ossified, metacarpals not ossified, metacarpals bipartite, and distal phalanx not ossified. The incidences of these effects in the control and low dose group were comparable.

Conclusions:

1. Rabbit Teratology Study

The core grade of this study can be raised to Core Minimum.

2. Rat Teratology Study

The core grade of this study can be raised to Core Mininum. 006761

Developmental NOEL = 10 mg/kg
Developmental LEL 70 mg/kg based upon an increased incidence of skeletal variations indicating delayed ossification.

reviews

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

MEMORANDUM

SUBJECT: Transmittal of DERs of a Rat and Rabbit Teratology Study Using Atrazine Required By Data Call-In Notice - EPA Registration No. 100-529 Accession No. 254979

Caswell No.: 63
TOX Br. Proj. No.: 7-0535

FFOM: Henry Spencer, Ph D., Pharmacologist HS (wsw for HS) 8-17-87
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Robert J. Taylor/Cynthia Giles, PM Team 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

wsw for ABK 8-17-87

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Review Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) 8/19/87

and

Theodore Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Background

A Data Call-In (DCI) Notice was issued by the Registration Division that included the requirement for both a rat and rabbit teratology study, using technical atrazine.

The present submission (Accession No. 254979) is the fulfillment of that portion of the DCI.

The following is the transmittal of the contractor-generated DERs for the two studies and the comments and conclusions by Toxicology Branch concerning the results of the reviews prepared by the contractor.

Comments and Conclusions

1. The rat teratology is Supplementary data, because a NOEL for developmental toxicity could not be established. The LEL was 10 mg/kg based on an increased incidence of runts.

   The study may be upgraded if data allow the establishment of a NOEL. Historical control data and chemical purity are required for the study.

   A maternal toxicity NOEL = 10 mg/kg. A maternal LEL was 70 mg/kg based on decreased body weight gain.

2. The rabbit teratology study exhibits a NOEL of 1 mg/kg and an LEL of 5 mg/kg for maternal toxicity based on reduced body weight gains during gestation and reduced food intake.

   The NOEL for developmental toxicity is 5 mg/kg and the LEL is 75 mg/kg based on increased resorptions, reduced fetal weights for both sexes, and increases in delayed ossification.

   The study is considered Supplementary and requires submission of the purity of the technical chemical employed. This study may be upgraded pending submission of the purity of the technical chemical used.

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STUDY REVIEW

Chemical: Atrazine
Test Material: Atrazine technical
Study/Action Type: Teratology study

STUDY IDENTIFICATION;

"A Teratology Study of Atrazine Technical in New Zealand White Rabbits"

Testing Facility: Safety Evaluation Facility, CIBA-GEIGY Corp., Summit, NJ
Project No.: 68-84
Report Date: 9-18-84
Study Director: Alan T. Arthur, Ph.D.
EPA Acession No.: 254979

Study Reviewed by: Helene B. Morgan, B.A. Hbm 8/11/87
Geraldine S. Danford, B.A.

BACKGROUND

The teratogenic potential of Atrazine technical in New Zealand White rabbits was investigated in a study conducted at the Safety Evaluation Facility, CIBA-GEIGY Corp., during the fall of 1983.

CONCLUSION

This study of the teratogenicity of atrazine in New Zealand White rabbits seems to meet all the requirements of the Standard Evaluation Procedure, Teratology Studies (EPA-540-9-85-018, June 1985). The dose levels meet the stated requirement and the data are well documented.

It is concluded that the teratology study of atrazine in rabbits (Safety Evaluation Facility, CIBA-GEIGY Corp., # 68-84) demonstrates the following:

Maternal No Observed Effect Level (NOEL): 1 mg/kg/day
Maternal Lowest Observed Effect Level (LOEL): 5 mg/kg/day

These values are based on a statisically significant reduction in body weight gain for gestional days 14-19 and a statistically significant reduction in food consumption on gestational days 17 and 19 in the 5 mg/kg/day group.

Conclusions and Recommendations

Developmental toxicity NOEL.	5 mg/kg/day
Developmental toxicity LOEL:	75 mg/kg/day

These values are based on a statistically significant increase in the number of resorptions in the high dose group, significantly decreased fetal weights (male and female) in this group, and an increase in skeletal variations, especially delayed ossification of appendicular skeletal elements.

PROCEDURES

Test material:	Atrazine technical
Vehicle:	3% aqueous corn starch containing 0.5% Tween 80
Dosage levels:	0, 1, 5, or 75 mg/kg/day by gavage
Period of administration:	Days 7-19 of gestation
Species:	New Zealand White rabbits

The protocol used in this study was in compliance with those recommended in the Standard Evaluation Procedure (SEP), Teratology Studies (EPA-540/9-85-018, June 1985).

