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

SU # 080803

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

August 6, 1991

MEMORANDUM:

Subject: ID# 100-542 Atrazine
Review and Discussion of Atrazine and Hydroxyatrazine Developmental Toxicity Studies in the Rat.

From: Myron S. Ottley, Ph. D.
Section IV
Toxicology Branch I
Health Effects Division (H7509C)

Tox Chem # 63
HED Proj.# 9-1412
Record # 244936

To: ~~Barbara Briscoe~~ PM50
Special Review and Reregistration Division (H7508W)

Thru: Marion P. Copley, D.V.M., D.A.B.T.
Section Head
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

Marion Copley 8/6/91

CONCLUSION

Toxicology Branch I has reviewed the developmental toxicity data on atrazine and hydroxyatrazine, as well as Dynamac's reviews of these data.

The studies are both classified as Core Supplementary, because the purity of the test compounds was not specified. Upon receipt of this information, the studies can be reclassified as Core Minimum.

No significant data gaps or areas of serious concern have bee identified at this time.

ACTION REQUESTED

To review 1) Developmental Toxicity Study of Atrazine in the Rat (Study # 382049; MRID #410652-01). See attached DER.

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2) **Developmental Toxicity Study of Hydroxyatrazine in the Rat (Study # 872202; MRID # 410652-02). See attached DER.**

SUMMARY Maternal and Developmental Toxicities Observed Following Treatment in Segment II Studies			
	Atrazine	Hydroxy-atrazine	Diaminochloro-triazine[1]
MATERNAL EFFECT(S)	Decreased food consump. Decreased body wt. gain.	Decreased food consump.	Decreased food consump. Decreased body wt. gain.
LOEL mg/kg/d	100	125	75
NOEL mg/kg/d	25	25	25
DEVELOPMENTAL EFFECT(S)	Increased delayed ossific. of skull bones.	Decreased body wt. Increased delayed ossific. of skull bones.	Decreased body weight. Increased delayed ossific. of skull and other bones.
LOEL mg/kg/d	100	125	25
NOEL mg/kg/d	25	25	2.5

DISCUSSION

As can be expected, the developmental toxicity profiles of atrazine and hydroxyatrazine are quite similar in terms of both

[1]Diaminochlorotriazine was reviewed in previous actions. Data are included here for comparison purposes only.

the nature of effects observed, and the dose levels at which they occur. In both cases, delayed ossification of certain skull bones was the most sensitive endpoint with LOELs being 10C and 125 mg/kg/d (decreased body was also observed at this level in the hydroxyatrazine treated offspring). At higher dose levels, an increase in the total number of external anomalies was observed in the hydroxyatrazine treated offspring.

The profile of developmental effects of diaminochloro-triazine is similar to that of atrazine and hydroxyatrazine. Diaminotriazine, reviewed in a separate action, produces similar types of effects in rats, but is toxic at lower dose levels than atrazine or hydroxyatrazine. The NOEL is lower by one order of magnitude (2.5 mg/kg/d vs. 25 mg/kg/d for atrazine and hydroxyatrazine).

In conclusion, these chemicals appear to be primary developmental toxicants in the rat via oral exposure. They exert significant effects on the conceptus at moderate dose levels.

003493

EPA: 68D80056
DYNAMAC No.: 232-A
TASK No.: 2-32A
May 6, 1991

DATA EVALUATION RECORD

ATRAZINE

Developmental Toxicity Study in Rats

STUDY IDENTIFICATION: Giknis, M. L. A teratology (segment II) study in rats. (Unpublished study No. 882049, conducted and submitted by Ciba-Geigy Corporation, Summit, NJ; dated February 23, 1989.) MRID No. 410652-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 5/6/91

1. CHEMICAL: 2-Chloro-4-ethylamino-6-isopropylamine-1,3,5-triazine.

2. TEST MATERIAL: Atrazine technical, batch No. FL 841802, was used; no other information was provided.

3. STUDY/ACTION TYPE: Developmental toxicity study in rats.

4. STUDY IDENTIFICATION: Giknis, M. L. A teratology (segment II) study in rats. (Unpublished study No. 882049, conducted and submitted by Ciba-Geigy Corporation, Summit, NJ; dated February 23, 1989.) MRID No. 410652-01.

5. REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
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6. APPROVED BY:

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Date: 5/6/91

Marion Copley, Ph.D.
EPA Reviewer and Section Head
Review Section II
Toxicology Branch I
(H-7509C)

Signature: Marion Copley
Date: 8/7/91

DATA EVALUATION RECORD 000493

STUDY TYPE: Developmental toxicity Guideline §83-3.

MRID NUMBER: 410652-01.

TEST MATERIAL: Atrazine.

SYNONYM(S): AAtrex, Farmco Atrazine, Gesaprim, Zeaphos.

STUDY NUMBER(S): 882049.

SPONSOR: Ciba-Geigy Corporation, Summit, NJ.

