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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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008349

May 9 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of a Developmental Toxicity Study in Rats
with Technical Diuron (Guideline 83-3)

TO: Lois Rossi
Registration Division, H7505C

FROM: David S. Liem, Ph.D. *David S Liem 4/19/91*
Section II, Toxicology Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head *K. Clark Swentzel 4/19/91*
Section II, Toxicology Branch II/HED (H7509C)
and
Marcia van Gemert, Ph.D., Branch Chief *M van Gemert 4/25/91*
Toxicology Branch II/HED (H7509C)

MRID No.: 402288-01 ID No.: 035505 DP BARCODE: D162091
CASWELL #: 410 HED Project #: 1-0808

ACTION REQUESTED

To review a developmental toxicity study of H-16035 (Diuron)
Administered by Gavage to Rats.

CONCLUSIONS

H-16035 was orally dosed to pregnant rats at levels of 0, 16, 80, or 400 mg/kg/day (25 rats/group) during gestation days 6-15. Maternal toxicity was evidenced by decreased body weight and food consumption in the 80 and 400 mg/kg group and increased mean liver/body weight ratio in the 400 mg/kg dose group. Developmental toxicity was evidenced by statistically significant increases in delayed ossification of the vertebrae and sternbrae and decreased fetal weights at the 400 mg/kg dose group.

Maternal Toxicity NOEL > 16 mg/kg.
Developmental Toxicity NOEL > 80 mg/kg.

CLASSIFICATION

Core-minimum. This study satisfies the guideline requirements (83-3) for a Developmental Toxicity study in rats. Although, the dosing concentration ranges were outside the acceptable range for several samples in the 80 mg/kg dose, this dose does not generally affect the reviewer's ability to set NOELs for maternal and developmental toxicity.

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Primary Review by: Deborah L. McCall, Review Section III, Toxicology Branch II, (H7509C) /HED *Deborah L. McCall* 4/16/91

Secondary Review by: James Rowe, Ph.D, Section Head, Review Section III, Toxicology Branch II, (H7509C) /HED *James N. Rowe* 4/17/91

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DATA EVALUATION RECORD

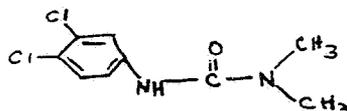
Study Type: Developmental Toxicity study in rats (583-3)

EPA Identification No.s: EPA MRID (Accession) No.: 402288-01
Caswell No.: 410
HED Project No.: 1-0807

Test Material: H-16035

Synonyms: Diuron

Chemical Structure:



Molecular Formula: $C_9H_{10}Cl_2N_2O$

Sponsor: E.I. duPont de Nemours and Company, Inc.

Study Number(s): HLO 410-86

Testing Facility: Argus Research Laboratories, Inc.
935 Horsham Road
Horsham, PA 19044
(215) 443-8710

Title of Report: Developmental Toxicity Study of H-16035 Administered by Gavage to Rats

Author(s): George E. Dearlove and Reviewed by Mildred S. Christian and Alan M. Hoberman.

Report Issued: June 16, 1986

Conclusions: H-16035 was orally dosed to pregnant rats at levels of 0, 16, 30, or 400 mg/kg/d (25 rats/group) during gestation days 6-15. Maternal toxicity was evidenced by decreased body weight and food consumption in the 30 and 400 mg/kg group and increased mean liver/body weight ratio in the 400 mg/kg dose group. Developmental toxicity was evidenced by statistically significant increases in delayed ossification of the vertebrae and sternbrae and decreased fetal weights in the 400 mg/kg dose group.

Developmental Toxicity NOEL \geq 30 mg/kg.

Maternal Toxicity NOEL \geq 16 mg/kg.

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Core Classification: Core Minimum

This study satisfies the Guideline requirements (83-3), Developmental Toxicity (Teratology) Study in rats. Although, the dosing concentration ranges were outside of the acceptable range for several samples in the 80 mg/kg dose, this dose does not generally affect the reviewers' ability to set NOELS for maternal and developmental toxicity.

1. MATERIALS AND METHODS

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A. Test Compound: Purity: 99%
Description: tan granular powder
Lot No. and Stability: no information was supplied

1. Vehicle(s): 0.5% aqueous hydroxypropyl methylcellulose (w/v)
(lot #52F-0103, Sigma Chemical Company).

[Reviewers note: All dosage calculations were corrected for the percentage of active ingredient.]