Graded doses of atrazine were administered by gavage to virgin female rabbits which had been artificially inseminated using semen collected from males of the same strain maintained at the research site. The oral route of dosing was chosen because potential human exposure is by this route.

Dosing occurred daily and was performed on days 7 through 19 of pregnancy with the day of artificial insemination being counted as day 0. This dosing period agrees with the recommendations of the SEP and covers the period of organogenesis in the rabbit.

Identification of individual rabbits, housing, food, water, environment, quarantine time, and group assignments were of standard experimental design. Dose level was based on the animals' body weight recorded on gestational days 7 and 14. Dosing was performed as indicated in Table 1.

Table 1: Dosing schedule

Group number	Number of females	Days of treatment	Dose (mg/kg/day)
1 (control)	19	7-19	0
2	19	7-19	1
3	19	7-19	5
4	19	7-19	75

The control group (vehicle control) received 5 ml/kg/day of 3% corn starch with Tween 80, which was a volume equivalent to that received by rabbits treated with atrazine.

According to the SEP, the highest dose of three different levels should induce overt maternal toxicity but not more than 10% maternal death. The highest dose (75 mg/kg/day) did induce overt maternal toxicity. A significant decrease in weight gain and food consumption were found during and after treatment. Significant increases were also found in vaginal bleeding and in little, none and/or soft stools. No deaths were found in this treatment group. The lowest dose is not supposed to induce evidence of toxicity. In the 1 mg/kg/day group there were three unexpected deaths. One female died after being dosed on day 17 of gestation. Another was found dead on day 19, apparently the result of a dosing accident. The third female was found dead on day 26 of gestation (possibly aborting). None of the deaths were dose related and thus are not considered significant. All other findings were similar to controls.

All does were examined daily for changes in appearance, behavior and food consumption. Individual body weights were recorded on days 0, 7, 14, 19, 21, 25, and 29. On day 29, all surviving females were sacrificed and examined for corpora lutea, uterine content, and gross morphological changes.

The fetuses were numbered in order of their positions in the uterus. Apparently viable fetuses were weighed and examined for gross abnormalities. Each fetus was examined viscerally using Staples' technique and its sex was determined. Following the visceral examination all fetuses were stained for skeletal examinations to determine malformations and/or variations.

RESULTS

A. MATERNAL EVALUATION

Maternal Mortality

The only three deaths in the entire study occurred in the lowest dose (1 mg/kg/day) group. None of the findings at necropsy indicated any relationship to the dose level, but rather to the process of dosing.

Clinical Observations

Stool changes were observed in low and intermediate dose group animals but were not considered to have been compound-related because the incidences were similar to that in the controls. All does in the high dose group exhibited stool changes. These changes were statistically significant and considered signs of maternal toxicity. Another statistically significant sign of toxicity in the high dose group was blood either on the vulva or in the cage in 4/19 females. (Table 2)

Incidental findings included alopecia, lacrimation, scabs, nasal discharge, vasodilation and decreased motor control. All of these findings did not appear to be dose-related.

Pregnancy Rates

The pregnancy rates for the 0, 1, 5, and 75 mg/kg/day groups were, respectively, 84.2%, 89.5%, 84.2%, and 94.7%. The pregnancy rates given are within acceptable ranges for rabbits. Woo and Hoar reported an 83% pregnancy rate among those artificially inseminated out of 11 pregnancies in NZW rabbits.[1] All does were examined for uterine contents following death, moribund sacrifice, or sacrifice on gestation day 29, showing the pregnancy rate is based on total number of females inseminated and not only on those surviving until sacrifice on gestation day 29. (Table 3)

Maternal Body Weight Data

Individual maternal body weights were recorded on days 0, 7, 14, 19, 21, 25, and 29 of presumed gestation. Prior to the dosing period (days 0-7), no differences in body weight gain were noted in any group. (Table 4)

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[1]Woo, D.C. and R.M. Hoar, Reproductive performance and spontaneous malformations in control New Zealand White rabbits: a joint study by MARTA, Teratology 25(2): 82A, 1982.

TABLE 2.
Summary of Clinical Observations in Does

Observations	Dose (mg/kg)			
	0	1	5	75
Stool: little, none and/or soft	9/19	4/19	10/19	19/19**
Blood on vulva/in Cage	0/19	1/19	0/19	4/19*
Vasodilation of Ears	0/19	0/19	0/19	1/19
Decreased Motor Activity	0/19	0/19	0/19	1/19
Alopecia	5/19	3/19	2/19	9/19
Lacrimation	3/19	0/19	1/19	3/19
Scab	1/19	1/19	2/19	0/19
Nasal Discharge	1/19	0/19	0/19	0/19
Abortion	0/19	0/19	1/19	2/19
Death	0/19	3/19	0/19	0/19

*Different from the control group at $p \leq 0.05$.
**Different from the control group at $p \leq 0.01$.

