9/27/90

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

TOX R 008113

SEP 27 1990

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Linuron - Evaluation of a Two-Generation Reproduction Study Provided as 6(a)(2) Data

Caswell No. 528
HED Project No. 0-1221
MRID No. 414634-01

FROM: Elizabeth A. Doyle, Ph.D. E.A. Doyle 8/21/90
Review Section I, Tox Branch II (HFAS) (H7509C)

TO: Carol Peterson, PM74
Reregistration Branch
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THRU: Yiannakis M. Ioannou, Ph.D., Section Head Y.M. Ioannou 8/21/90
Review Section I, Tox Branch II (HFAS) (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Tox Branch II (HFAS)
Health Effects Division (H7509C) M van Gemert 8/22/90

Registrant: E. I. du Pont de Nemours and Company

Action Requested: Review of a 2-generation reproduction study in rats provided by the registrant as 6(a)(2) data, indicating the occurrence of a previously unreported lesion in rats, that is, corneal opacity and lens degeneration in F_1 adult males.

The subject study has been reviewed and the ocular effects reported by the registrant appear to be legitimate, treatment related effects, occurring in F_1 adult males from a treatment group receiving a diet containing 625 ppm of linuron (≈31.25 mg/kg/day). No treatment related effects on fertility or reproductive performance were reported at treatment levels up to 625 ppm. The systemic NOEL for this study was 12.5 ppm and the LOEL was 100 ppm based on a decrement in body weight gain. This study was classified as "Guideline".

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beth A. Doyle, Ph.D E. A. Doyle 8/21/90
Toxicology Branch II (HFAS) (H7509C)
Reviewer: Yiannakis M. Ioannou, Ph.D., Section Head
Toxicology Branch II (HFAS) (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat (Guideline 83-4)

MRID NUMBER: 414634-01

TEST MATERIAL: Linuron; Caswell No. 528

SYNONYMS: IN Z326-118; Urea, N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl; 3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea; CAS No. 330-55-2

STUDY NUMBER(S): MR 6511-001

SPONSOR: Agricultural Products Department
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19805

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: Reproductive and Fertility Effects with IN Z326-118 (Linuron) Multigeneration Reproduction Study in Rats

AUTHOR(S): Linda S. Mullin

DATE REPORT ISSUED: March 29, 1990

CONCLUSIONS: Linuron had no effect on fertility or reproductive performance at doses of 12.5, 100 or 625 ppm in diet (= 0.625, 5.0 and 31.25 mg/kg/day). F_1 adult males in the 625 ppm group exhibited testicular and epididymidal abnormalities and ocular abnormalites consisting of mineralization of the cornea and lens degeneration.

Reproductive NOEL > 625 ppm
Systemic NOEL = 12.5 ppm
Systemic LOEL = 100 ppm based on body weight gain decrement

Core Classification: Guideline

This study satisfies the guideline requirements (83-4) for a "Multigeneration Reproduction Study in Rats".

I. PROTOCOL

A. Materials

1. Test Material: Technical grade herbicide, Purity: 96.2%, Batch No.: 16,569.

2. Test species: 43-day old male and female Crl:CDBR rats were obtained for the first parental generation of the study from Charles River Laboratories, Inc., Kingston, New York. The rats were acclimated for a period of 17 days before they were placed into the study.

3. Diet preparation: Rats were fed a diet of irradiated Purina Certified Rodent Chow #5002 meal containing 0, 12.5, 100 or 625 ppm test material. All diets were prepared weekly.

   Test diets were analyzed for homogeneity of mixtures. Chemical stability in dietary mixtures was using grab samples stored at room temperature for 7 days, refrigerated for 14 days or at room temperature for 14 days.

B. Procedures and Study Design

1. Mating: Males were caged individually with females from the same test group until extruded or intravaginal copulation plugs were observed or until three weeks had elapsed.

   All females, with or without evidence of successful mating, were individually housed in polypropylene pans with bed-o'cobs bedding on day 14 of gestation. They were observed twice daily for signs of delivery and offspring.

2. Mating schedule: The F_0 parental animals were given test diets for 72 days before they were mated, and the F_1 parental animals were not mated until 75 days after they were selected from the F_{1A} litters. Selection of parents for the F_1 generation was made when the pups were 21 days of age, and the mated animals in the study were approximately 105 days of age at mating.

