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

APR 19 1991

008339

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

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

TO: Lois Rossi
Registration Division, H7505C

FROM: David S. Liem, Ph.D. David Liem 4/10/91
Section II, Toxicology Branch II/HED (H7509C)

THROUGH: K. Clark Swentzel, Section Head K. Clark Swentzel 4/10/91
Section II, Toxicology Branch II/HED (H7509C)
and
Marcia van Gemert, Ph.D., Branch Chief m van Gemert 4/15/91
Toxicology Branch II/HED (H7509C)

MRID No.: 402288-02 ID No.: 035505 DP BARCODE: D162261

CASWELL #: 410 HED Project #: 1-0807

ACTION REQUESTED

To review a developmental toxicity study of H-16035 (Diuron) Administered by Gavage to New Zealand White Rabbits

CONCLUSIONS:

Four groups of 24, 24, 25, and 25 pregnant rabbits were given technical diuron in 0.5% hydroxypropyl methylcellulose, by oral gavage, at dose levels of 0, 2, 10, and 50 mg/kg/day, respectively, from day 7 to day 19 of gestation.

The maternal toxicity NOEL is determined to be 10 mg/kg/day. The maternal toxicity LOEL is 50 mg/kg/day based on decreased body weight and food consumption values during the dosing period (days 7 to 19 of gestation).

The developmental toxicity NOEL is determined to be 50 md/kg/day (highest dose tested). The developmental toxicity LOEL is more than 50 mg/kg/day.

It is noted that the lot number and the stability of the test material were not provided in the study report. The gravid uterine weights were not recorded and the net body weights (Body Weight - Gravid Uterine Weight) were not calculated.

CLASSIFICATION: Core-minimum. This study satisfies the guideline requirements (83-3) for a Developmental Toxicity study in rabbit.

008339

Primary Reviewer: David S. Liem, Ph.D. David Liem 4/8/91
Section II, Toxicology Branch II/HED
Secondary Reviewer: K. Clark Swentzel, Section Head K. Clark Swentzel 4/19/91
Section II, Toxicology Branch II/HED

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity GUIDELINE: 83-3

Test Animal: New Zealand Rabbit

EPA Identification Nos.: MRID No.: 402288-02
ID No.: 035505 DP BARCODE: D162261
CASWELL #: 410 HED Project #: 1-0807

Test Material: Diuron (H-16035) with a 99% active ingredient

Synonym: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

Dosages: 0, 2, 10, and 50 mg/kg/day

Sponsor: E.I. du Pont de Nemours & Co., Inc., Agriculture Product Department.

Study Number: HLO 332-86

Testing Facility: Argus Research Laboratories, Inc., 935 Horsham Rd., Horsham, PA 19044

Title of Report: Developmental Toxicity Study of H-16035 (Diuron) Administered by Gavage to New Zealand White Rabbits

Author: George E. Dearlove

Report Issued: May 16, 1986

CONCLUSIONS:

Four groups of 24, 24, 25, and 25 pregnant rabbits were given technical diuron in 0.5% hydroxypropyl methylcellulose, by oral gavage, at dose levels of 0, 2, 10, and 50 mg/kg/day, respectively, from day 7 to day 19 of gestation.

The maternal toxicity NOEL is determined to be 10 mg/kg/day. The maternal toxicity LOEL is 50 mg/kg/day based on decreased body weight and food consumption values during the dosing period (days 7 to 19 of gestation).

The developmental toxicity NOEL is determined to be 50 mg/kg/day (highest dose tested). The developmental toxicity LOEL is more than 50 mg/kg/day.

It is noted that the lot number and the stability of the test material were not provided in the study report. The gravid uterine weights were not recorded and the net body weights (Body Weight - Gravid Uterine Weight) were not calculated.

CLASSIFICATION: Core-minimum. This study satisfies the guideline requirements (83-3) for a Developmental Toxicity study in rabbit.

Study Title: Developmental Toxicity Study of H-16035 (Diuron) Administered by Gavage to New Zealand White Rabbits

Author: George E. Dearlove

Report Date: May 6, 1986

Study No.: HLO 332-86

Study Period: March 2 - April 15 (group 1 to 4), and March 23 to June 2 (group 5) 1986

Testing Facility: Argus Research Laboratories, Inc., 935 Horsham Rd., Horsham, PA 19044

Test Material: Diuron (H-16035); 3-(3,4-dichlorophenyl)-1,1-dimethylurea with a 99% active ingredient

Test Animal: New Zealand White Rabbit

A. OBJECTIVE

The objective of this study was to assess the developmental toxicity of diuron administered by gavage to pregnant rabbits during the period of major organogenesis (gestation days 7-19).