TABLE 3.
Summary of Reproductive Parameters

	Treatments (mg/kg/day)			
	0	1	5	75
No Pregnant	16	17	16	18
% Pregnant	84.2	89.5	84.2	94.7
Mean No. Corpora Lutea/Litter	13.6	13.1	12.9	14.3
Mean No. Implantations/Litter	10.1	10.2	10.5	10.4
No. Litters Examined	16	14	15	15
Mean No. Embryonic Resorptions	.40	.30	1.0	1.6
Mean No. Fetal Resorptions	.90	1.1	.40	3.2
Mean Number of Resorptions	1.3	1.4	1.4	4.8**
Mean Number of Dead Fetuses	0.0	0.0	0.0	0.0
Pre-Implantation Loss (No.)	3.6	2.9	2.5	3.9
Pre-Implantation Loss (%)	26.1	21.6	18.4	26.5
Post-Implantation Loss (%)	12.0	11.4	13.0	42.6**
Mean Number of Live Fetuses	8.8	8.9	9.1	5.9*
Fetal Sex Ratio (% Males)	48.6	47.6	44.1	51.7

*Different from the Control at $p \leq 0.05$.
**Different from the Control at $p \leq 0.01$.

TABLE 4.
Summary of Maternal Body Weight (grams)

Days of Gestation	Treatments (mg/kg/day)			
	Control (0)	1	5	75
0	3852 ± 81 (16)[a]	3798 ± 86 (14)	3854 ± 56 (15)	3999 ± 91 (15)
7	4038 ± 85 (16)	3972 ± 88 (14)	4019 ± 52 (15)	4181 ± 94 (15)
14	4151 ± 91 (16)	4049 ± 93 (14)	4083 ± 47 (15)	3659 ± 88* (15)
19	4[illegible]0 ± 100 (16)	4153 ± 104 (14)	4128 ± 43 (15)	3454 ± 84* (15)
21	4316 ± 104 (16)	4192 ± 105 (14)	4171 ± 44 (15)	3545 ± 96* (15)
25	4337 ± 95 (16)	4225 ± 102 (14)	4238 ± 49 (15)	3903 ± 81* (15)
29	4363 ± 86 (16)	4261 ± 98 (14)	4280 ± 52 (15)	4012 ± 82* (15)
29U[b]	3779 ± 100 (16)	3685 ± 95 (14)	3711 ± 43 (15)	3605 ± 88 (15)

[a]Numbers in parenthesis ( ) equal number of animals used in mean.
[b]Day 29U = (Term.) Body Weight less uterus, placentas and fetuses.
*Different from the Control at $p \leq 0.01$.

Statistically significant reductions in maternal bod weights were observed in the high dose group for gestational day 14-29 (Table 5). Also, weight gains in the high dose group wer significantly reduced at some intervals during and followin treatment. During the treatment (days 7-14 and days 14-19), bod weight losses rather than body weight gains were found. Th total body weight gain for the high dose group for the entir gestational period (days 0-29U) was also significantly reduced.

In the intermediate group (5 mg/kg/day) the body weight gai was significantly reduced for gestational days 14-19. Othe weight variations in this group are not considered significant.

Any changes in the weights of does in the low dose grou were not significant, as the average body weight of this grou was never less than 97% of the average body weight of does in th control group.

Maternal Food Consumption Data

Maternal toxicity, as shown by a statistically significan reduction in food consumption during treatment, was observed i the high dose (75 mg/kg/day) group. (Table 6) Followin treatment, evidence of recovery was observed as food consumptio increased. This increase was statistically significant for day 24-28.

Although slight reductions in food consumption were als found in the intermediate group (5 mg/kg/day), these reduction were statistically significant only for gestational days 17 an 19.

Food consumption was very slightly reduced in the low dos group both before and during the dosing period, the reductio being statistically significant only on day 13 of gestation Because these reductions also occurred before the dosing period they were not considered to have been compound-related.

Abortion

There were three confirmed abortions in the present study One intermediate dose female on day 21 and two high dose female on days 20 and 25 were killed because they were aborting.

One of the females who died in the low dose group wa thought to possibly be aborting on day 26.

