7/2/92

CASWELL FILE

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

JUL -2 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Linuron - Developmental Toxicity in the Rat (Guideline 83-3)

ToxChem No.: 528

MRID No. 00018167
HED Project Nos.:

FROM: Susan L. Makris, M.S. Susan L Makris 6/26/92
Review Section III
Toxicology Branch II
Health Effects Division (H7509C)

TO: Mr. Walter Waldrop/ Ms. Carol Peterson (PM-71)
Special Review Branch
Special Review and Reregistration Division (H7508W)

THRU: James N. Rowe, Ph.D., Section Head James N. Rowe 6/26/92
Review Section III
Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (H7509C) Marcia van Gemert 6/30/92

Registrant: E.I. du Pont de Nemours and Company
Elkton Road. P.O. Box 50
Newark, Delaware 19714

Action Requested: Reevaluate the developmental toxicology study in rats with Linuron based upon a request from the RfD Committee.

The following materials were evaluated:

1. Report for Du Pont study No. HLR 33-79, MRID No. 00018167

1 of 1

Printed on Recycled Paper

2. Copies of raw study data for study No. HLR 33-79, provided by Du Pont representative, Nancy Lomax, as an individual data supplement to HLR 33-79
3. EPA Data Evaluation Report Document No. 000676, dated December 12, 1979

Summary/Conclusions:

Linuron was administered in the diet to pregnant female Crl:CD^R rats at levels of 50, 125, and 625 ppm on Days 6-15 of gestation, inclusive (with gestation Day 1 defined as the day sperm was found).

Maternal systemic toxicity: **NOEL = 125 ppm (12.1 mg/kg/day)**
LOEL = 625 ppm (49.8 mg/kg/day)
Based upon decreased maternal body weight and food consumption values.

Developmental Toxicity: **NOEL = 125 ppm (12.1 mg/kg/day)**
LOEL = 625 ppm (49.8 mg/kg/day)
Based upon an increase in postimplantation loss and in litter/fetal incidence of resorptions.

Recommendation:

1. The previous Agency review of this study (Document No. 000676, dated 12/10/79) did not cite the deficiencies in procedure and data analysis as described above. The CORE Classification of "Guideline" given at the time of that review infers that all guideline recommendations are met. Due to the many discrepancies with §83-3 recommendations, the CORE Classification is being changed to MINIMUM.

2. In the previous Agency review, maternal systemic effects, specifically "reduced body weight gain and food consumption" were noted at the 125 ppm dose level, and this level was determined to be the maternal Low-Effect-Level. Based upon a closer evaluation of the data, a consistent, dose-related effect on maternal body weight or food consumption results cannot be identified for the 125 ppm dose level. Therefore, it is recommended that 125 ppm be listed as the maternal NOEL and 625 ppm as the maternal LOEL.

3. No changes to the assessment of developmental toxicity are suggested. (Based upon an increase in fetal resorptions, the LOEL = 625 ppm and the NOEL = 125 ppm.)

cc: G. Ghali (H7509C)

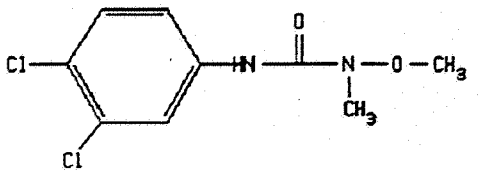
Primary Review by: Susan Lynn Makris, M.S. 6/26/92
Toxicologist, Review Section III, HED, Toxicology Branch II (H7509C)
Secondary Review by: James N. Rowe, Ph.D. 6/26/92
Section Head, Review Section III, HED, Toxicology Branch II (H7509C)

DATA EVALUATION RECORD

Study Type: Developmental Toxicity (Teratology) in the Rat (Guideline: 83-3)
Replacement DER for HED Document No. 000676, dated December 10, 1979

EPA Accession Nos.: MRID No. 00018167
Caswell No. 528

Test Material: 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea



Synonyms: Linuron, Lorox®

Sponsor: Agricultural Products Department
E.I. du Pont de Nemours and Company, Inc.
P.O. Box 80038
Wilmington, Delaware 19880

Study Numbers: Haskell Laboratory Report No. 33-79
Medical Research Project No. 3055

Testing Facility: E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714

Title of Report: Teratology Study of 3-(3,4-Dichlorophenyl)-1-Methoxy-1-Methylurea in Rats

Author: Rudolph Culik, Ph.D.