TESTING FACILITY: Ciba-Geigy Corporation, Summit, NJ.

TITLE OF REPORT: A Teratology (Segment II) Study in Rats.

AUTHOR: Giknis, M. L.

REPORT ISSUED: February 23, 1989.

CONCLUSIONS: In a developmental toxicity study in which groups of mated female rats were administered, by gavage, atrazine at dose levels of 0, 5, 25, and 100 mg/kg/day, maternal toxicity as evidenced by reduced body weight gain and food consumption was observed at the high-dose level. The NOEL and LOEL for maternal toxicity were 25 and 100 mg/kg/day, respectively.

Developmental toxicity, evidenced by increased incidences of delayed ossification of bones of the skull, was observed at the highest dose level. Consequently, the NOEL and LOEL were 25 and 100 mg/kg/day, respectively.

Classification: CORE Supplementary data. This study may be reclassified if the purity of the test material is provided.

A. MATERIALS:

Test Compound: Purity: Not reported.
Description: Not reported.
Lot No.: Not reported, batch No. FL 841802.
Contaminants: Not reported.

Vehicle(s): Three percent aqueous cornstarch containing 0.5% Tween 80.

Test Animals: Species: Rat.
Strain: Sprague-Dawley [Crl:COBS CD(SD)BR].
Source: Charles River Breeding Laboratories, Kingston, NY.
Age: Approximately 2.5 months of age at mating.
Weight: Ranged from 237 to 310 g at initiation of dosing.

B. STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of atrazine technical when administered by gavage from gestation days 6 through 15, inclusive.

Mating: Female rats were mated 2:1 with sexually mature male rats approximately 4.5 months of age of the same strain. Day 0 of gestation was designated as the day on which sperm were detected following vaginal lavage.

Group Arrangement: Females were randomly assigned to groups using the Teros computer system. The method was not reported.

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Test group	Dose level (mg/kg/day)	Number assigned per group
Control	0	26
Low dose	5	26
Mid dose	25	26
High dose	100	26

Dosing: All doses were administered in a volume of 10 mL/kg body weight/day and prepared once during the dosing period. The dosing solutions were analyzed for concentration prior to study initiation. The test material was reportedly stable for 24 hours at room temperature and 36 days at 2-8°C. Therefore, the test suspensions were stored at 2-8°C during the study. Dosing was based on the most recently recorded body weight measured on gestation day (GD) 6, 8, or 12.

Observations: The animals were checked twice daily for mortality and once daily for changes in appearance and behavior. Body weight and food consumption were measured on GD 0, 6, 8, 12, 16, and 20. Any female rats found dead were subjected to a gross necropsy. Surviving animals were sacrificed on GD 20. Examination at necropsy consisted of:

- A gross necropsy that included an inspection of the thoracic and abdominal cavities;
- Measurement of uterine weight;
- Inspection of uterine contents and determination of the number of live and dead fetuses and resorptions; and
- Determination of the number of corpora lutea.

The viable fetuses were examined in the following manner:

- Sex was determined;
- Fetal weights were measured;
- Gross abnormalities were recorded;

- Approximately half of the fetuses were placed in Bouin's solution and subjected to a visceral inspection using the method of Monie, Kho, and Morgan[1]; and

- Skeletal abnormalities of the remaining fetuses were detected using the method of Staples and Schnell.[2]

Statistical Analysis: The following analyses were conducted:

- Maternal body weight, body weight gain, and food consumption; ANOVA with Bartlett's test and Dunnett's test.

- Fetal weight; Healy analysis.

- Number of corpora lutea, implantations, resorptions, viable fetuses, postimplantation losses, and fetal sex ratios; Mantel's trend test with the Blom conversion factor, Chi-bar test.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated March 16, 1989, was provided.

- A signed Statement of Compliance with EPA GLPs, dated February 23, 1989, was provided.

- A signed Quality Assurance Statement, dated February 23, 1989, was provided.

C. RESULTS:

1. Dose Analysis: Analysis of the concentrations of dosing suspensions revealed that actual concentrations ranged from 96 to 100% of target concentrations. The test material was homogeneously suspended in the vehicle.

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[1]Monie, I., W. Kho, and J. Morgan. 1965. Dissection procedures for rat fetuses permitting Alizarin Red staining of skeleton and histological study of viscera. Supplement to Teratology Workshop Manual. Berkeley, CA, January 25-30.

[2]Staples, R.E. and V.L. Schnell. 1964. Refinements in rapid clearing technique in the KOH-Alizarin Red S method for fetal bone. Stain Technology 39: 62.

2. Maternal Toxicity:

Mortality: One pregnant female from the high-dose group died on GD 20. No abnormal findings were noted at necropsy. No other deaths were observed.

Abortion: No abortions occurred during the study. One female from the mid-dose group delivered prematurely on GD 19. No other premature deliveries occurred.