TABLE 5.
Summary of Maternal Weight Gain (grams)

Days of Gestation	Treatments (mg/kg/day)			
	Control (0)	1	5	75
0-7	185 ± 19 (16)[a]	174 ± 24 (14)	166 ± 16 (15)	162 ± 20 (15)
7-14	113 ± 13 (16)	76 ± 13 (14)	63 ± 18 (15)	-522 ± 19** (15)
14-19	120 ± 15 (16)	105 ± 17 (14)	45 ± 23* (15)	-204 ± 26** (15)
19-21	46 ± 16 (16)	39 ± 10 (14)	43 ± 10 (15)	108 ± 27 (14)
21-25	21 ± 22 (16)	33 ± 26 (14)	67 ± 13 (15)	304 ± 37** (13)
25-29	26 ± 21 (16)	36 ± 11 (14)	42 ± 16 (15)	109 ± 25* (15)
0-29U[b]	-73 ± 52 (16)	-113 ± 64 (14)	-143 ± 42 (15)	-393 ± 28** (15)

[a] Numbers in parenthesis ( ) equal number of animals used in mean.
[b] Day 29U = Day (Term.) Body Weight less uterus, placentas and fetuses.
*Different from the Control at $p \leq 0.05$.
**Different from the Control at $p \leq 0.01$.

TABLE 6.
Summary of Maternal Food Consumption (grams)

Days of Gestation	Treatments (mg/kg/day)			
	Control (0)	1	S	TS
7	183 ± 11 (13)[a]	164 ± 10 (12)	185 ± 6 (15)	76 ± 8** (15)
8	200 ± 11 (15)	180 ± 8 (13)	183 ± 7 (15)	80 ± 7** (15)
9	182 ± 10 (12)	172 ± 11 (12)	175 ± 6 (14)	20 ± 6** (13)
10	201 ± 12 (13)	179 ± 10 (13)	176 ± 5 (15)	12 ± 3** (14)
11	177 ± 12 (15)	177 ± 8 (13)	170 ± 6 (14)	8 ± 3** (15)
12	182 ± 9 (14)	170 ± 8 (13)	162 ± 6 (15)	2 ± 1** (14)
13	182 ± 8 (13)	155 ± 8* (14)	163 ± 6 (15)	1 ± 1** (15)
14	175 ± 10 (13)	156 ± 13 (10)	156 ± 7 (15)	2 ± 1** (15)
15	181 ± 13 (12)	149 ± 10 (11)	157 ± 10 (15)	2 ± 1** (15)
16	177 ± 17 (14)	159 ± 13 (11)	142 ± 15 (13)	3 ± 1** (15)
17	182 ± 10 (12)	157 ± 14 (11)	134 ± 15* (14)	6 ± 2** (15)
18	167 ± 11 (9)	154 ± 11 (12)	137 ± 16 (14)	8 ± 4** (15)
19	164 ± 9 (14)	136 ± 10 (11)	129 ± 12* (14)	14 ± 7** (14)
20	161 ± 10 (14)	144 ± 13 (12)	136 ± 11 (14)	80 ± 16** (12)
21	148 ± 10 (15)	128 ± 11 (13)	138 ± 10 (15)	118 ± 18 (12)
22	142 ± 13 (16)	119 ± 15 (12)	132 ± 10 (14)	162 ± 18 (13)
23	129 ± 11 (16)	110 ± 14 (13)	115 ± 9 (13)	162 ± 14 (11)
24	107 ± 9.. (14)	100 ± 12 (13)	104 ± 8 (14)	174 ± 9* (13)
25	92 ± 11 (16)	95 ± 11 (13)	95 ± 6 (13)	174 ± 10* (13)
26	91 ± 10 (15)	93 ± 11 (12)	95 ± 7 (13)	172 ± 11* (12)
27	89 ± 9 (13)	94 ± 9 (12)	100 ± 6 (11)	192 ± 15* (11)
28	91 ± 9 (14)	91 ± 11 (12)	87 ± 9 (11)	193 ± 15* (10)

[a]Numbers in parenthesis ( ) equal number of animals used in mean.
*Different from the Control at $p \leq 0.05$.
**Different from the Control at $p \leq 0.01$.

Reproduction Data at C-Section

The results of cesarean section, including ovarian, uterine, and litter data, are presented in Table 3.

The mean number of corpora lutea, uterine implantations, and the mean implantation efficiency were comparable for all groups examined. For the 0, 1, 5, and 75 mg/kg/day groups the mean number of resorptions were, respectively, 1.3 (12.0%), 1.4 (11.4%), 1.4 (13.0%), and 4.8 (42.6%). The 75 mg/kg/day values were statistically significantly higher ($p<0.01$) than the control values. Except for the number of resorptions in the high dose group, the results compare favorably with control data in NZW rabbits compiled by Woo and Hoar.[2]

The mean number of live fetuses per litter was 8.8 (control), 8.9 (1 mg/kg/day), 9.1 (5 mg/kg/day), and 5.9 (75 mg/kg/day). The high dose group had a reduced number of fetuses, which is statistically significant ($p<0.05$) when compared to the control group.

B. DEVELOPMENTAL TOXICITY EVALUATION

Fetal Data

The fetal data collected at cesarean section are summarized in Table 3.

