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FINAL

DATA EVALUATION REPORT

PROMETRYN

Study Type: Developmental Toxicity - Rat

Prepared for:

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Guideline Series 83-3: Developmental Toxicity

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DATA EVALUATION REPORT

STUDY TYPE: Developmental toxicity in - Rat (83-3)

EPA IDENTIFICATION NUMBERS

Tox Chem. No.: 097

PC No.: 080805

MRID No.: 404575-17

TEST MATERIAL: 2,4-Bis(isopropylamino)-6-(methylthio)-s-triazine

SYNONYMS: Prometryn, Prometryne, Prometrex, Primatol Q, G-34161

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

STUDY NUMBER: MIN 862228 REPORT NUMBER: 87030

TESTING FACILITY: Pharmaceutical Division, Ciba-Geigy Corporation, Summit, NJ

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

AUTHORS: J. Weissenborn, E.J. Levy, M.L.A. Giknis, and E.T. Yau

REPORT ISSUED: December 18, 1987

CONCLUSIONS: A developmental toxicity study was conducted in which Sprague-Dawley rats were administered prometryn via gavage at 0, 10, 50, or 250 mg/kg/day during gestational days (GD) 6-15, inclusive.

Maternal NOEL - 50 mg/kg/day
Maternal LOEL - 250 mg/kg/day (salivation and decreased body weight and food consumption)

Developmental Toxicity NOEL - 50 mg/kg/day
Developmental Toxicity LOEL - 250 mg/kg/day (decreased fetal body weight and increased incomplete ossification of sternebrae, metacarpals)

CLASSIFICATION: Core Supplementary Data - Upgradable. This study does not meet the requirements set forth under EPA Guideline Series 83-3 for a developmental toxicity study in rats because the following information was not submitted: purity of the compound, homogeneity analysis of the test

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compound in the vehicle, frequency of preparation and storage of dose suspensions, and data to confirm the results of the stability analysis. If these data are submitted and found to be acceptable, the study may be upgraded.

A. MATERIALS

Test compound

Purity:	Not reported
Solubility:	Water and organic solvents
Melting point:	118°-120°C
Description:	White crystalline
Lot number:	FL 841559
Contaminants:	Not reported

Vehicle: 3% aqueous cornstarch with 0.5% Tween 80 (source not reported)

Test animals

Species:	Rat
Strain:	Crl:COBS CD (SD) BR Sprague-Dawley
Source:	Charles River Laboratories, Kingston, NY
Age:	Not reported
Weight:	218-281 g on GD 0
Males used:	Resident, stock males, weight--453-744 g

B. STUDY DESIGN

This study was designed to assess the potential of prometryn to cause developmental toxicity in rats when administered daily via gavage from GD 6 through 15, inclusive.

Mating: Following approximately 1 week of acclimation, 140 females were mated with 60 males of the same strain and source. The day on which mating was confirmed (presence of sperm in the vaginal washing) was designated day 0 of gestation.

Animal husbandry: Food (#5002 Purina® Certified Rodent Chow) and tap water were available ad libitum throughout the study. A 14:10-hour light/dark cycle was maintained. Temperature and humidity ranges were 23°C ± 3° and 50% ± 20%, respectively.

Group arrangement: Animals were assigned to dose groups using computer-generated random numbers as follows.

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Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	26
Low dose	10	26
Mid dose	50	26
High dose	250	26

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

Dose administered: Doses were administered daily via gavage on GD 6 through 15 in a volume of 10 mL/kg. The most recently recorded body weights were used to calculate the concentration of the doses. Dosing suspensions were not adjusted for active ingredient. Purity and stability of the test compound were analyzed by the sponsor; homogeneity and stability of the test material in the vehicle as well as concentrations of the dosing suspensions were determined during or after the study by the testing laboratory.

Observations: Animals were observed twice daily for mortality, moribundity, and overt signs of toxicity. Body weight was recorded on GD 0, 6-16, and 20; food consumption was recorded on GD 0 and 6 and daily thereafter. On GD 20, dams were sacrificed by asphyxiation with CO_2, and litters were delivered by cesarean section. Examination of the dams at sacrifice included the following:

- Gross pathology observations of visceral organs
- Gravid uterine weights
- Number of corpora lutea
- Number of implantation sites
- Numbers of resorptions (early and late) and live and dead fetuses

Uteri from apparently nonpregnant animals were not confirmed for pregnancy status (by staining with 10% ammonium sulfide solution to detect early embryo loss or by using press plates).

Examination of live fetuses included the following:

- Individual fetal uterine position, weight, and sex
- External anomalies
- Visceral anomalies for approximately one-half of the fetuses using the method of Monie, Kho, and Morgan (1965)

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- Skeletal anomalies for the remaining half of the fetuses using the method of Staples and Schnell (1964)

Statistical analysis: The following methods were used.