Clinical Observations: Salivation was observed in 18/26 high-dose females. The incidence of alopecia was slightly increased in the high-dose group (from 1/26 in controls to 5/26 in high-dose animals). Incidences of clinical signs were similar among control rats and rats from the low- and mid-dose groups.

Body Weight: The investigators supplied the following data: body weights from high-dose dams were significantly reduced ($p \leq 0.05$) when compared with controls on GD 8, 12, and 16. Corrected body weight (minus uterine weight) for the entire gestation period (GD 0-20) was also significantly decreased ($p \leq 0.05$) in high-dose dams when compared with controls. A summary of body weight gains and corrected body weight gain is presented below.

TABLE 1. Body Weight Gains and Corrected Body Weight Gains(g)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-16)	Post-Dosing Period (GD 16-20)	Entire Gestation Period (GD 0-20)	Corrected Body Weight Gain for Entire Gestation[b] (GD 0-20)
0	33.6 ± 9.57[c]	57.2 ± 11.27	63.5 ± 10.27	154.3 ± 20.96	79.0 ± 13.41
5	32.9 ± 7.96	55.9 ± 12.22	65.4 ± 9.01	154.2 ± 18.73	76.4 ± 14.98
25	33.0 ± 8.19	56.4 ± 12.26	66.8 ± 8.31	157.6 ± 19.84	76.4 ± 15.24
100	33.1 ± 7.86	47.0 ± 10.44*	68.5 ± 10.56	149.1 ± 20.52	63.1 ± 16.85*

[a]Data were extracted from study No. 882049, Table 6.5, and Appendices 7.5-7.8.

[b]Corrected body weight gain = Total body weight gain minus uterine weight.

[c]Mean ± S.D.

*Significantly different from controls ($p \leq 0.05$).

Significant reductions ($p \leq 0.05$) in body weight gain were observed in dams from the high-dose group on GD 6-8 and 6-16 when compared with that of controls. Corrected body weight gain for the entire gestation period was also significantly reduced ($p \leq 0.05$) for high-dose females when compared with controls. No significant changes in body weight or body weight gain were observed in the low- and mid-dose dams compared with controls.

Food Consumption: Significant reductions ($p \leq 0.05$) in food consumption were observed in high-dose dams during GD 6-8 and 8-12. Food consumption was also significantly decreased ($p \leq 0.05$) in high-dose dams during the entire dosing period (GD 6-16) (Table 2). Food consumption was similar among the control and low- and mid-dose groups.

Gross Pathological Observations: One dam from the low-dose group had hollow, discolored kidneys. Another female from the mid-dose group had a fluid-filled, hollow right kidney. No other abnormalities were reported.

Cesarean Section Observations: A summary of gestational parameters is presented in Table 3. The pregnancy rate of the high-dose group was decreased as compared with controls. However, the number of implantations and live fetuses per dam were significantly increased ($p \leq 0.05$) when compared to controls. All other parameters were similar among control and test groups.

3. Developmental Toxicity

External Examinations: External variations and malformations were found only in control fetuses. One control fetus had anophthalmia and microphthalmia. One fetus from a second control litter had multiple external malformations, and another fetus from this litter had ectrodactyly and a filament tail.

Visceral Examinations: Several kidney-related variations, i.e., short or absent renal papillae, dilated ureters, and pitted kidneys, were observed with similar incidences in all groups, including controls. Malformations were observed only in control and low-dose fetuses. One fetus from a control litter had multiple malformations that included agenesis of the kidney, ureters, adrenal gland, and diaphragm; irregularly shaped liver and spleen; and an additional lung lobe. A second fetus in this litter had small stomach, spleen, and kidneys. One low-dose fetus had an irregularly shaped heart, unilobular lung, and a fused pulmonary artery and aorta. No other visceral malformations were observed.

TABLE 2. Food Consumption Data (g/interval)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-16)	Post-Dosing Period (GD 16-20)
0	135 ± 14.1	253 ± 25.8	118 ± 11.3
5	133 ± 11.7	243 ± 22.7	117 ± 8.6
25	135 ± 11.4	245 ± 25.7	118 ± 10.9
100	134 ± 22.3	221 ± 20.7*	116 ± 22.0

[a]Data were extracted from study No. 882049, Table 6.3, and Appendices 7.3 and 7.4.

*Significantly different from controls ($p \leq 0.05$).