B. MATERIALS AND METHODS

Test Compound: Light brown (tan) granular solid; 99.0% purity
Source: E.I. du Pont de Nemours
Lot no.: Not provided
Stability: Not provided
Storage: Room temperature
Vehicle: Hydroxypropyl methylcellulose (Lot# 52F-0103 from Dow Chemical Co.)

Test Animals: Species: New Zealand White [Hra:(NZW)SPF]
Source: Hazelton Research Animals, Denver, PA 17517
Acclimation period: About 4 weeks before mating
Age: About 5 months old on arrival (11/06/85)
Body Weight: Females = 3.28 to 4.34 kg prior to artificial insemination
Caging: In individual stainless steel cages
Feed: Approximately 180 gm/day of Purina Certified Rabbit Chow #5322 (lot# AUG12 85 2B and OCT03 85 1A) and water ad libitum

Environmental Parameter: Air temperature = 64-71°F; Relative Humidity = 34% - 64%; Photoperiod: 12/12 hours light/dark cycle.

Dose Selection

Dosages for this study were based on a pilot study of five groups of five pregnant rabbits each, administered by gavage, suspensions of diuron in hydroxypropyl methylcellulose (vehicle) at dose levels of 0 (vehicle), 50, 125, 250 or 350 mg/kg/day on days 7 through 19 of gestation. The 350 mg/kg/day dose group was terminated on day 12 of gestation due to severe maternal toxicity. Dose-related increases of mottled liver and liver weights were observed at dosages of 125, 250 and 350 mg/kg/day, thin-walled stomach and/or gastric ulceration and death at 250 and 350 mg/kg/day groups. Decreased fetal body weights were observed at dosages of 250 and 350 mg/kg/day, and reduced maternal food consumption and increased dried feces were observed at all treated groups (50, 125, 250 and 350 mg/kg/day). Based on these findings, dosages of 0(vehicle), 2, 10, and 50 mg/kg/day were selected for this study (p. 112-116 of the study report).

Group Arrangement:

Out of the 104 female rabbits received, 99 were identified as acceptable and were used for this study. Within a week prior to artificial insemination, animals were assigned to the study using a weight ordered randomization method as follows:

Dose Groups	Dose Level (mg/kg/day)	Number of Animals
Control(Vehicle)	0	24
Low Dose	2	25[a]
Mid Dose	10	25
High Dose	50	25[b]

a = one female was transferred to the high dose group see "b"
b = one female, which delivered live pups prior to scheduled insemination was removed from study and replaced by one female originally assigned to the low-dose group

Mating:

Female rabbits were given pregnil (human chorionic gonadotropin) intravenously (20 USP Units/kg) and then artificially inseminated. Five males served as sires. The day of insemination was considered as day 0 of presumed gestation.

Dosing:

All doses were given via gavage to the rabbits in a volume of 5 ml/kg of body weight/day. Dose suspensions were prepared by mixing diuron in 0.5% hydroxypropyl methylcellulose daily during the dosing period. The dose administered daily to each animal was based on the animal's body weight on that day.

Dose Analyses:

The concentration and homogeneity of simulated dosing suspensions were analyzed (p. 231-235 of the study report).

Clinical Observations

The animals were checked twice daily for viability during the acclimation period and on days 0 through 6. Observations were made several times each day for mortality, moribundity and signs of toxicity during the remainder of the study period. On day 29 all surviving females were sacrificed using UTHOL injection.

Maternal Body Weights and Food Consumption Data

Individual body weights were taken five times during the acclimation period, on days 0 and daily on days 7 through 29 of gestation. Food consumption was recorded daily throughout the study, from day 0 to day 29 of gestation.

Postmortem data

On day 29, all surviving females were killed with T-61 euthanasia, and internal organ abnormalities were evaluated macroscopically. Lungs were examined to detect damage due to gavaging, and the liver was removed and weighed. The uteri and ovaries were examined and the number of implantation sites, corpora lutea, resorptions, and live/dead fetuses were recorded.