Date Report Issued: May 8, 1979

Summary/Conclusion: Linuron was administered in the diet to pregnant female Crl:CD^R rats at levels of 50, 125, and 625 ppm on Days 6-15 of gestation, inclusive (with gestation Day 1 defined as the day sperm was found).

Maternal systemic toxicity: NOEL = 125 ppm (12.1 mg/kg/day)
LOEL = 625 ppm (49.8 mg/kg/day)
Based upon decreased maternal body weight and food consumption values.

Developmental Toxicity: NOEL = 125 ppm (12.1 mg/kg/day)
LOEL = 625 ppm (49.8 mg/kg/day)
Based upon an increase in postimplantation loss and in litter/fetal incidence of resorptions.

CORE Classification: MINIMUM

A. Materials

Test substance: 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (Linuron, Lorox®)
Purity: Approximately 97%
Description: White, crystalline solid; melting point of 89-91°C
Lot No.: INZ-326-118, N.B. 7673-8
Haskell No. 10,720, MR-3055-001

Test animals: Species: rat
Strain: Crl:CD^R
Age: Not provided
Pregnancy status: Primigravida females
Source: Charles River Laboratories
North Wilmington, Massachusetts

B. Study Design

A copy of the methods presented in report No. 33-79 is appended as Attachment 1. This study was conducted to assess the developmental toxicity potential for Linuron when administered to Crl:CD^R Sprague-Dawley rats in the diet on Days 6-15 of gestation inclusive, at dose levels of 50, 125, and 625 ppm.

Group Assignment and Dosage Levels:

Time-mated rats were received from the Charles River Laboratories. The day in which sperm were found was counted as Day 1 of gestation, and the rats were received at Haskell Laboratory "as three and two days pregnant." The animals were assigned to study as follows:

Group No.	Dose (ppm)	No. per Group
1 (Control)	0	27
2 (Low)	50	27
3 (Mid)	125	27
4 (High)	625	27

Test Material Formulation and Administration:

No information on the method or frequency of formulation of the test substance in the diet was provided in the study report.

The test material was administered to the study animals in the diet on Days 6-15 of gestation. Control animals received basal diet.

No analyses were performed to determine the stability of the test substance in the diet under the conditions of the study, nor the homogeneity and concentration of the test substance in the diet mixes provided to the study animals.

Observations:

Observations for clinical signs of toxicity and changes in behavior were made daily. Body weights were recorded on the day of arrival and on Days 6, 10, 16, and 21 of gestation. Food consumption was measured and recorded at the same intervals as body weights.

Dams were sacrificed and necropsied on Day 21 of gestation. Corpora lutea were counted, and uterine contents were examined for pregnancy, number and placement of implantations, early and late resorptions, and live and dead fetuses.

Following removal from the uterus, the fetuses were weighed individually and examined for gross abnormalities. Crown-rump length was recorded for each live fetus. Approximately one-half of the fetuses in each litter were processed in Bouin's solution and examined for soft tissue alterations using a modification of Wilson's sectioning technique; the remainder of the fetuses were eviscerated, macerated, stained with alizarin red-S, cleared, and examined for skeletal alterations.

Statistical analysis:

The following description was excerpted from report No. 33-79, p. 4:

"For statistical evaluation of the data, the litter was considered the experimental unit of treatment and observation. The Fisher Exact Probability Test was used to evaluate the incidence of resorptions and abnormalities among litters. Maternal and fetal body weights and fetal crown-rump measurements were treated statistically by analysis of variance and least significant difference (LSD) tests. The number of corpora lutea, implantations and live fetuses per litter were analyzed by the Wilcoxon rank sum test. In all cases, two-tailed significance tests were performed, and significance was judged at the 0.05 probability level."

Historical control data were not provided.

Compliance: The following signed and dated statements were not provided:
- Flagging Statement
- Statement of No Confidentiality Claim
- GLP Compliance Statement
- Quality Assurance Statement

C. Results

1. Maternal Toxicity

Mortality and Clinical Observations:

All adult females survived to scheduled sacrifice. Other than chromodacryorrhea noted in one rat at 625 ppm on Days 10 and 16 of gestation, no clinical signs of toxicity were noted.