3. Animal assignment: F_0 animals were randomly assigned to test groups as follows:

Test groups No.	Designation	Dose (ppm) *	Animals per group ** Males	Females
1	Control	0	30	30
2	Low (LDT)	12.5	30	30
3	Mid (MDT)	100	30	30
4	High (HDT)	625	30	30

* Diets were administered from the beginning of the study until the animals were sacrificed.

** The same number of animals were picked from the F_1 litters as parents for the F_2 generation.

C. Observation Schedule

1. Parental animals: Observations and the schedule for those observations is summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	All	Once a day during premating and growth periods.
Detailed clinical observations	All	Once a week during growth and breeding periods.
Body weight	All	At beginning of study and weekly through growth and mating periods.
	Maternal animals	Days 0, 7, 14 and 21 of gestation; days 0, 7, 14 and 21 post partum; and weekly until sacrifice.
Food consumption	All	Weekly during premating period and gestation.

2. Reproductive performance: Parental reproductive performance was assessed from breeding and parturition records of animals in the study. The following indexes were calculated:

$$\text{Mating index (\%)} = \frac{\text{No. females copulating}}{\text{No. cohoused}} \times 100$$

$$\text{Female fertility index (\%)} = \frac{\text{No. females bearing litters}}{\text{Total no. females mated}} \times 100$$

$$\text{Gestation index (\%)} = \frac{\text{No. live litters born}}{\text{No. pregnancies}} \times 100$$

3. Litter observations: According to the report, the following litter observations were made:

Observation	Time of observation (lactation day) Birth	Day 4	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X
Pup weight	X	X	X	X	X
External alterations	X				
Number of dead pups	X				
Sex of each pup	X				

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

The following indices were calculated:

$$\text{Pups born live (\%)} = \frac{\text{No. live pups born}}{\text{No. live + dead pups born}} \times 100$$

$$\text{Viability Index (\%)} = \frac{\text{No. live pups at day 4}}{\text{No. live pups born}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{No. live pups at day 21}}{\text{No. live pups at day 4}} \times 100$$

$$\text{Litter survival (\%)} = \frac{\text{No. litters weaned}}{\text{No. viable litters delivered}} \times 100$$

4. Necropsy

a. Parental animals: All surviving parental males were sacrificed as soon as possible after the last litters in each generation were produced. Maternal animals were sacrificed after the last litter of each generation was weaned. These animals were subjected to post mortum examinations as follows:

Animals examined	Macroscopic	Microscopic
Found dead	all	all
Unscheduled sacrifice	all	all
Scheduled sacrifice	all	all

b. Offspring: The F1 and F2 offspring were sacrificed at 21 days of age. These animals were subjected to post mortum examinations as follows:

Animals examined	Macroscopic	Microscopic
Found dead	all	none
Scheduled sacrifice	20	20

c. Necropsy observations: Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

The following tissues were prepared for microscopic examination:

X	Ovaries	X	Epididymides
X	Uterus	X	Prostate
X	Unusual lesions	X	Seminal vesicles
X	Vagina/cervix	X	Testes

Additional tissues prepared for microscopic examination included coagulating gland, mammary gland (F1), pituitary gland and eyes (F1).

D. Statistical analyses: "Body weights, body weight gains, food consumption, organ weights, and gestation length were analyzed by a one-way analysis of variance. When the test for differences among test groups (F test) was significant, pairwise comparisons between test and control groups were made with the Dunnett's tests. The Bartlett's test for homogeneity of variances was performed on the organ weight and, when significant (alpha = 0.005), was followed by nonparametric procedures.

"Incidence of clinical observations was evaluated by the Fisher's Exact test with Bonferroni correction and, if significant, was followed by Cochran-Armitage test for trend. Incidences of gross and microscopic pathological lesions were analyzed by the Fisher's Exact test. Measures of reproduction and lactation performance were evaluated with either the Fisher's Exact test (mating, fertility, and gestation indices and survival) or the Mann-Whitney U test (pup numbers, survival, weights, viability index, and lactation index).

"Except for Bartlett's test, all other significance was judged at alpha = 0.05."

II. REPORTED RESULTS

A. Analysis of test diets: The homogeneity of the test diets was evaluated in grab samples of treated diet prepared on 7/14/88. Samples were taken from the top, middle and bottom of each diet. The concentrations obtained upon analysis were 94-98%, 93-95% and 89-91% of the nominal concentrations for the low, mid and high concentration diets, respectively.

Stability of the test material in diet was evaluated under multiple storage conditions. These were fresh frozen, and after 7 days at room temperature, 14 days at room temperature or 14 days under refrigeration. The test material in diet was stable under all storage conditions for a period sufficient for conduct of this study.