Each fetus was killed by Pentobarbitol Sodium (UTHOL), and was subjected to external macroscopic examination, sexed, and weighed. The fetal thoracic and abdominal cavities were dissected and internal organs were examined macroscopically. The brain of each fetus was free-hand transverse-sectioned and examined. All fetuses were fixed in buffered 10% formalin for skeletal staining (alizarin red) and evaluation.

Female rabbits that died or were sacrificed in a moribund condition were also examined macroscopically as described above. No maternal tissues were retained.

Statistical analysis

A number of statistical methods were used and they are appended as Appendix B.

Compliance

- o A signed Statement of Confidentiality Claim was provided.
- o A signed Statement of compliance with EPA GLP's was provided
- o A signed Quality Assurance Statement was provided.

C. RESULTS AND DISCUSSIONS

a. Dosing Suspension Analysis

Stability data of the test material was not provided. Diuron in the dosing suspensions were between 91% and 103% of nominal concentrations. Two 0.4 mg/mL solutions of 12/20/85 yielded average values of 55% and 61%. Absolute recoveries of diuron added to control dosing solutions were 100% and 99%.

b. Maternal Mortality

One control female (#10222), convulsed and died on day 0 of gestation, due to an anaphalatic reaction to insemination. It was not replaced, thus only 23 control rabbits remained. One high dose doe, which delivered pups prior to scheduled insemination was removed from study. It was replaced by one female originally assigned to the low-dose group. Therefore, only 24 females remained in the low-dose group, and 25 females were used for the high dose group. No other test animals died during the study.

c. Maternal Clinical Observations

One high dose doe (#10295) aborted on day 26 of gestation. This doe had nine implantation sites. Eight aborted pups were partially cannibalized, and the ninth pup was not found and presumably cannibalized (see attached Appendix B). This doe lost weight (-0.68 kg) on days 12 to 25 of gestation. The food consumed by this doe ranged from 0 to 5 gm/day on days 13 through 24 as compared to 179-184 gm/day on days 0 to 10 of gestation. Dried feces was observed on days 14 though 21 and days 24 and 25 of gestation The historical control rate of rabbit abortions in the testing facility was noted as 2.1%. There is a possibility that this one abortion was related to body weight loss and food consumption reduction. No other clinical signs observed were related to treatment.

d. Maternal Body Weight and Body Weight Change Data

As seen from Appendix C, the mean maternal body weights for predosing period (days 0-7) were generally comparable among the four groups. On days 10-13, 13-16 and 7-20 of gestation, the high dose mean body weight gain was statistically decreased as compared to the control. The weight loss of the high dose group on days 13-20, resulted in significantly decreased mean maternal body weight on day 20 of gestation, as compared to the control. Towards the end of the study, the mean body weight gain in the high dose group rebounded and on day 29 of gestation it was comparable to the control. The observed mean maternal body weight reduction in the high dose group is considered to be related to treatment. The gravid uterine weights (GUW) were not recorded and the net body weights (BW - GUW) were not calculated.

e. Maternal Food Consumption Values

Group mean food consumption values are presented in the attached Appendix D. As seen from this Appendix, reduced absolute and relative mean food consumption values were observed in the high dose group during the dosing period, as compared to the control. This effect was statistically significant for days 13-16 and 16-20 of gestation, as well as throughout the dosing period, days 7 to 20 of gestation. The food consumption rebounded during the post-dosing period in the high dose group, and on days 24 to 29 of gestation, the absolute and relative food consumption values were increased, as compared to the control. Reduction of maternal food consumption in the high dose group during the dosing period is considered to be related to treatment.

f. Gross Maternal Observations and Fetal Development

The maternal gross pathological observations and fetal development data are presented in the attached Appendix E.

The pregnancy rates were comparable among the groups, 91.6%, 91.7%, 92.0%, and 92% for the control, low, mid, and high dose groups, respectively.

The mean absolute and relative (liver/body weight ratios) liver weights were comparable among the groups.

The caesarean section observation data, including the numbers of corpora lutea, implantation sites, fetal resorptions, number of live fetuses, and the mean fetal weight were comparable among the groups. There is no evidence that any of these findings are related to treatment. It is noted that the gravid uterine weight and fetal crown/rump length were not recorded.

g. Fetal Alteration Data

A total of 141, 149, 161, and 166 fetuses from 21, 22, 23, and 22 litters of the control, low-, mid-, and high-dose groups, respectively, were examined. The summary fetal alteration data is presented in the attached Appendix F.