TABLE 3. Cesarean Section Observations[a]

Parameter:	Dose Group (mg/kg/day) 0	5	25	100
No. animals assigned	26	26	26	26
No. animals pregnant	26	25	25	22
Pregnancy rate (%)	100	96	96	85
Maternal wastage				
No. died	0	0	0	1
No. died/pregnant	0	0	0	1
No. nonpregnant	0	1	1	4
No. aborted	0	0	0	0
No. premature delivery	0	0	1	0
Total corpora lutea	459	442	422	384
Corpora lutea/dam	17.7 ± 2.10[b]	17.7 ± 2.10	16.9 ± 1.99[c]	18.3 ± 2.37
Total implantations	364	365	366	350
Implantations/dam	14.0 ± 2.50	14.6 ± 1.96	14.6 ± 2.22[c]	15.9 ± 2.62[d]*
Total live fetuses	349	345	347	324
Live fetuses/dam	13.4 ± 2.50	13.8 ± 2.10	14.5 ± 1.84	15.4 ± 2.89*
Total resorptions	15	20	12	14
Early/dam	0.54	0.80	0.50	0.62
Late/dam	0.04	0	0	0.05
Resorptions/dam	0.58 ± 0.70	0.80 ± 1.00	0.50 ± 0.93	0.67 ± 0.86
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Fetal weight (g)	3.4 ± 0.19	3.5 ± 0.24	3.5 ± 0.23	3.4 ± 0.19
Preimplantation loss (%)*	19.8 ± 15.49	17.2 ± 0.09	11.2 ± 9.38	11.8 ± 8.54
Postimplantation loss (%)	4.1 ± 5.28	5.5 ± 6.85	3.4 ± 6.83	4.5 ± 5.77
Sex ratio (% male)	50	45	49	58

[a]Data were extracted from study No. 882049, Tables 6.6 and 6.7, and Appendices 7.12 and 7.13.

[b]Mean ± S.D.

[c]Includes one female that delivered prematurely.

[d]Includes one pregnant female that died during the study.

*Calculated by the reviewers.

*Significantly different from controls ($p \leq 0.05$).

Skeletal Examinations: No skeletal malformations were observed. However, significant increases ($p \leq 0.05$) in the fetal and litter incidence of incomplete ossification of the hyoid, occipitals, and parietals were reported at the high-dose level (Table 4). In addition, a significant increase ($p \leq 0.05$) in the fetal and litter incidences of incomplete ossification of the interparietals was reported at the low-, mid-, and high-dose levels when compared with control incidences. The incidences of other visceral variations were similar among control and test groups.

D. DISCUSSION/CONCLUSION:

1. Maternal Toxicity: The maternal body weight and food consumption of high-dose dams were significantly reduced compared with control animals. In addition, clinical signs of toxicity (salivation) were observed at the high-dose level. Based on these effects, the NOEL and LOEL for maternal toxicity are 25 and 100 mg/kg/day, respectively.

   The pregnancy rate of high-dose females was reduced compared with controls. However, since administration of the test material began on GD 6, this effect was not considered to be compound related.

2. Developmental Toxicity:

   a. Deaths/Resorptions: A slight, nonsignificant increase in the number of resorptions/dam was observed in low- and high-dose groups when compared with controls. However, since the uteri of females that appeared nonpregnant were not stained with ammonium sulfide to determine the presence of resorption sites, we were not able to assess the significance of these data. The number of implantations/dam and the number of live fetuses/litter were slightly higher for the low- and mid-dose groups and significantly higher at the high-dose level when compared with controls. However, this is not considered to be compound-related since dosing was initiated after completion of implantation on GD 6. These increases are probably due to low values in the control groups.

   b. Altered Growth: Fetal body weight was similar among control and test groups, although the number of live fetuses/litter was significantly higher at the high-dose level.

TABLE 4. Summary of Fetal Skeletal Variations[a]

Observation	Dose Level (mg/kg/day) 0	5	25	100
No. litters (fetuses) examined	26 (181)	25(178)	24(179)	21(166)
Interparietals not completely ossified				
Fetal (%) incidence	16 (8.8)	42 (23.6)*	43 (24.0)*	73 (44.0)*
Litter (%) incidence	10 (38.5)	15 (60.0)*	14 (58.3)*	20 (95.2)*
Parietals not completely ossified				
Fetal (%) incidence	4 (2.2)	9 (5.1)	7 (3.9)	14 (8.4)*
Litter (%) incidence	3 (11.5)	6 (24.0)	3 (12.5)	9 (42.9)*
Occipitals not completely ossified				
Fetal (%) incidence	14 (7.7)	26 (14.6)	22 (12.3)	35 (21.1)*
Litter (%) incidence	10 (38.5)	13 (52.0)	10 (41.7)	16 (76.2)*
Hyoid not completely ossified				
Fetal (%) incidence	20 (11.0)	26 (14.6)	27 (15.1)	36 (21.7)*
Litter (%) incidence	10 (38.5)	9 (36.0)	13 (54.2)	15 (71.4)*

[a]Data were extracted from study No. 882049, Table 6.8, and Appendices 7.13 and 7.18.

*Significantly different from controls ($p \leq 0.05$).