- Maternal body weight, body weight change, and food consumption--ANOVA, Bartlett's test for homogeneity of variance, and Dunnett's test
- Fetal weight--Healy analysis
- Numbers of corpora lutea, implantation sites, resorptions, and viable and dead fetuses; percent postimplantation loss; and fetal anomalies--Mantel's trend test with equally spaced linear scores, using a continuity correction of one-half the difference between adjacent scores (if over two-thirds of the dams from all treatment groups pooled together were totally unaffected) or Chi-square test with conversion to normal scores using Blom's method (if at least one-third of the dams were affected)
- Fetal sex ratios--Mantel's trend test using equally spaced linear scores (two-sided version)

Compliance

- A signed Statement of No Data Confidentiality Claim, dated December 21, 1987, was provided.
- A signed Statement of Compliance with EPA and OECD GLPs, dated December 21, 1987, was provided.
- A signed Quality Assurance Statement, dated December 18, 1987, was provided.

C. RESULTS

1. Test Material Analysis

Dosing suspensions for concentration (all dose levels included) ranged from 99% to 106% of target. The vehicle was reported to be stable for 36 days in storage at room temperature or at 2°-8°C; the test compound in the vehicle was reported to be stable for 24 hours at room temperature and for 33 days at 6°C (qualitative statements only; no data submitted for verification). Homogeneity data were not submitted.

2. Maternal Toxicity

Mortality: No mortalities were observed.

Abortion: No abortions were observed.

Clinical observations: Compound-related clinical signs, observed at 250 mg/kg/day, were manifested as a nonsignificant increase in the incidence of salivation (0, 0, 0, and 4 animals at 0, 10, 50, and 250 mg/kg/day respectively). Additional incidental clinical signs, noted

at all dose levels, included alopecia, chromodacryorrhea, crooked teeth, rales, and sores and were not considered treatment related.

Body weight: A summary of maternal body weight gain for selected intervals is presented in Table 1; detailed results are presented in the text. Compound-related decreases in body weight and weight change were observed at 250 mg/kg/day.

Maternal body weight (data not shown) decreased significantly from GD 7 to 16 (4-10%) at 250 mg/kg/day and from GD 8 to 10 (4-5%) at 50 mg/kg/day. While these decreases were considered to be due to treatment, at 50 mg/kg/day they were <5% and not biologically relevant. Corrected body weight on GD 20 also decreased significantly at 250 mg/kg/day (5%).

Maternal body weight gain decreased significantly at 250 mg/kg/day during GD 6-8 (-6.0 g versus +8.6 g in the control group, data not shown); GD 8-12 (7.5 g at 250 mg/kg/day versus 18.7 g in the control group, data not shown); GD 6-16 (55%, Table 1); GD 0-20 (10%, Table 1); and GD 0-20 (corrected weight gain) (80%, Table 1). During the post-dosing period (GD 16-20), body weight gain increased significantly at 250 mg/kg/day (128%). These effects were all considered to be due to treatment.

Food consumption: A summary of food consumption data (g/animal/interval) is presented in Table 2; detailed results are presented in the text. A compound-related decrease in food consumption was observed at 250 mg/kg/day.

Food consumption for the following intervals decreased significantly at 250 mg/kg/day: GD 6-7 (63%), GD 7-8 (54%), GD 8-9 (67%), GD 9-10 (67%), GD 10-11 (60%), GD 11-12 (75%), GD 12-13 (80%), and GD 13-14 (78%). Also at 250 mg/kg/day, but for the entire dosing period (GD 6-16), food consumption decreased significantly (73%); for the post-dosing period (GD 16-20) and for GD 18-19, it increased significantly (109% and 118%, respectively).

Gross pathology observations: No compound-related gross pathology findings were observed. Incidental observations included two dams at 10 mg/kg/day, one with an enlarged spleen and uterus filled with brownish fluid, the other with fused placenta.

Cesarean section observations: A summary of cesarean section data is presented in Table 3. Compound-related effects were observed at 250 mg/kg/day. Fetal body weight for both males and females decreased significantly at this dose level. Incidental (but statistically significant) decreases were noted in the number of resorptions (and consequently in percent postimplantation loss) at 50 and 250 mg/kg/day.

3. Developmental Toxicity

Summaries of visceral and skeletal anomalies are presented in Tables 4 and 5; detailed results are presented in the text. Compound-related effects were observed at 250 mg/kg/day.

External examinations: No external malformations or variations were observed.

Visceral examinations: One incidental visceral malformation was observed (Table 4). One fetus at 50 mg/kg/day exhibited dilated lateral ventricles.