STABILITY OF IN Z326-118 IN DIETS PREPARED 7/14/88
(ppm, % of Nominal Concentration)

Sample Type	Nominal Concentration (ppm) 0		12.5		100		625	
Fresh Frozen	0	-	12.9	(103)[a]	94	(94)	576	(92)
7 Days Room Temp.	-	-	11.0	(88)	88	(88)	548	(88)
14 Days Room Temp.	-	-	12.2	(98)	85	(85)	530	(85)
14 Days Refrigerated	-	-	12.0	(96)	92	(92)	548	(88)

[a]Values in parentheses represent percent of nominal concentration

B. Parental animals

1. Mortality and clinical signs: No treatment related effects on mortality were reported in either F0 or F1 females.

2. Body weight and food consumption: Body weights were significantly reduced in F_0 males and females from the high dose group during the premating period. The decreases relative to the control were evident beginning at the day 7 weighing and persisted throughout the premating period. The body weights of mid dose F_0 males were reduced intermittently during the premating period. Weighings at days 21, 28, 35, 42 and 63 produced body weight values that were significantly reduced relative to the control. Body weight gains were significantly reduced in high dose F_0 males.

Food consumption was significantly reduced in high dose F_0 males beginning with the first treatment week and remained lower than the control throughout the entire premating period. Food consumption by mid dose males was significantly reduced relative to the control for isolated weeks only.

Food consumption by this group tended to be slightly but nonsignificantly lower than the control group throughout the premating period. Average daily food consumption for the entire premating period was significantly reduced relative to the control in the mid and high dose groups.

F_0 females had reduced food consumption in the high dose group only. The reduction was statistically significant when averaged over the entire premating period and at all individual measurement points except for weeks 6 and 10.

Observation and study week	Dose group			
	Control	Low	Mid	High
F_0 Generation Males - Premating				
Mean body weight (g)				
0	330.9	332.7	332.9	330.5
10	606.9	604.1	575.2	493.8*
Mean weight gain (g)				
0 - 10	276.2	271.4	242.4*	163.3*
Mean food consumption (g/rat/day)				
1	29.2	28.7	27.1*	19.2*
2	29.1	28.1	26.7	22.8*
10	29.3	29.9	27.3*	23.9*
0 - 10	30.0	29.6	28.2*	23.9*
F_0 Generation Females - Pre-mating				
Mean body weight (g)				
0	202.3	205.3	199.9	202.7
10	292.2	306.4	281.5	252.3*
Mean weight gain (g)				
0 - 10	89.8	100.6	81.6	49.6*
Mean food consumption (g/rat/day)				
1	18.7	19.2	17.6	13.0*
2	19.3	19.8	17.5*	15.7*
4	19.5	20.3	18.8	16.8*
6	18.5	19.9	18.8	16.5*
8	19.8	20.2	19.3	17.3*
10	19.6	19.1	18.8	17.0
0 - 10	19.3	19.9	18.6	16.5*

* Statistically significantly different from control, $p<0.05$.

At the start of the premating period, F_1 males from the low, mid and high dose groups weighed 99.3, 92.9 and 67.5% of the control. Mid and high dose males weighed significantly less than the control for the entire premating period. However, no adverse treatment related effect was reflected by this significant difference because the relative difference did not increase during the premating period but rather decreased slightly. At the end of the premating period (week 15), the low, mid and high dose groups weighed 99.5, 92.9 and 75.6% of the control, respectively.

Similarly, mid and high dose F_1 females had significantly lower initial body weights than the control. Low, mid and high dose F_1 female rats weighed 99.5, 91.8 and 67.5% of the control group, respectively, at the beginning of the premating period. By the end of the premating period, the mid and high dose females continued to have body weights significantly lower than the control. However, similar to the F_1 males, the actual differences in body weights were reduced by the end of the premating period even though the difference remained statistically significant. At the end of the premating period, the low, mid and high dose rats weighed 100.3, 91.6 and 75.3% of the control, respectively.

Mean body weight gains were significantly lower in mid and high dose F_1 males than in the control group throughout the premating period. However, this is consistent with the lower starting weights and does not indicate an adverse effect due to the test material. Mean body weight gains were also lower in mid and high dose F_1 females although not to a statistically significant extent at individual time points. When body weight gain for F_1 males and females are normalized to starting weight, the body weight gain expressed as the final body weight at the end of the premating period is comparable for all treatment groups.