Delayed ossification of several bones of the skull was observed in fetuses from all dose groups as compared with controls. The author reported significant increases in the fetal and litter incidences of incomplete ossification of the interparietals in the low-, mid-, and high-dose groups and incomplete ossification of parietals, occipitals, and hyoid in the high-dose group when compared with controls. Upon comparison with the laboratory's historical control data, it is evident that both fetal and litter incidences of incomplete ossification of the interparietals in the low- and mid-dose groups were within the normal range. They appeared elevated because the concurrent controls were low (fetal range: 9-31%, mean: 21 ± 7%; litter range: 30-78%, mean: 58 ± 13%). The incidences of incomplete ossification in the skull bones in high-dose groups were, in addition to being statistically significantly elevated, outside the normal range. This was considered to be a compound-related effect.

d. Developmental Anomalies: No compound-related effects were observed.

Developmental toxicity, as evidenced by increased incidences of delayed ossification of bones in the skull, was observed at 100 mg/kg/day. Therefore, the NOEL and LOEL for developmental toxicity were 25 and 100 mg/kg/day, respectively.

3. Study Deficiencies: The following deficiencies were noted.

a. No protocol was presented; details of the methods used were limited.

b. The purity of the test material was not reported.

c. No analytical methods were reported. Therefore, the adequacy of the methods used could not be assessed.

d. The uteri of females that did not appear pregnant were not reported as being stained to determine the presence of early resorptions. Therefore, the pregnancy rate may be inaccurate.

e. Individual data on maternal necropsy observations were not presented. Only abnormal observations were reported.

E. CLASSIFICATION: CORE Supplementary Data.

Maternal NOEL = 25 mg/kg/day.
Maternal LOEL = 100 mg/kg/day.
Developmental Toxicity NOEL = 25 mg/kg/day.
Developmental Toxicity LOEL = 100 mg/kg/day.

F. RISK ASSESSMENT: Not applicable.

[illegible]
[illegible]
[illegible]

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EPA: 68D80056
DYNAMAC No.: 232-B
TASK No.: 2-32B
May 6, 1991

DATA EVALUATION RECORD

HYDROXYATRAZINE

Developmental Toxicity Study in Rats

STUDY IDENTIFICATION: Giknis, M.L.A. Hydroxyatrazine technical. A toxicology (segment II) study in rats. (Unpublished study No. 872202 conducted and submitted by Ciba-Geigy Corporation, Summit, NJ; dated February 14, 1989.) MRID No. 410652-02.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 5/6/91

1. CHEMICAL: Hydroxyatrazine.

2. TEST MATERIAL: Hydroxyatrazine technical, batch No. FL 870869, was used. No other information was presented.

3. STUDY/ACTION TYPE: Developmental toxicity study in rats.

4. STUDY IDENTIFICATION: Giknis, M.L.A. Hydroxyatrazine technical. A toxicology (segment II) study in rats. (Unpublished study No. 872202 conducted and submitted by Ciba-Geigy Corporation, Summit, NJ; dated February 14, 1989.) MRID No. 410652-02.

5. REVIEWED BY:

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Principal Reviewer
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Date: May 6, 1991

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Signature: Pia Lindström
Date: 5/6/91

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

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Date: 5/6/91

Marion Copley, D.V.M.
D.A.B.T.
EPA Reviewer and
Section Head
Review Section II
Toxicology Branch I
(H-7509C)

Signature: Marion Copley
Date: 8/7/91

DATA EVALUATION RECORD

STUDY TYPE: Developmental toxicity; Guideline §83-3.

MRID NUMBER: 410652-02.

TEST MATERIAL: Hydroxyatrazine technical.

SYNONYM(S): None found.

STUDY NUMBER(S): 872202.

SPONSOR: Ciba-Geigy Corporation, Agricultural Division, Greensboro, NC.

TESTING FACILITY: Ciba-Geigy Corporation, Toxicology/Pathology Division, Summit, NJ.

TITLE OF REPORT: Hydroxyatrazine Technical. A Teratology (Segment II) Study in Rats.

AUTHOR: Giknis, M.L.A.

REPORT ISSUED: February 14, 1989.

CONCLUSIONS: In a developmental toxicity study in which mated female rats were administered 0, 5, 25, or 125 mg/kg/day of hydroxyatrazine by gavage on days 6 to 15 of gestation, maternal toxicity as evidenced by decreases in food consumption was observed at the high-dose level. The NOEL and LOEL for maternal toxicity were 25 and 125 mg/kg/day, respectively.

Developmental effects that included decreased fetal body weight and incomplete ossification of several skull bones were observed at 125 mg/kg/day. Therefore, the NOEL and LOEL for developmental toxicity were 25 and 125 mg/kg/day, respectively.

Classification: CORE Supplementary data. This study can be reclassified if purity of the test material is provided.

A. MATERIALS:

Test Compound: Purity: Not reported.
Description: Not reported.
Lot No.: Not reported; batch No. FL 870869.
Contaminants: Not reported.

Vehicle(s): Three percent aqueous cornstarch containing 0.5% Tween 80.