As noted earlier, only in the high dose group was a significant change in the litter size observed. No dead fetuses were found in the control or any of the treated groups. Also, no variations in fetal sex ratio were detected.

Only in the 75 mg/kg/day group was a statistically significant reduction of mean fetal weight observed. The male fetal weight was 35.7 grams ($p<0.01$) and the female fetal weight was 35.8 grams ($p<0.01$). These values were compared to 46.1 grams (males) and 44.0 grams (females) for the control fetuses. These data are shown in Table 7.

[2]Woo and Hoar, op. cit.

Table 7: Summary of Fetal Weights (grams)

Parameter	Treatments (mg/kg/day): 0	1	5	75
Fetal Weight - Male	46.1	44.0	43.2	35.7*
Fetal weight - Female	44.0	43.3	43.1	35.8*

*Different from the control group at P<0.01.

The reviewers investigated a possible increase in the number of runts (body weight less than 30 grams) in the treated groups. The findings are presented in the table in Attachment I. A definite increase was found in the high dose group. A slight increase was found in both the 1 mg/kg/day and 5 mg/kg/day fetuses; however, no increase over controls was found in the number of litters containing runts for either of these two groups. Seven of the 9 runts in the 5 mg/kg/day dose group were in very large litters containing 11 or 12 fetuses, which often results in low birth weight. The other 2 were in a litter consisting of 9 fetuses. Additional calculations gave the mean total litter weight (combined weights of all fetuses in a litter added for dose level divided by total number of litters) for controls to be 387.8 grams and that for the 5 mg/kg/day group to be 384.75 grams. Many teratologists advocate the litter approach over the fetal approach in handling statistical data for determining the effect of an agent.[3-5] Information from researchers working with New Zealand White rabbits indicated that the control values of 4 runts in 4 of 16 litters was unusually high and the problem might be with the rabbits supplied for the study. Without support of other signs of fetal toxicity at the 5 mg/kg/day dose level, the reviewers conclude that, under the conditions of this study, no biologically significant effect of atrazine on the fetus at the intermediate dose level is evident.

[3] Walter, H., Choice of number of sampling units in teratology. Teratology 9: 257-258, 1974.

[4] Staples, R.E., and J.K. Haseman, Selection of appropriate experimental units in teratology, Teratology 9: 259-260, 1974.

[5] Weil, C.S., Selection of the valid number of sampling units and a consideration of their combination in toxicological studies involving reproduction, teratogenesis or carcinogenesis, Food and Cosmetics Toxicology 8: 177-182, 1970.

Malformation and Variations

Of the 489 fetuses produced in this study, two were externally malformed, one had a visceral abnormality, and one had a skeletal malformation. (Table 8) The external anomalies were omphalocele (control group) and ablepharia (high dose group). The only visceral malformation was the absence of a gallbladder in one intermediate dose fetus. The only skeletal malformation was in one control fetus which exhibited ectromelia. All of these were considered spontaneous and not dose related.

A slight increase in skeletal variation was found with increasing dose levels. The parameters most increased were those concerning delayed ossification. The high dose group had statistically significant increases in delayed ossification. These findings are indicative of fetal toxicity secondary to severe maternal toxicity, a conclusion consistent with reduced fetal body weights in the high dose group. (Table 9)

Discussion

These reviewers are in general agreement with the more important conclusions of this study. The means and standard deviations were found to be correct after spot checking.

Administration of technical atrazine to NZW rabbits from days 7-19 of gestation resulted in maternal toxicity during the treatment period at doses of 5 and 75 mg/kg/day. Does in the 75 mg/kg/day group did not recover from symptoms of this toxicity during the period after dosing. Signs of maternal toxicity in the intermediate dose group were decreased food consumption and decreased body weight. Signs of meternal toxicity in the high dose group included blood on vulva or in cage, decreased food consumption, abnormal stools, and decreased body weight and weight gain. In the lowest dose level (1 mg/kg/day) no dose related toxicity was observed. It can be concluded that a maternal NOEL in rabbits can be set at 1 mg/kg/day. The maternal LOEL in this study is 5 mg/kg/day.

The increased number of resorptions in the high dose group was statistically significant and was not observed in any of the other groups. No dead fetuses were observed in any of the groups. Fetal weights were normal in all groups except the high dose group. In this group the weights of both the male and female fetuses were significantly reduced. No compound-related malformations were observed. Skeletal variations, especially delayed ossification of appendicular skeletal elements, were found more frequently in the high dose group. Embryotoxicity and fetotoxicity in the high dose group was considered to have been secondary to maternal toxicity. These reviewers agree that the developmental toxicity (embryo/fetotoxicity) NOEL in rabbits is 5 mg/kg/day and conclude the LOEL is 75 mg/kg/day.