Body Weight and Food Consumption:

Maternal body weight and food consumption data are summarized in Table 1. Due to the receipt of time-mated animals, no Day 1 body weight data were recorded. A significant treatment-related decrease in mean body weight was noted for the high-dose (625 ppm) rats at Days 10, 16, and 21 of gestation. (Three of the pregnant females actually had a slight weight loss at the Day 10 measurement; at Day 21 cesarean section, one of these females was noted to have 7 early resorptions, representing total litter loss.) Treatment-related reductions in high-dose mean food consumption values were also noted for Days 6-10 and 10-16 of gestation. In the mid-dose (125 ppm) group, significant reductions in the mean body weight value at Day 6 and in the mean food consumption value for Days 6-16 were observed. These differences were not judged to be treatment-related.

Based upon body weight and food consumption data, the average amount of test substance consumed per dam during Days 6-16 of gestation was calculated to be 5.0, 12.1, and 49.8 mg/kg/day for the 50, 125, and 625 ppm dose levels, respectively.

Table 1. Mean Maternal Body Weight and Food Consumption During Gestation (± S.D.)

Interval	0 ppm	50 ppm	125 ppm	625 ppm
Body Weight (g)				
Day 6	204 ± 10	205 ± 13	198 ± 10*	208 ± 11
Day 10	230 ± 11	234 ± 14	227 ± 11	216 ± 9*
Day 16	283 ± 14	287 ± 18	275 ± 12	252 ± 16*
Day 21	355 ± 24	365 ± 26	347 ± 17	330 ± 25*
Food Consumption (g/day)				
Prior to Day 6	19.3 ± 2.0	19.0 ± 2.0	18.3 ± 2.5	19.5 ± 1.9
Days 6-10	21.3 ± 1.7	21.4 ± 2.4	20.2 ± 2.1	15.2 ± 2.2*
Days 10-16	26.6 ± 1.5	26.6 ± 2.3	24.7 ± 2.0*	20.2 ± 2.2*
Days 16-21	30.6 ± 2.1	30.8 ± 3.1	29.6 ± 2.0	29.0 ± 3.3

* Significantly different from control value, $p \leq 0.05$.
Note: Data were extracted from report No. 33-79, pages 8-9.

Observations Noted at Cesarean Section:

No gross pathological findings were reported for maternal rats.

The results of the examination of uterine contents at cesarean section are presented in Table 2. There appeared to be no treatment-related effects on the number of corpora lutea, implantation sites, live or dead fetuses, or late resorptions. Mean fetal weight values, mean crown-rump length, fetal viability, and fetal sex ratio were similar between control and treated groups.

There is a slight increase in the number of litters with early resorptions, the number of resorptions per litter with resorptions, and the mean postimplantation loss value (calculated by Reviewer) for Group 4 (625 ppm). No statistical differences among groups were reported for these data.

2. Developmental Toxicity

Anomalies noted at external, visceral, and skeletal evaluation of fetuses are presented in Table 3. Neither the incidence nor distribution of fetal alterations indicated a clear treatment-related effect. There was, however, an apparent increase in the number of fetuses and litters (7 of each) with bipartite thoracic vertebral centra at the high-dose (625 ppm) level as compared to the concurrent control group (in which none were observed), although statistical significance was not attained. In addition, the observation of unapposed sternebrae was noted in 3 fetuses of 3 litters at the high dose level. These observations are indicative of developmental delays.

Table 2. Summary of Cesarean Section Data

Parameter	0 ppm	50 ppm	125 ppm	625 ppm
No. animals assigned No. pregnant (%)	27 25(92.6)	27 23(85.2)	27 25(92.6)	27 22(81.5)
Maternal deaths	0	0	0	0
Corpora lutea/dam[a]	11.2±1.9	11.4±1.9	11.0±1.5	11.0±2.3
Implantations/dam[a]	8.9±2.5	10.0±2.6	9.4±1.9	9.7±1.4
Live fetuses/dam[a]	8.5±2.5	9.6±2.7	9.0±2.2	8.5±2.6
Litters with early resorptions (%) Litters with late resorptions (%) Litters totally resorbed (%) Resorptions per litter with resorptions[a]	9(36.0) 0 (0.0) 0 (0.0) 1.6±0.8	4(17.4) 0 (0.0) 0 (0.0) 1.6±0.5	9(36.0) 0 (0.0) 0 (0.0) 1.2±0.4	13(59.1) 1 (4.5) 1 (4.5) 2.1±1.8
Litters with dead fetuses	0	0	0	0
Mean fetal weight (g)[b]	4.2±0.4	4.2±0.3	4.2±0.5	4.0±0.3
Fetal crown-rump length (cm)[b]	3.5±0.1	3.5±0.2	3.5±0.2	3.5±0.1
Preimplantation loss (mean %)[c] Postimplantation loss (mean %)[d]	22.0±19.5 5.8±11.5	13.7±19.5 3.5±8.4	13.5±16.9 4.4±8.3	10.7±9.8 14.0±24.0

a Mean ± standard deviation.
b Mean of litter means ± standard deviation.
c Calculated by Reviewer; group mean of:
% (per litter) = (total corpora lutea - total implantation sites) / (total corpora lutea) x 100
d Calculated by Reviewer; group mean of:
% (per litter) = (total resorptions + total dead fetuses) / (total implantations) x 100

Note: Data were extracted from report No. 33-79 (page 11) and from copies of the raw cesarean section data provided by the Registrant.