Variations (Table 4) consisting of short and/or absent renal papilla(e), dilated ureter, and pitted kidneys were observed. The incidence of short renal papilla(e) at 250 mg/kg/day increased significantly above control. This was considered to be a secondary effect of the test compound owing to decreased fetal body weight.

Skeletal examinations: Rudimentary 13th rib(s) occurred in 1 fetus from the control group, 9 (7 litters) from the 10 mg/kg/day group, 3 (3 litters) from the 50 mg/kg/day group, and 1 from the 250 mg/kg/day group (Table 5).

Variations (Table 5) included incomplete ossification; bipartite/irregular vertebrae and/or sternebrae, wavy and/or rudimentary ribs; and misaligned/bipartite/irregular sternebrae. Fetal (but not litter) incidences of "sternebrae not completely ossified" and "metacarpals not ossified" were significantly increased at 250 mg/kg/day. This was considered to be a compound-related effect. All other variations were observed at similar incidences in all dose groups.

D. DISCUSSION/CONCLUSIONS

1. Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) to be included with the evaluation of the study. Criterion 6 was only partially satisfied (see Test Material Analyses below for discussion). All other criteria were satisfied.

2. Test Material Analyses

Concentrations of the test material in the vehicle were within ±6% of target. Stability data were not submitted and therefore the qualitative statement could not be verified. Homogeneity was not reported.

3. Maternal Toxicity

Compound-related maternal toxicity was observed at 250 mg/kg/day and was manifested as an increased incidence of clinical signs and decreased body weight, weight gain, and food consumption during the dosing period. At 50 mg/kg/day, body weight was also significantly decreased during the dosing period. However, the decrease was ≤5% and no other signs of toxicity were noted at this dose level. Therefore, this was not considered to be biologically significant.

Based on these results, the maternal NOEL and LOEL were 50 and 250 mg/kg/day, respectively.

4. Developmental Toxicity

a. Deaths/resorptions: No compound-related effects were observed.

b. Altered growth: At 250 mg/kg/day, fetal body weight was significantly decreased, and incomplete ossification in the sternebrae and metacarpals was observed at significantly increased fetal incidences. These fetal growth retardations were considered to be compound-related effects.

c. Developmental anomalies: No compound-related malformations or variations were observed.

Based on altered growth, the developmental NOEL and LOEL were 50 and 250 mg/kg/day, respectively.

5. Study Reporting Deficiencies

Food consumption was only reported as g/animal/interval and no statistical analyses were applied to consumption for the entire gestation period. It would have been more informative if the food consuption data had been reported as food efficiency/day and if an analysis for GD 0-20 was included.

Homogeneity data were not reported, although the protocol states that such analyses were conducted.

Stability data were not submitted, and the report did not state how frequently the dosing suspensions were prepared. Consequently, the qualitative statement that the compound was stable in the vehicle cannot be verified.

Age of the animals was not reported.

Uteri from apparently nonpregnant animals were not confirmed for pregnancy status (by using press plates at time of necropsy or by staining with ammonium sulfide to detect early resorption). Therefore, the pregnancy rates that are reported by the study authors cannot be verified.

Table 1. Mean Body Weight Gain (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)	Gestation Period (GD 0-20)	Corrected Body Weight Change[b]
0	34.5 ± 7.6 (23)[c]	58.5 ± 9.5 (23)	51.7 ± 10.2 (23)	144.7 ± 20.4 (23)	67.6 ± 16.5 (23)
10	32.0 ± 8.0 (23)	56.0 ± 9.1 (23)	51.4 ± 16.4 (24)	138.9 ± 22.4 (24)	63.3 ± 16.6 (24)
50	30.0 ± 5.5 (20)	55.5 ± 7.2 (20)	56.6 ± 10.0 (20)	142.1 ± 16.7 (20)	64.9 ± 12.4 (20)
250	32.2 ± 8.6 (22)	31.9 ± 20.3* (22)	66.0 ± 14.1* (22)	130.1 ± 15.0* (22)	54.0 ± 13.1* (22)

[a]Data were extracted from Study No. MIN 862228, Table 6.4.

[b]Weight gain during gestation minus gravid uterine weight

[c]Number of animals used in mean

*Significantly different from control ($p \leq 0.05$)

Table 2. Mean Food Consumption (g/animal/interval ± 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
0	136.7 ± 14.9 (23)[b]	250.6 ± 29.9 (22)	107.2 ± 13.1 (23)
10	127.8 ± 12.7 (23)	237.8 ± 16.9 (23)	107.2 ± 11.8 (24)
50	127.5 ± 11.1 (20)	232.8 ± 14.7 (19)	111.4 ± 12.5 (20)
250	132.4 ± 12.9 (22)	182.2 ± 30.8* (22)	117.3 ± 14.6* (22)

[a]Data were extracted from Study No. MIN 862228, Table 6.2.