B. Test Animal(s): Species: Rat, nulliparous female
Strain: Sprague-Dawley (CrI:COBS[®]CD[®] Br)
Source: Charles River Breeding Laboratories, Inc.
Kingston, New York
Age: ♂ 73 days, ♀ 70 days
Weight: ♂ 275 - 360 g, ♀ 183 - 266 g

C. Study Design: This study was designed to assess the developmental toxicity potential of H-16035 when administered by the oral route to rats on gestation days 6 through 15, inclusive.

1. Dose Selection: H-16035 was administered by gavage to naturally bred rats (8/group) during gestation days 6 - 15. The dose levels were 0, 100, 200 or 400 mg/kg in aqueous suspensions of 0.5% hydroxypropyl methylcellulose. No deaths or abortions were noted during the study. Maternal body weight gain and food consumption was decreased for all test groups as compared with the control group. An apparent dose-related increase was noted in the incidence of early resorptions in the 400 mg/kg group as compared with the control group. Average fetal body weights were reduced in the 400 mg/kg as compared to the control group. However, H-16035 did not have any effect on the number of resorptions, implantation incidences, litter size, fetal alterations or fetal sex ratios.

2. Mating: Nulliparous female rats were naturally-breed with males of the same stock and strain. The rats were placed in cohabitation for a maximum of 4 days on a one to one basis. Upon observation of either spermatozoa in a vaginal smear and/or a vaginal plug that was considered to be day '0' of gestation.

3. Group Arrangement: The presumed pregnant females were randomized to experimental and control groups by computer-generated random (weight-ordered) numbers into 4 dose groups of 25 rats each. The animals were identified by Monel[®] self-piercing ear tags and placed in individual cages.

Test Group	Dose Concentration (mg/mL)	Dose Level (mg/kg)	No. of Rats Assigned
Control	0	0	25
Low	3.2	16	25
Mid	16	80	25
High	80	400	25

4. Dosing: Oral suspensions of 0, 16, 80, 400 mg/kg/d were administered at a dose volume of 5 mL/kg once daily to the rats on gestation days 6 through 15, inclusive. Dosing was based on daily body weights. The suspensions were prepared twice weekly, and two reserve samples from each batch were retained (frozen) for analysis.

5. Observations: The animals were checked for mortality, signs of abortion, and abnormal conditions twice daily. Dams were sacrificed on day 20 of gestation by carbon dioxide. Examinations at sacrifice consisted of: macroscopic pathology, liver weights, number of corpora lutea, number and location fetuses (live/dead) in each horn, number of intrauterine resorptions and uteri of nonpregnant rats were stained with ammonium sulfide to detect early resorptions.

The fetuses were examined by: gross inspection, sex-determination, and individual fetal body weights. Historical control data were provided to allow comparison with concurrent controls (see Appendix B).

D. Statistical analysis: A copy of the statistical methods used for the data analysis is attached (Appendix A).

E. Compliance: A Quality Assurance Statement and a Statement of Compliance with FIFRA Good Laboratory Practice Standards were signed and dated June 10, 1987.

2. RESULTS

A. Analyses of Suspensions: Methods and results of analyses were provided. The nominal concentrations for the samples ranged from 88 - 112%. The low dose group (16 mg/kg) ranged from 97 - 112%, mid dose group (80 mg/kg) ranged from 42 - 124%, and the high dose group (400 mg/kg) ranged from 63 - 154% of nominal concentrations.

The dose ranges for several samples are outside of the acceptable ranges for the 80 mg/kg dose group; however this dose does not generally affect the reviewers ability to set NOELS for maternal and developmental toxicity.

B. Maternal Toxicity

1. Mortality: No animals were reported to have died during the study.

2. Clinical Observations: Cage-side observations were thin, urine-stained fur, alopecia, soft or liquid feces and crooked snout. The only treatment-related observation was thin appearance in one high dose rat. All other observations were not treatment-related or statistically significant.

3. Body Weight: The animals were weighed on days 0, and 6 through 20 of gestation. The investigators supplied the following data: group mean and individual animal data. Corrected maternal body weight data was not presented in the report.