1. Fetal External Observation

As seen from Appendix F, external fetal variations were observed in one control and in two different mid dose fetuses. One control fetus had a kinked tail. One mid-dose fetus (10252-F11) had multiple alterations (acephaly, gastroschisis, short tail, and "clubbed" fore- and hind-paws), and the other one (10266-F2) had an umbilical hernia. Skeletal examination showed that the acephaly was due to agenesis of all skull bones, and the clubbed paws were due to the absence of phalanges. There is no evidence that these findings are related to treatment.

2. Fetal Visceral Data

Three high-dose fetuses of three different litters, two fetuses of two different litters of the controls and each of the low- and mid-dose groups were observed with subcorneal hemorrhage in one eye. None of these fetuses had any other developmental alteration. The other visceral alterations observed were agenesis of the intermediate lobe of the lung (one each in the control, low- and high-dose fetuses), and an umbilical hernia observed in one fetus in the mid-dose group. All observed fetal visceral alterations observed are not judged to be related to treatment.

3. Fetal Skeletal Variations and Malformations

Noteworthy fetal/litter skeletal variation data are as follows:

Four fetuses from two high dose litters had an irregularly shaped fontanelle, and 3 fetuses from 3 litters of the mid-dose group had small in one or both parietal skull bones, as compared to none in the control. These observations were not statistically different as compared to the control. It was also noted that these incidences were within the historical range of the same strain of rabbits observed in the testing facility. Other observed skull bone alterations were scattered among the dose groups. None of the skull bone variations are judged to be treatment-related.

One or both alae of the hyoid were angulated in two fetuses from two litters of the control, five low-dose fetuses from four litters, six mid-dose fetuses from five litters, and in one high-dose fetus from one litter. Thickened ossification areas of the ribs were observed in three fetuses from three litters of the control, in six mid-dose fetuses from six litters, and in two fetuses from two litters. Unossified xiphoid was observed in seven fetuses from four litters of the control, five low-dose fetuses from three litters, nine mid-dose fetuses from five litters, and six high-dose fetuses from five litters. Since no dose related trends were observed, these observations were not judged to be related to treatment.

Other low incidences (one or two fetuses from one or two litters per group) of non-treatment related skeletal variations were scattered among the dose groups. These include variations of the vertebrae, ribs, sternebrae, manubrium, claviculae, scapulae, and phalanges.

None of the incidences of fetal skeletal variations observed in the treated groups were statistically different from those of the controls and no clear dose-related trends were evident. Thus these differences are not considered compound related effects.

CONCLUSIONS

Four groups of 24, 24, 25, and 25 pregnant rabbits were given, by oral gavage, technical diuron in 0.5% hydroxypropyl methylcellulose at dose levels of 0, 2, 10, and 50 mg/kg/day, respectively, from day 7 to day 19 of gestation.

The maternal toxicity NOEL is determined to be 10 mg/kg/day. The maternal toxicity LOEL is 50 mg/kg/day based on decreased body weight and food consumption values during the dosing period (days 7 to 19 of gestation).

The developmental toxicity NOEL is determined to be 50 mg/kg/day (highest dose tested). The developmental toxicity LOEL is more than 50 mg/kg/day.

It is noted that the lot number and the stability of the test material were not provided in the study report. The gravid uterine weights were not recorded and the net body weights (Body Weight - Gravid Uterine Weight) were not calculated.

CLASSIFICATION: Core-minimum. This study satisfies the guideline requirements (83-3) for a Developmental Toxicity study in rabbit.

APPENDICES

APPENDIX A: Statistical Analysis Used in the Study (Copied from p.23-24 of the study report)

APPENDIX B: Summary Clinical Sign Observations (Copied from p. 36-37 of the study report)

APPENDIX C: Summary Mean Body Weights and Mean Body Weight Changes (Copied from p. 38-39 of the study report)

APPENDIX D: Summary Mean Absolute Food Consumption (g/day) and Mean Relative Food Consumption (g/kg/day) Data (Copied from p. 40-41 of the study report)

APPENDIX E: Summary Gross Maternal Observations and Fetal Developmental Data (Copied from p. 42 of the study report)

APPENDIX F: Summary of Fetal Alteration Data (Copied from p. 43-47 of the study report)

Diuron

Page_____ is not included in this copy.

Pages 13 through 24 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.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.