D. Study Deficiencies:

1. Formulation: No information on the method or frequency of formulation of the test substance in the diet was provided in the study report.

2. Stability, homogeneity, and concentration analyses: Analyses were not performed to determine the stability of the test substance in the diet under the conditions of the study, as recommended in §80-3 (b)(2)(v). Neither the homogeneity nor concentration of the test substance in the diet mixes provided to the study animals were determined [§80-3(b)(2)(vi)].

Table 3. Summary of Fetal Anomalies [No. of Fetuses (Litters)]

Finding	0 ppm	50 ppm	125 ppm	500 ppm
EXTERNAL ANOMALIES				
No. fetuses (litters) examined	208(25)	221(23)	225(25)	187(21)
BODY - Subcutaneous hematoma	11(10)	9(7)	9(8)	6(6)
BODY - Petechial hemorrhage	3(2)	6(4)	5(4)	1(1)
BODY - Runted	0(0)	3(3)	3(3)[b]	0(0)
BODY - Umbilical hernia	0(0)	0(0)	2(2)[b]	1(1)
JAW - Agnathia	0(0)	1(1)	0(0)	0(0)
VISCERAL ANOMALIES				
No. fetuses (litters) examined	97(25)	108(23)	107(25)	88(21)
BODY - Subcutaneous edema	0(0)	5(3)	0(0)	0(0)
EYE - Hemorrhage between lens and cornea	1(1)	0(0)	0(0)	0(0)
EYE - Anophthalmia	0(0)	1(1)[a]	1(1)[b]	0(0)
BRAIN - Hydrocephalus	0(0)	0(0)	0(0)	1(1)
HEART - Great vessel irregularity	0(0)	1(1)[a]	0(0)	1(1)
LIVER - Peliosis	2(2)	0(0)	0(0)	1(1)
KIDNEY - Hydronephrosis and/or hydroureter	7(7)	10(5)	10(7)	4(3)
GONADS - Undescended testis	5(5)	3(2)	3(2)	2(2)
GONADS - Anovarism	0(0)	0(0)	1(1)[b]	0(0)
SKELETAL ANOMALIES				
No. fetuses (litters) examined	111(25)	113(23)	118(25)	99(21)
SKULL - Enlarged fontanel	0(0)	0(0)	1(1)	0(0)
STERNEBRAE - Unossified	21(11)	18(12)	25(13)	15(11)
STERNEBRAE - Asymmetrical (one or more)	0(0)	0(0)	0(0)	3(3)
RIB(S) - 14th rudimentary	56(21)	38(17)	47(20)	32(15)
RIB(S) - pair full 14th	0(0)	1(1)	1(1)	0(0)
RIB(S) - Wavy ribs	2(2)	0(0)	3(2)	2(2)
VERTEBRAE - Bipartite centra (thoracic)	0(0)	2(2)	1(1)	7(7)

a,b Observed in the same fetus.

Note: Data were extracted from report No. 33-79 (pages 11 and 12) and from copies of the raw fetal evaluation data provided by the Registrant.

3. Route of test substance administration: The test material was administered to the study animals in the diet on Days 6-15 of gestation. Dietary administration is generally not recommended for a developmental toxicity study [see §83-3 (g)(5)] due to inconsistencies in individual maternal dosage. Since systemic toxic effects were noted in the dams at the high dose level (625 ppm), dietary administration of the test substance was deemed adequate.

4. Treatment interval: The dams were treated with the test substance on Days 6-15 of gestation, the time period recommended when the day sperm is found is considered to be Day 0 [§83-3 (g)(4)]. In this study, however, the day sperm was found was called Day 1, and the treatment period should have been Days 7-16 of gestation. Nevertheless, since the dams were exposed to the test substance through a majority of the period of organogenesis, including the most sensitive phases of neural and cardiovascular development in the rat, it is considered unlikely that this error in dosing regimen would adversely affect the study outcome.