Maternal weight gains were significantly ($P \leq 0.01$) reduced in the 80 and 400 mg/kg group during gestation days 6-16 and in the 80 mg/kg group during gestation days 6-10 as compared with the control group (see Table 1). Significant body weight loss was noted during the first several days of dosing (gestation days 6-9) for the 80 and 400 mg/kg dose group. Body weight gains for the control group were +4.5 g; compared to the 80 mg/kg group with a weight loss of -11.3 g and the 400 mg/kg group with a weight loss of -29.7 g during gestation days 6-9.

Upon completion of dosing, a significant increase in mean body weights gains occurred in the 80 and 400 mg/kg dose groups of +67.9 and +78.2 grams, respectively. However, despite this rebound effect the 80 and 400 mg/kg dose groups had maternal body weight gains which were significantly ($P \leq 0.01$) less than the control group during gestation days 0-20.

4. Food Consumption: A certified standard diet (Ralston Purina Certified Rodent Chow Meal® #5002M) and local water from stainless steel automatic watering system was provided ad libitum. Food consumption was recorded 0, 6, 10, 16, and 20.

A statistically significant ($P \leq 0.01$) decrease in mean food consumption was noted in the 80 and the 400 mg/kg dose groups during gestation days 6-16 compared to controls (see Table 2). The most noticeable effect occurred in the 400 mg/kg group during the first days of dosing (gestation days 6-10). Upon completion of dosing (gestation days 16-20) a statistically significant increase in the mean food consumption occurred in the 80 and 400 mg/kg dose groups compared with the controls. This rebound effect correlates with the same increases seen in the body weight gains. However, despite these increases in the 80 and 400 mg/kg groups, the mean food consumption was significantly ($P \leq 0.01$) less for the entire gestation period compared with the controls. Also, a similar effect was noted in mean feed consumption per kg body weight.

C. Gross Pathological Observations: The liver of each dam was removed and weighed. The mean liver weight to body weight ratio was significantly increased ($P \leq 0.01$) for the 400 mg/kg group compared to the

Table 1 - Selected Maternal Body Weights Changes

Maternal Body Weight Change (g)	0 mg/kg	16 mg/kg	80 mg/kg	150 mg/kg
Days 6-9	4.5 ± 7.1	0.5 ± 9.5	-11.3 ± 10.1**	-29.7 ± 13.9**
Days 9-12	13.0 ± 4.2	14.6 ± 6.5	4.0 ± 9.6**	-2.8 ± 13.3**
Days 16-20	57.0 ± 10.1	59.7 ± 10.7	67.9 ± 18.7**	78.3 ± 16.1**
Days 6-10	8.8 ± 5.9	6.4 ± 6.6	-9.0 ± 10.0**	-31.4 ± 15.9**
Days 6-16	39.1 ± 9.7	37.9 ± 9.4	14.6 ± 14.5**	-12.2 ± 21.6**
Days 6-20	96.2 ± 16.8	97.6 ± 16.7	82.5 ± 25.8*	66.0 ± 30.4**
Days 0-20	129.4 ± 18.6	129.7 ± 18.1	111.8 ± 26.5**	98.4 ± 30.8**

* = Significantly different from controls by Mann-Whitney U test at P<0.05.
 ** = Significantly different from controls by Mann-Whitney U test at P<0.01.
 Only pregnant rats were included in this table.

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Table 2 - Selected Mean Maternal Food Consumption in G/Day

Maternal Food Consumption (g/day)	0 mg/kg	16 mg/kg	80 mg/kg	400 mg/kg
Days 6-10	20.8 ± 4.4	20.1 ± 3.5	14.6 ± 2.9**	7.6 ± 4.2**
Days 10-16	22.8 ± 2.5	23.0 ± 2.3	17.0 ± 3.0**	12.3 ± 5.1**
Days 16-20	24.7 ± 2.3	26.1 ± 2.1*	27.1 ± 3.2**	26.9 ± 5.5**
Days 6-16	22.0 ± 1.9	21.8 ± 2.3	16.0 ± 2.5**	10.4 ± 3.2**
Days 6-20	22.8 ± 1.8	23.0 ± 2.0	19.2 ± 1.7**	15.2 ± 3.1**
Days 0-20	22.3 ± 2.1	22.5 ± 2.2	19.6 ± 1.9**	17.0 ± 2.2**

Only pregnant rats were included and rats with 100% resorptions were excluded.
 * = Significantly different from controls by Mann-Whitney U Test at $P \leq 0.05$.
 ** = Significantly different from controls by Mann-Whitney U Test at $P \leq 0.01$.
 + = Significantly different from controls by Dunnett's Test at $P \leq 0.01$.

controls (see Table 3). However, the liver weight and liver to body weight ratio of the four dosage groups were not significantly different. This observation appears to be treatment-related due to the significant decrease in body weight gains.