Test Animals: Species: Rat.
Strain: Sprague-Dawley [Crl:COBS CD(SD)BR].
Source: Charles River Breeding Laboratories, Kingston, NY.
Age: Females--1.5 months and males--6 months at mating.
Weight: Females--205-289 g at initiation of treatment.

B. STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of hydroxyatrazine technical when administered by gavage from gestational days (GD) 6 through 15, inclusive.

Mating: A total of 120 females were paired with 55 sexually mature males of the same strain (No other information was provided). Day 0 of gestation was designated as the day on which sperm was detected following vaginal lavage.

Group Arrangement: Sperm-positive females were randomly assigned to dose groups. The randomization method was not reported. The dose groups were assigned as follows:

Test group	Dose level (mg/kg/day)	Number assigned per group
Control	0	26
Low dose	5	26
Mid dose	25	26
High dose	125	26

Dosing: All doses were administered in a volume of 10 mL/kg of body weight/day. No information on the frequency of dose preparation was reported. The dosing solutions were analyzed for concentration, homogeneity, and stability prior to initiation of dosing. Dosing was based on the most recent body weight recorded on GD 6, 8, or 12.

Observations: Animals were observed twice daily for mortality and once daily for abnormal condition. Body weight and food consumption were measured on GD 0, 6, 8, 12, 16, and 20. Dams were sacrificed on GD 20. Examinations at sacrifice consisted of:

- Inspection of contents of the thoracic and abdominal cavities;
- Inspection of the internal structure of the kidney;
- Measurement of gravid uterine weight;
- Determination of numbers of corpora lutea and implantations, including live and dead fetuses and resorptions; and
- Position of fetuses in the uterus.

The fetuses were examined in the following manner:

- Sex of individual fetuses was determined;
- Individual body weight of viable fetuses was measured;
- Gross (external) anomalies were recorded;

- About half of the fetuses were placed in Bouin's solution and examined viscerally using the method of Monie, Kho, and Morgan[1]; and

- The remaining half of the fetuses was examined to determine skeletal anomalies using the method of Staples and Schnell.[2]

Statistical Analysis: The following statistical analyses were used:

- Maternal body weight, body weight gain, and food consumption--ANOVA, Bartletts test, and Dunnett's test.

- Fetal body weight--Healy analysis.

- Number of corpora lutea, implantations, resorptions and viable fetuses, postimplantation loss, and fetal sex ratios--Mantel's trend test with the Blom conversion factor and Chi-bar test.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated March 14, 1989, was provided.

- A signed Statement of Compliance with EPA GLPs, dated February 14, 1989, was provided.

- A signed Quality Assurance Statement, dated February 14, 1989, was provided.

C. RESULTS:

1. Test Material Analysis: Analysis of dose solutions revealed that actual concentrations ranged from 92 to 98% of target concentrations. Stability tests revealed that

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[1]Monie, I., Kho, W., and Morgan, J. 1965. Dissection procedures for rat fetuses permitting Alizarin Red staining of skeleton and histological study of viscera. Supplement to Teratology Workshop Manual, Berkeley, CA, January 25-30.

[2]Staples, R.E. and Schnell, V.L. 1964. Refinements in rapid clearing techniques in the KOH-Alizarin Red S method for fetal bone. Stain Technology 39: 62.

the test material was stable in the vehicle for 24 hours at room temperature and for 47 days at 6°C. The dosing suspensions were stored at 2 to 8°C.

2. Maternal Toxicity:

Mortality: No deaths occurred during the study.

Abortion: No abortions or premature deliveries were observed during the study.

Clinical Observations: Alopecia of both forelimbs was observed in one mid-dose female; no other abnormal clinical observations were noted. The water delivery system apparently malfunctioned on 2 consecutive days prior to initiation of dosing, resulting in temporary weight loss (as judged by clinical observation, not measurement of body weight) in several animals from each group on those days.

Body Weight: No statistically significant changes in body weight (data not presented) or body weight gain (Table 1) were observed during the study.

TABLE 1. Mean Body Weight Gains (g ± S.D.)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-16)	Post-Dosing Period (GD 16-20)	Corrected Body Weight Gain During Gestation Period[b] (GD 0-20)
0	38 ± 11.1	58 ± 12.1	55 ± 10.5	77 ± 15.3
5	39 ± 12.3	58 ± 10.5	61 ± 16.3	82 ± 18.2
25	34 ± 10.4	54 ± 13.1	54 ± 11.8	71 ± 12.6
125	35 ± 16.9	56 ± 17.2	52 ± 12.0	69 ± 20.9

[a]Data were extracted from study No. 872202, Table 6.5 and Appendix 7.5.

[b]Corrected Body Weight = body weight gain for entire gestational period minus gravid uterine weight.