5. Body weight data: Day 0 body weight data were not collected due to the use of time-mated animals that were received at the laboratory on Day 2 or 3 of gestation. This lack of Day 0 body weight (and food consumption feed-in) data, while although it may not be technically interpreted as a deviation from standard protocol procedures as described in §83-3 (g)(7)(vi), precludes calculation of pretreatment body weight gain and food consumption values as well as overall maternal body weight gain through gestation.

6. Fetal evaluation data and analyses: Due to the fact that 1) fetal anomalies were not classified as malformations or variations and 2) laboratory procedures did not dictate that each fetus retain individual numerical identification during processing for visceral and skeletal examination, it was not possible to calculate selected informative summary incidences, such as: the number and percent of fetuses or litters-with-fetuses with any alteration (or malformation or variation) observed, or the mean percent of fetuses with any alteration (or malformation or variation) per litter. Because the observations noted at fetal evaluation did not suggest a treatment-related developmental or teratogenic effect, the lack of such information does not compromise the results of this study.

7. Report format: The study report was not formatted according to the example in 49 FR (188) 37596 (9/26/84). The cover page did not contain required information about the study conduct, the report was not signed by the Study Director, and the following signed and dated statements were not provided:
   a. Flagging Statement
   b. Statement of No Confidentiality Claim
   c. GLP Compliance Statement
   d. Quality Assurance Statement

D. Discussion/Conclusions

a. Maternal Toxicity:

Dietary administration of Linuron to Crl:CD^R female rats on Days 6-15 of presumed gestation

(where Day 1 is the day mating was confirmed) at dose levels of 50, 125, and 625 ppm (5.0, 12.1, and 49.8 mg/kg/day, respectively) produced evidence of maternal systemic toxicity at the 625 ppm (high-dose) level. A significant treatment-related decrease in mean body weight was noted for the high-dose rats at Days 10, 16, and 21 of gestation, and treatment-related reductions in high-dose mean food consumption values were noted for Days 6-10 and 10-16 of gestation.

Maternal NOEL = 125 ppm (12.1 mg/kg/day)
Maternal LOEL = 625 ppm (49.8 mg/kg/day)

b. Developmental Toxicity:

The examination of uterine contents at cesarean section revealed no treatment-related effects on numbers of corpora lutea, implantations, live and dead fetuses, or late resorptions. A biologically significant increase in the number and percent of litters with early resorptions was noted at the 625 ppm (high-dose) level as compared to control; this was substantiated by an increase in the postimplantation loss value. Fetal body weight, crown-rump length, and viability were similar between control and treated groups.

No fetal malformation or variation revealed by gross external, visceral, or skeletal evaluation was unequivocally attributed to administration of Linuron. In a study published by K.S. Khera (Toxicology and Applied Pharmacology, Volume 45, 1978) it was claimed that 200 mg/kg of Linuron (Lorox 50% WP) produced incidences of nonapposed sternebrae in rat fetuses. Khera judged this response to be teratogenic; however, nonapposed sternebrae are generally classified as variations that are the result of retardation in fetal developmental. Although there was a slight incidence of nonapposed sternebrae noted in fetuses of the 625 ppm dose group of this study (3 fetuses, in 3 litters), this was not considered to indicate a teratogenic response.

Developmental Toxicity NOEL = 125 ppm (12.1 mg/kg/day)
Developmental Toxicity LOEL = 625 ppm (49.8 mg/kg/day)

E. Recommendations:

1. The previous Agency review of this study (Document No. 000676, dated 12/10/79) did not cite the deficiencies in procedure and data analysis as described above. The CORE Classification of "Guideline" given at the time of that review infers that all guideline recommendations are met. Due to the many discrepancies with §83-3 recommendations, the CORE Classification is being changed to MINIMUM.

2. In the previous Agency review, maternal systemic effects, specifically "reduced body weight gain and food consumption" were noted at the 125 ppm dose level, and this level was determined to be the maternal Low-Effect-Level. Based upon a closer evaluation of the data, a consistent, dose-related effect on maternal body weight or food consumption results cannot be identified for the 125 ppm dose level. Therefore, it is recommended that 125 ppm be listed as the maternal NOEL and 625 ppm as the maternal LOEL.

3. No changes to the assessment of developmental toxicity are suggested. (Based upon an increase in fetal resorptions, the LOEL = 625 ppm and the NOEL = 125 ppm.)

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11

Linuron

Page _____ is not included in this copy.

Pages 12 through 14 are not included in this copy.

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.

X 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.