TABLE 8.
Summary of Fetal Malformations

Location	Parameter	Treatments (mg/kg/day) 0	1	5	75
	Total Number of Fetuses Examined	140	124	136	89
	Total Number of Litters Examined	16	14	15	15
External	Omphalocele	1	0	0	0
	Ablepharia	0	0	0	1
Visceral	Gallbladder-Absent	0	0	1	0
Skeletal	Ectromelia	1	0	0	0

TABLE 9.
Summary of Fetal Skeletal Variations (by Fetus)

Parameter	Treatments (mg/kg/day) 0	1	5	75
Number of Fetuses with Variations	94	80	99	75
Number of Fetuses with Variations Excluding Forepaw and Hindpaw	92	76	97	68

Attachment I.

Number of Runts (Body weight less than 30 grams)

Treatment mg/kg/day	0	1	5	75
Total litters	16	14	15	15
Male Runts	1	4	7	9
Female Runts	3	4	2	12
Total Runts	4	8	9	21
Number of litters with Runts	4	3	4	9

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STUDY REVIEW

Chemical: Atrazine
Test Material: Atrazine Technical
Study/Action Type: Teratology study

STUDY IDENTIFICATION:

"A Teratology Study of Atrazine Technical in Charles River Rats"

Testing Facility: The Safety Evaluation Facility, CIBA-GEIGY Corp., Summit, New Jersey
Project No.: 60-84
Report Date: 9-18-84
Study Director: Robert N. Infurna, Ph.D.
EPA Accession No.: 264979

Study Reviewed by: Geraldine S. Danford, B.A. 2/11/87.
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BACKGROUND

The Safety Evaluation Facility, CIBA-GEIGY Corp., Summit, New Jersey, conducted this teratology study of Atrazine Technical in Charles River rats during the fall of 1984.

CONCLUSION

It is concluded that the teratology study of atrazine technical in rats (CIBA-GEIGY Corporation, # 60-84) demonstrates the following:

Maternal No Observed Effect Level (NOEL): 10 mg/kg/day
Maternal Lowest Observed Effect Level (LOEL): 70 mg/kg/day

These values are based on a statistically significant decrease in bod. weight gain during the first half of the dosing period and a statistically significant reduction in food consumption for the first two days of agent administration in the 70 mg/kg/day group. The maternal mortality at the highest dose tested, 700 mg/kg/day, was 77.8%, making this group unsatisfactory for evaluation.

(See supplemental memo),

Developmental Toxicity LOEL: 10 mg/kg/day

This value is based on a three-fold increase over controls in the number of litters containing runts. No historical control data was supplied in the report to show if the control group in this particular study experienced an unusually low rate of runting.

From the data furnished, under the conditions of this study, a Developmental Toxicity NOEL cannot be set.

PROCEDURES

Test material:	Atrazine Technical
Vehicle:	3% aqueous corn starch containing 0.5% Tween 80
Dosage levels:	0, 10, 70, 700 mg/kg/day by gavage
Period of administration:	Days 6-15 of gestation
Species:	Charles River CD rats

The protocol used in this study was in compliance with those recommended in the Standard Evaluation Procedure (SEP), Teratology Studies (EPA-540/9-85-018, June 1985).

Atrazine was administered once daily by gastric intubation to females mated to males of the same strain. The study was composed of four groups and the number of females per group was 27. This number was more than adequate as the minimum recommended number is 20. The oral route of dosing was chosen because potential human exposure is by this route.

Dosing occurred daily and was performed on days 6 through 15 of presumed gestation, the period of organogenesis in the rat. The day of mating was determined by the presence of sperm in the vaginal washing and was designated as day "0" of gestation.

Identification of individual rats, housing, food, water, environment, quarantine time, and group assignments were of standard experimental design. Dosing was performed as indicated in Table 1.

Table 1: Dosing schedule

Group number	Number of females	Days of treatment	Dose (mg/kg/day)
1 (control)	27	6-15	0
2	27	6-15	10
3	27	6-15	70
4	27	6-15	700

The control group received 10 ml/kg/day of 3% corn starch containing 0.5% Tween 80 which was a volume equivalent to that received by treated rats. The volume of suspension of compound or vehicle to be administered to each animal was determined by the animal's body weight recorded on gestational days 6, 10, and 14.

According to the SEP, the highest dose of three different levels should induce overt maternal toxicity but not more than 10% maternal death. The highest dose in this study does not meet this requirement as 21 of the 27 females died during gestation.

All dams were observed daily for changes in appearance and behavior. Females were weighed on days 0, 6, 10, 14, 18, and 20 of gestation. Food consumption measurements were taken daily for gestational days 5 to 20. On day 20, the surviving dams were sacrificed and necropsied. A detailed examination and recording was made of the uterine contents. The females were examined for gross pathology and any maternal gross lesions were excised for microscopic evaluation.