[b]Number of animals used in mean

*Significantly different from control ($p \leq 0.05$)

Table 3. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	10	50	250
No. animals mated	26	26	26	26
No. animals pregnant	23	24	20	22
Pregnancy rate (%)	88.5	92.3	76.9	84.6
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	3	2	6	4
No. aborted	0	0	0	0
No. premature delivery	0	0	0	0
No. litters examined	23	24	20	22
Total corpora lutea[b]	369	404	333	379
Corpora lutea/dam[c]	16.0 ± 1.9	16.8 ± 2.0	16.7 ± 2.7	17.2 ± 2.7
Total implantations[b]	333	346	278	330
Implantations/dam[c]	14.5 ± 2.0	14.4 ± 3.4	13.9 ± 2.1	15.0 ± 1.5
Total live fetuses[b]	313	316 (23)[d]	272	318 (21)
Live fetuses/dam[c]	13.6 ± 2.5	13.7 ± 3.9	13.6 ± 2.1	14.5 ± 1.7*
Total resorptions[b]	20	30 (23)	6	12 (21)
Early	20	30	6	12
Late	0	0	0	0
Resorptions/dam[c]	0.9 ± 0.9	1.3 ± 2.3	0.3 ± 0.6*	0.6 ± 0.8*
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Fetal weight/litter (g)[c]				
Males	3.61 ± 0.05	3.64 ± 0.05	3.69 ± 0.06	3.37 ± 0.05*
Females	3.46 ± 0.05	3.44 ± 0.05	3.48 ± 0.05	3.23 ± 0.05*
Preimplantation loss (%)[b]	9.4	14.6	16.9	11.7
Postimplantation loss (%)	6.6	8.5	2.1*	3.7*
Sex ratio (% male)	50.8	45.2	48.2	53.8

[a]Data were extracted from Study No. MIN 862228, Tables 6.7 and 6.8 and Appendix 7.12.

[b]Calculated by the reviewers using individual animal data

[c]Mean ± S.D

[d]Number of litters

*Significantly different from control ($p \leq 0.05$)

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Table 4. Incidences of Fetal Visceral Anomalies[a]

Findings[b]	Dose Level (mg/kg/day) 0	10	50	250
No. fetuses (litters) examined	151 (23)	152 (24)	131 (20)	155 (22)
Malformation				
Brain, lateral ventricles dilated	0	0	1	0
Total no. fetuses (litters) with visceral malformations	0	0	1	0
Variations				
Renal papilla(e) short	37 (14)	22 (14)	41 (15)	54* (18)
Renal papilla(e) absent	5 (2)	3 (3)	11 (5)	3 (2)
Dilated ureter(s)	28 (9)	27 (10)	20 (10)	11 (6)
Pitted kidney	0	0	1	0
Total no. fetuses (litters) with visceral variations	50 (16)	37 (15)	50 (15)	58* (19)

[a]Data were extracted from Study No. MIN 862228, Tables 6.9, 6.10, and 6.11.

[b]More than one type of anomaly may be found in one fetus.

Table 7. Incidences of Fetal Skeletal Malformations and Selected Variations[a]

Findings[b]		Dose Level (mg/kg/day) 0	10	50	250
No. fetuses (litters) examined		162 (23)	164 (24)	141 (20)	163 (20)
Malformation					
Rudimentary 13th rib		1	9 (7)	3 (3)	1
Total no. fetuses (litters) with skeletal malformations		1	9 (7)	3 (3)	1
Variations					
Centrum/vertebrae,	bipartite	2 (2)	2 (2)	3 (3)	4 (3)
	irregular	1	0	0	0
Ribs,	wavy	2 (2)	5 (4)	2 (2)	0
	rudimentary 14th	1	0	1	2 (2)
Sternebrae,	bipartite	2 (2)	1	0	1
	irregular	1	1	0	0
	misaligned	16 (9)	9 (8)	13 (8)	11 (10)
	not completely ossified	86 (22)	98 (24)	84 (20)	115* (22)
Metacarpals,	not ossified	41 (17)	45 (14)	42 (15)	86* (20)
Total no. fetuses (litters) with skeletal variations		162 (23)	164 (24)	141 (20)	163 (22)

[a]Data were extracted from Study No. MIN 862228, Tables 6.9, 6.12, and 6.13.

[b]More than one type of anomaly may be found in one fetus.

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

ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	YES	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group).
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	Y/N	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	YES	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	YES	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with a * are supplemental, may not be required for every study.

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