Food Consumption: A significant reduction ($p<0.05$) in food consumption during GD 8-12 (data not shown) and 6-16 was observed for the high-dose group (Table 2). In addition, food consumption during GD 16-20 was significantly increased for the low-dose group.

TABLE 2. Food Consumption Data (g/animal/day)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period[b] (GD 6-16)	Post-Dosing Period (GD 16-20)	Entire Gestation Period[b] (GD 0-20)
0	21 ± 1.8	23 ± 2.5	25 ± 2.5	23 ± 2.1
5	21 ± 1.7	24 ± 2.1	27 ± 2.9*	24 ± 1.9
25	20 ± 1.7	22 ± 2.1	26 ± 2.8	23 ± 1.7
125	21 ± 1.9	21 ± 3.1*	25 ± 3.1	22 ± 2.6

[a]Data were extracted from study No. 8672202, Table 6.3 and Appendix 7.3.

[b]Calculated by the reviewers; statistical analysis performed using ANOVA and Dunnett's test.

*Significantly different from controls ($p<0.05$).

Gross Pathological Observations: Two females from the high-dose group had enlarged, mottled kidneys. Hollow kidneys were observed in one control female. Other findings included fused placentas in a mid-dose female and excessive blood in the uterus of a control female. No histological examinations were conducted.

Cesarean Section Observations: No significant differences in any gestational parameters were observed between control and test groups (Table 3). One mid-dose dam delivered a litter with no viable fetuses. The body weights of male and female fetuses from high-dose dams were significantly reduced ($p<0.05$) when compared with controls.

3. Developmental Toxicity:

External Examinations: One fetus from a high-dose litter had gastroschisis, and another fetus from a second high-dose litter had an umbilical hernia. No control litters were affected. The incidence of total external malformations was significantly increased ($p<0.05$) for high-dose litters when compared with controls.

TABLE 3. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	5	25	125
No. animals assigned	26	26	26	26
No. animals pregnant	25	23	23[b]	22
Pregnancy rate (%)	96	89	89	85
Maternal wastage				
No. died	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	1	3	3	4
No. aborted	0	0	0	0
No. premature delivery	0	0	0	0
Total corpora lutea	410	408	399	378
Corpora lutea/dam	16.4 ± 3.32[c]	17.7 ± 2.47	17.4 ± 2.79	17.2 ± 2.40
Total implantations	348	331	306	327
Implantations/dam	13.9 ± 3.17	14.4 ± 2.92	13.3 ± 4.34	14.9 ± 1.52
Total live fetuses	335	306	283	311
Live fetuses/dam	13.4 ± 3.39	13.3 ± 3.34	12.3 ± 4.76	14.1 ± 1.67
Total resorptions	13	25	23	16
Early/dam	0.5 ± 0.65	1.1 ± 1.81	1.0 ± 1.40	0.7 ± 0.83
Late/dam	0	0	0.4 ± 0.21	0
Resorptions/dam	0.5 ± 0.65	1.1 ± 1.81	1.0 ± 1.38	0.7 ± 0.83
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Mean fetal weight (g)				
Male	3.6 ± 0.04	3.7 ± 0.04	3.6 ± 0.05	3.5 ± 0.04*
Female	3.4 ± 0.05	3.5 ± 0.05	3.4 ± 0.05	3.3 ± 0.05*
Preimplantation loss (%)[d]	16.1 ± 12.00	19.0 ± 14.42	24.4 ± 22.06	12.6 ± 9.53
Postimplantation loss (%)	5.6 ± 10.62	7.4 ± 12.69	11.6 ± 22.59	4.9 ± 5.55
Sex ratio (% male)	50	51	52	50

[a]Data were extracted from study No. 872202, Table 6.6 and Appendix 7.12.

[b]One female had a litter with no viable fetuses.

[c]Mean ± S.D.

[d]Calculated by the reviewers.

*Significantly different from controls ($p<0.05$).

Visceral Examinations: No visceral malformations were observed in fetuses from control or test groups. The incidences of visceral variations were similar among control and test groups (Table 4).

Skeletal Examinations: Cleft palate was observed in one fetus from the mid-dose group. No other skeletal malformations were observed. Significant increases ($p<0.05$) in fetal and litter incidences of incomplete ossification of the hyoid and interparietals and lack of ossification of proximal phalanges and metacarpals of the forepaw were observed in the high-dose group when compared with controls (Table 4).

D. DISCUSSION/CONCLUSIONS:

1. Maternal Toxicity: Food consumption for high-dose dams was significantly reduced only during the dosing period and was similar to controls during the pre- and postdosing intervals and during the entire gestation period. Body weight gain for this dose group was slightly decreased during the gestational period, but the changes were not significantly different from controls. In fact, the dams may have tolerated a higher dose than 125 mg/kg/day of hydroxyatrazine without overt toxicity.

   A nonsignificant trend towards reduced pregnancy rate was observed in the test groups when compared with controls (Fisher's exact test and Cochran Armitage trend test). The biological significance of this is unclear because mating and implantation occurred before dosing was initiated.