The fetuses were numbered in order of their positions in the uterus from the ovarian end of the left horn to the ovarian end of the right horn. Apparently viable fetuses were weighed and the fetuses examined for gross abnormalities. Approximately 1/2 of the fetuses were fixed for visceral examination and 2/3 were prepared for skeletal examination.

RESULTS

A. MATERNAL EVALUATION

Maternal Mortality

A high rate of maternal death (21/27) occurred in the 700 mg/kg/day group. This was 77.8% of the females in this group. No deaths were observed in the control, 10, or 70 mg/kg/day groups.

Clinical and Pathological Observations

No maternal toxicity was observed in the control group and the only toxic sign recorded in the 10 mg/kg/day group was rales in one dam. Although alopecia was statistically significant in the intermediate group it was not considered to be biologically significant since it is commonly observed in control animals at The Safety Evaluation Facility. Statistically significant symptoms observed in the high dose (700 mg/kg/day) group included: salivation in 13/27, oral/nasal discharge in 12/27, ptosis in 11/27, swollen abdomens in 8/27 and blood on the vulva in 7/27. Incidental findings in this group included alopecia and swollen hindleg. (Table 2)

At necropsy high-dose females were found to have a statistically elevated increase in enlarged stomachs (26/27), enlarged adrenals (12/27), and discolored lungs (3/27).

Pregnancy Rates

The pregnancy rates for the 0, 10, 70, and 700 mg/kg/day groups were, respectively, 88.9%, 85.2%, 92.6%, and 96.3%. The pregnancy rates given are within acceptable ranges for rats. Woo and Hoar reported a 90% pregnancy rate for pooled data on 2452 Charles River CD rats in control studies.[1] All females were examined for uterine contents following early death or sacrifice on gestation day 20, showing the pregnancy rate is based on total number of females mated and not only on those surviving until sacrifice on gestation day 20. (Table 3)

Maternal Body Weight Data

Individual maternal body weights were recorded on days 0, 6, 10, 14, 18, and day 20 of presumed gestation. (Table 4)

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[1]Woo, D.C., and R.M. Hoar. Reproductive performance and spontaneous malformations in control Charles River CD rats: a joint study by MARTA, Teratology 19: 54A, 1979.

Prior to the dosing period, no differences in body weig gain were noted in any group. (Table 5) In the low dose group, statistically significant increase in body weight gain was fou during days 6-10 of gestation. In the intermediate group, bo weight gains were significantly reduced during the first half agent administration (days 6-10). Statistically signific reductions in body weights were observed in the high dose gr on days 14, 18, and 20 of gestation. (Table 4) The body wei changes for the high dose group were also significantly redu for gestation days 10-14, 14-18, 18-20, and 6-20. (Table 5)

Maternal liver weights in the high dose group w significantly reduced: however, this was not conside biologically significant because no significant dose-rela differences were found for liver weight as a percentage of day 20 body weight.

Maternal Food Consumption

Statistically significant reductions in food consump were observed in the high dose (700 mg/kg/day) group during dosing and post-dosing periods. This is considered a sig severe maternal toxicity. The intermediate group (70 mg/kg/ also had a significant reduction in food consumption for first two days of compound administration (days 6 and 7). Statistically significant increases in food consumption observed on gestation day 9 in the low dose group ar gestational day 17 (post-dosing) in the intermediate dose gr (Table 6)

Reproduction Data at C-Section

The results of cesarean section, including ovarian, ute and litter data, are presented in Table 7.

The mean number of corpora lutea, uterine implantatio resorptions, and mean implantation efficiency were compara all groups examined, except for some discrepancies found i for the high dose group. These discrepancies seem to resul the high number of maternal deaths in this group and the v times during pregnancy at which the necropsy was performed reported results compare favorably with control data in C River CD rats compiled by Woo and Hoar.[2] The mean number o fetuses per litter was 12.7 (control), 13.7 (10 mg/kg), 14 mg/kg), and 12.4 (700 mg/kg).

[2]Woo and Hoar, op. cit.

B. DEVELOPMENTAL TOXICITY EVALUATION

Fetal Data

The fetal data collected at cesarean section are summarized in Table 7.

As noted earlier, no significant change in the litter size was observed. No dead fetuses were found in the control, low or intermediate dose groups. In the high dose group only two fetuses in the same litter were found dead. No variations in fetal sex ratio were detected in any group.

Table 7: Summary of Fetal Weights (grams)

Parameter		Treatments (mg/kg/day) 0	10	70	700
Fetal weight	Male	3.4	3.6	3.4	1.9*
Fetal weight	Female	3.3	3.3	3.2	1.8*

* Different from the control group at P<0.01.