Table 3 - Maternal Liver Weights

	0 mg/kg	16 mg/kg	80 mg/kg	400 mg/kg
Mean Liver Weight (g)	15.99 ± 1.4	16.30 ± 1.70	15.43 ± 1.73	15.87 ± 1.64
Mean Liver/Body Weight Ratio (%)	4.06 ± 0.34	4.07 ± 0.38	4.06 ± 0.33	4.37 ± 0.23**

** = Sign. different from the controls by the Mann-Whitney U test ($P \leq 0.01$). (Includes only pregnant rats.)

D. Cesarean Section Observations: Approximately one-half of all fetuses from each litter were examined for visceral alterations by the Staples technique. These fetuses were decapitated, and then fixed in Bouin's solution for free-hand sectioning. All remaining fetuses were eviscerated and stained with alizarin red-s.

H-16035 did not adversely effect the number of corpora lutea, incidence of pregnancy, number of implantation sites, litter size compared to the control group (see Table 4). However, two dams had total resorptions at the 400 mg/kg dose level which was possibly dose-related. The average fetal body weight for the 400 mg/kg dose group was significantly decreased ($P \leq 0.01$) compared to the control group and considered to be dose-related.

E. Developmental Toxicity:

1. External Observations: No external observations were noted in the 16 or 80 mg/kg dose group. One fetus each in the control and 400 mg/kg dose group had external observations. The control fetus had micrognathia and agenesis of the external nares. Skeleton examination revealed fused nasals, short and fused mandibles and an incompletely ossified palate. The 400 mg/kg dose fetus had a depressed eye bulge, agenesis of the tongue and external nares, a cleft palate and micrognathia. Skeleton examination revealed unossified hyoid bone, incompletely ossified palate, short, fused mandibles and nasals, and small orbit. Neither of these observations are considered to be dose-related or biologically significant by the reviewer.

2. Visceral Examination: One-half of the fetuses/litter were examined using the Staples' technique. No malformations were noted in any of the groups.

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Table 4: Cesarean Section Observations^a

Dose (mg/kg)	Control	37.5	75	150
# Animals Assigned	25	25	25	25
# Nonpregnant Pregnancy Rate %	3 88%	2 92%	3 88%	3 88%
<u>Maternal Wastage</u>				
# Died	-	-	-	-
# Aborted	-	-	-	-
# Premature	-	-	-	-
Total # of Litters	22	23	22	20
Total Corpora Lutea Corpora Lutea/Dam	383 17.4 ± 3.2	414 18.0 ± 4.0	360 16.4 ± 4.6	361 ^b 18.0 ± 2.1
Total Implantations Implantations/Dam	317 14.4 ± 3.3	337 14.6 ± 3.8	313 14.2 ± 5.0	316 ^b 15.8 ± 2.7
Total Live Fetuses Live Fetuses/Dam	288 13.1 ± 3.5	306 13.3 ± 3.3	297 13.5 ± 4.7	279 14.0 ± 2.7
<u>Total Resorptions</u>				
Early	29	31	16	37 ^b
Late	-	-	-	-
Resorptions/Dam	1.3 ± 1.4	1.4 ± 1.4	0.7 ± 1.0	3.2 ± 4.8
# Dams w/ resorpts.	13 (59.1%)	18 (78.3%)	10 (45.4%)	18 (81.8%)
# Dams w/ 100% resorptions	-	-	-	2
Total Dead Fetuses	-	-	-	-
<u>Fetal Body Weight</u>				
Males/Litter	47.9%	54.4%	57.7%	52.1%
Females/Litter	52.1%	45.6%	42.3%	47.8%
Mean Litter Fetal Wgt (g)	3.36 ± 0.2	3.42 ± 0.2	3.35 ± 0.3	3.07 ± 0.4**
Preimplantation Loss (%) ^{b,c}	17.2	18.6	13.1	12.5
Postimplantation Loss (%) ^{b,c}	9.1	9.2	5.1	11.7
(%) Male/Litter	47.9 ± 15.2	54.4 ± 10.6	57.7 ± 16.2	52.1 ± 13.1

^a = Excludes nonpregnant rats.^b = Excludes two dams with 100% resorptions (#'s 28877 & 29992).^c = Calculated by the reviewer.