   Based on a significant decrease in food consumption during the dosing period, the maternal LOEL and NOEL were 125 and 25 mg/kg/day, respectively.

2. Developmental Toxicity:

   a. Deaths/Resorptions: The number of resorptions was increased in dams from the low- and mid-dose groups when compared with controls. This was due mainly to the complete or partial loss of one litter in each group. In the low-dose group, one dam had a total of eight resorptions and six live fetuses. The entire litter (five fetuses) of the mid-dose dam was resorbed. Furthermore, the number of resorptions was similar among control and high-dose litters. Therefore, the increased resorptions observed at the low- and mid-dose levels were not considered to be compound related.

TABLE 4. Summary of Fetal Malformations and Variations[a]

Findings	Dose Level (mg/kg/day) 0	5	25	125
Visceral				
No. fetuses (litters) examined	158 (24)[b]	147 (23)	136 (21)[b]	151 (22)
Renal papilla (short)				
Fetal (%) incidence	26 (16)	20 (14)	11 (8)	7 (5)
Litter (%) incidence	11 (46)	8 (35)	6 (29)	6 (27)
Dilated ureters				
Fetal (%) incidence	10 (6)	14 (10)	7 (5)	5 (3)
Litter (%) incidence	5 (21)	6 (26)	5 (24)	2 (9)
Skeletal				
No. fetuses (litters) examined	177 (25)	159 (23)	147 (22)	160 (22)
Cleft palate				
Fetal (%) incidence	0	0	1 (1)	0
Litter (%) incidence	0	0	1 (5)	0
Interparietals not completely ossified				
Fetal (%) incidence	35 (20)	57 (36)	42 (29)	70 (44)*
Litter (%) incidence	14 (56)	19 (83)	14 (64)	20 (91)*
Parietals not completely ossified				
Fetal (%) incidence	9 (5)	17 (11)	16 (11)	15 (9)
Litter (%) incidence	7 (28)	8 (35)	9 (41)	8 (36)
Occipitals not completely ossified				
Fetal (%) incidence	25 (14)	29 (18)	27 (18)	37 (23)
Litter (%) incidence	10 (40)	10 (43)	12 (55)	15 (68)
Hyoid not completely ossified				
Fetal (%) incidence	11 (6)	26 (16)	14 (10)	27 (17)*
Litter (%) incidence	5 (20)	9 (39)	8 (36)	12 (55)*

(Continued)

TABLE 4. Concluded

Findings	Dose Level (mg/kg/day) 0	5	25	125
Proximal phalanges not ossified				
Fetal (%) incidence	169 (96)	153 (96)	139 (95)	159 (99)*
Litter (%) incidence	24 (96)	23 (100)	22 (100)	22 (100)*
Metacarpals not ossified				
Fetal (%) incidence	67 (39)	58 (36)	61 (41)	96 (60)*
Litter (%) incidence	21 (84)	18 (78)	19 (86)	21 (95)*

[a]Data were extracted from study No. 872202, Tables 6.8 and 6.9 and Appendices 7.16 and 7.17.

[b]One control and one mid-dose dam had litters with one viable fetus which was processed for skeletal examination.

*Significantly different from controls ($p<0.05$).

b. Altered growth: The body weights of both male and female fetuses were significantly reduced at the high-dose level.

Significant increases in the fetal and litter incidences of incomplete ossification of the hyoid and interparietals were observed at the high-dose level. These incidences were also outside the range of the historical control data. In addition, significantly increased incidences of incomplete ossification were observed in the proximal phalanges and metacarpals at the highest dose level. However, the incidences of concurrence and historical control data are too high (up to 100%) for a meaningful analysis, and the reviewers consider these results not to be of biological importance.

c. Developmental Anomalies: No compound-related findings were observed.

Based on decreased fetal body weight and increased incidences of incomplete ossification in the hyoid and interparietals at the high-dose level, the developmental toxicity NOEL and LOEL were 25 and 125 mg/kg/day, respectively.

3. Study Deficiencies: The following deficiencies were noted:

a. The purity and description of the test material were not presented.

b. No analytical methods for determining concentration, homogeneity, and stability of the dosing suspensions were reported. Therefore, the appropriateness of the methods used could not be assessed.

c. Individual data on maternal necropsy observations were not presented. Only abnormal observations were reported. Therefore, the summary data could not be verified.

d. The authors did not report staining the uteri with ammonium sulfide to detect the presence of early resorptions. Therefore, unless press-plates were used instead, the pregnancy rate may be inaccurate.

E. CLASSIFICATION: CORE Supplementary data.

Maternal NOEL = 25 mg/kg/day.
Maternal LOEL = 125 mg/kg/day.
Developmental Toxicity NOEL = 25 mg/kg/day.

F. **RISK ASSESSMENT**: Not applicable.