Mean fetal weights in the low and intermediate groups are similar to the weights observed in the control group. (Table 7) In the control, fetal weights were 3.4 grams (males) and 3.3 grams (females). These values are comparable with those compiled by Wood et al[3] for 24 day fetuses (3.5 grams). The high dose group exhibited a statistically significant decrease in fetal weight (p<0.01). The weights were 1.9 grams (males) and 1.8 grams (females).

The reviewers studied a possible increase in the number of runts (body weight less than 2.5 grams) found in the treated groups. The results of this study are presented in the table in Attachment 1. In the 700 mg/kg/day group, 27 viable fetuses were produced, and all but 2 of these were runts. A trend toward runting was found in 10 mg/kg/day and 70 mg/kg/day groups in both the number of fetuses and the number of litters affected. These increases occurred in a dose-related manner.

[3] Wood and Hoar, op, cit.

Malformations and Variations

External and visceral findings in all litters of this study are consistent with the data compiled by Woo and Hoar. In an examination of 28,142 fetuses they found only 49 (0.2%) exhibited external malformations. The incidences of visceral and skeletal malformations were 1.2% and 0.7%, respectively.[4] Banerjee and Durloo also agreed that a low rate of malformations was observed in the Charles River CD rat and reported that no pronounced visceral anomalies were observed except for hydroureter (1.36%).[5]

Occurrence of malformations and variations are recorded in Table 8 and Table 9. Skeletal examinations were not conducted in the high dose group because fetal size and weight were severely reduced. Fetotoxicity, attributable to maternal toxicity, was observed in the intermediate group as exhibited by increased skeletal variations, considered developmental delays. While results of individual endpoints of ossification delay showed statistically significant increases in this group, these data were not significant when total number of fetuses with variations were compared on a fetal and litter basis. Four types of skeletal malformations were observed. Polydactyly and rib agenesis occurred in separate fetuses of the low dose group. Rudimentary thirteenth ribs occurred in six control, one low dose and two intermediate dose fetuses. Centrum/vertebrae agenesis occurred in three control fetuses. These malformations were not dose-related and are not considered compound related. Visceral variations in the treated groups were not increased significantly over those in the control groups. Thus there was no indication in this study that atrazine was teratogenic in the rat.

Discussion

Administration of atrazine technical to Charles River CD rats from days 6-15 of gestation resulted in maternal toxicity during and after the treatment period at the 700 mg/kg/day dose level. Signs of toxicity in the high dose level group included death (21 of 27 dams), reduced food consumption, reduced weight gain, salivation, ptosis, swollen abdomen, oral/nasal discharge, and bloody vulva. Maternal toxicity was also found at the 70 mg/kg/day dose level. Toxicity signs in this group included reduced food consumption, reduced body weight, and reduced weight gain. No maternal toxicity was observed in the 10 mg/kg/day or control groups. It can be concluded that a maternal NOEL in rats can be set at 10 mg/kg/day. The maternal LOEL in this study is 70 mg/kg/day.

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[4] Woo and Hoar, op. cit.

[5] Banerjee, B.N. and R.S Durloo, Incidence of teratological anomalies in control Charles River C-D strain rats, Toxicology 1: 151-154, 1973.

The number of resorptions was not dose related in any of the groups. Only two fetuses were found dead and both of these were found in the high dose group; however, this was not statistically significant. The mean number of live fetuses and the sex ratio were unaffected by increasing dosage in the 0, 10, 70, and 7[illegible] mg/kg/day groups. Fetal size and body weight were severely reduced in the high dose group. This fetal toxicity was considered to occur as a result of severe maternal toxicity. The body weights of all other treatment groups were similar to the weights of the control group; however, a trend toward runting was observed in all three treated groups, occurring in a dose-related manner. The increase in total number of runts is not as significant as the increase in the percent of litters containing runts.* In this study, the 10 mg/kg/day group had 3 litters (13[illegible] of the litters containing runts, while the control group had 1 litter (4.3% of the litters) containing runts. No skeletal examination was performed on the high dose fetuses due to their small size; however, a statistically significant increase in skeletal variations was observed in the intermediate group. This was considered the result of developmental delays. This type of developmental delay is usually not permanent and therefore not considered a malformation. No dose-related malformations were observed in any of the treatment groups. These reviewers conclude that, under the conditions of this study, the developmental toxicity LOEL of atrazine in rats is 10 mg/kg/day based on an increase in runting. A developmental NOEL cannot be determined from this study from the data provided.

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* [illegible] the valid number of sampling units and [illegible] consideration [illegible] studies [illegible] teratogenesis or carcinogenesis. [illegible] 1970.