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3. Skeletal examination: Skeletal assessment was performed on all of the fetuses/litter (see Table 5). Dose-related significant ($P \geq 0.05$) increases were noted in the incidence of delayed ossification of the vertebrae and sternebrae for the 400 mg/kg dose group, as compared to control incidences.

F. Discussion/Conclusions:

1. Maternal Toxicity: Evidence of maternal toxicity was seen in the 80 and 400 mg/kg dose group by decreased body weight gain and food consumption and increased mean liver/body weight ration in the 400 mg/kg group.

Maternal Toxicity NOEL \geq 16 mg/kg.

2. Developmental Toxicity: At a dose of 400 mg/kg, H-16035 appeared to have effects on developmental toxicity by an increases in delayed ossification of the vertebrae and sternebrae.

- a. Deaths/Resorptions: No treatment-related effects were noted.
- b. Altered Growth: Decreased mean fetal weights were noted in the 400 mg/kg dose.
- c. Developmental Anomalies: Statistically significant increases in delayed ossification of sternebrae and vertebra in the 400 mg/kg group.
- d. Malformations: No treatment-related effects were noted.

Developmental Toxicity NOEL \geq 80 mg/kg.

G. Study Deficiencies: On page 38 the statistical tests were not identified, it was assumed to be Mann-Whitney U test with a significance level of $P \leq 0.01$.

Number of fetuses in the low dose group totaled 306 on page 82, but the total number fetuses used in most the tables (ex. table 8) is 305, please explain this discrepancy.

No information was supplied on the stability or the lot number of the compound.

H. Core Classification: Core Minimum

This study satisfies the Guideline requirements (83-3), Developmental Toxicity (Teratology) Study in rats. Although, the dosing concentration ranges were outside of the acceptable range for several samples in the 80 mg/kg dose, this dose does not generally affect the reviewers' ability to set NOELS for maternal and developmental toxicity.

Table 5: Selected Skeletal Examinations

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Dose (mg/kg)	0	16	80	400
Litters examined	22	23	22	20
Fetuses examined	288	306 ^a	297	279
<u>Hyoid:</u>				
not ossified				
affected fetuses	31	32	23	17
affected litters ^b	14	10	11	6 ⁺⁺
<u>Vertebrae:</u>				
thoracic, centra not ossified				
affected fetuses	-	-	-	4 ⁺
affected litters ^b	-	-	-	3 ⁺
<u>Ribs:</u>				
wavy				
affected fetuses ^b	2	9	10	-
affected litters ^b	1	5 ⁺	4	-
incompletely ossified (hypoplastic)				
affected fetuses ^b	-	5	2	-
affected litters ^b	-	4 ⁺	2	-
<u>Sternebrae:</u>				
not ossified				
affected fetuses ^b	3	3	2	14
affected litters ^b	3	3	2	5
incompletely ossified				
affected fetuses ^b	3	5	1	16
affected litters ^b	3	3	1	9*
<u>Xiphoid:</u>				
not ossified				
affected fetuses ^b	1	3	1	13
affected litters ^b	1	3	1	4

^a = Discrepancy in number of fetuses, reviewer counted 306 and table 8 shows 305.

^b = Some observations may be grouped together;

[%] = Percent per litter;

⁺ = Increased from control value, at $P \geq 0.05$ to $P \leq 0.10$.

⁺⁺ = Significantly less than control group value, at $P \leq 0.05$.

^{*} = Significantly different from control value, at $P \leq 0.05$.

(Data was extracted from Table 8, pg 41-42.)

Diuron

Page ___ is not included in this copy.

Pages 13 through 20 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
- FIFRA registration data.
- The document is a duplicate of page(s) _____.
- The document is not responsive to the request.

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