Gestational body weights and body weight gains for high dose females were indicated to be significantly lower than the control at all weighings for F_0 and F_1 females. However, the indication of statistical significance, although mathematically correct, is misleading in that the indicated females began gestation with body weights that were significantly lower than the control. When body weight gains were normalized to the beginning body weight for gestation, the percentage weight gains for all groups were similar. For control, low, mid and high dose F_0 females, body weight gains represented 32.8, 31.3, 33.1 and 32.4% of the final gestational body weight, respectively. For F_1 females, body weight gains during gestation represented 27.1, 30.0, 30.2 and 31.2% of the final gestational body weights, respectively. No body weight decrement occurred due to treatment in either generation during gestation.

Similarly, lactational body weights for mid and high dose groups at the beginning of lactation were significantly lower than the control in both F_0 and F_1 females. At the end of lactation, body weights from the mid dose group were comparable to control. The high dose group remained statistically lower than the control. Lactational body weight gains in F0 and F_1 mid and high dose groups were significantly greater than the control. F_1 low dose females had lactational body weight gains that were greater than the control but not to statistically significant extent. Rats from

Observation and study week	Dose group: Control	Low	Mid	High
F_1 Generation Males - Premating				
Mean body weight (g)				
0	59.0	58.6	54.8*	39.8*
15	610.5	607.7	574.1*	461.7*
Mean weight gain (0 - 15) (g)	551.5	549.1	519.3*	421.9*
(% of final body wt.)	90.3	90.3	90.5	91.4
Mean food consumption (g/rat/day)				
1	12.3	12.7	12.4	9.8*
5	28.8	29.1	27.1*	24.2*
10	31.5	30.6	29.4*	26.4*
15	30.7	30.2	29.4	25.2*
0 - 15	28.0	27.9	26.4*	23.2*
F_1 Generation Females - Premating				
Mean body weight (g)				
0	56.3	56.0	51.7*	38.0*
15	312.3	313.2	286.0*	235.2*
Mean weight gain (0 - 15) (g)	256.0	257.1	234.3*	197.2*
(% of final body wt.)	81.9	82.1	81.9	83.8
Mean food consumption (g/rat/day)				
1	11.9	12.7	11.6	9.2*
5	21.7	20.7	19.4*	17.7*
10	21.5	21.0	20.2	17.7*
15	21.0	21.1	20.2	17.9*
0 - 15	20.3	20.2	19.0*	16.7*

* Statistically significantly different from control, $p<0.05$.

the treated groups demonstrated a greater net increase in body weight during lactation than the control group in both generations.

Selected group mean body weights and food consumption values for pregnant or nursing dams were summarized in the report as follows:

servation and study time	Dose group Control	Low	Mid	High
	F_0 Generation			
Mean body weight (g)				
Day 1 of gestation	286.1	306.3*	278.7	244.6*
Day 21 of gestation	422.3	445.9*	416.3	361.8*
Day 1 of lactation	332.8	339.2	314.9*	268.3*
Day 21 of lactation	330.4	335.9	326.0	279.2*
Mean body weight gain (g)				
Days 1-21 of gestation	138.6	139.6	137.7	117.2*
Day 1-21 of lactation	-2.4	-3.2	11.1*	10.9*
	F_1 Generation			
Mean body weight (g)				
Day 1 of gestation	312.3	306.3	285.1*	233.9*
Day 21 of gestation	428.2	437.7	411.7	334.5*
Day 1 of lactation	345.5	339.2	317.3*	268.4*
Day 21 of lactation	343.6	346.3	335.0	288.6*
Mean body weight gain (g)				
Days 1-21 of gestation	115.9	131.4	124.6	104.5
Day 1-21 of lactation	-1.9	7.1	17.7*	20.1*

* Statistically significantly different from control, $p<0.05$.

Test Substance Intake: Based on food consumption, body weight, and dietary analyses results, the doses expressed as mg test substance/kg body weight/day were as calculated for the pre-mating period.

Reproductive performance: No evidence of treatment related effects on mating were reported in either parental generation. The fertility index from the F_0 parents was notably lower than for any of the treated parents. The fertility index for the F_1 control parents was similar to that for the F_0 controls, but the indices for the treated groups were highly variable, with no indication of dose response. The low fertility index for the F_1 high dose group (53.6%) may be an indication of a treatment related effect; however, due to the variability in the data, this conclusion can not be unequivocally defended.

COMPOUND INTAKE (mg/kg/day)

Week	Dose levels (ppm) Males 12.5	100	625	Females 12.5	100	625
			F_0 Generation			
1	0.942	7.31	36.5		5.28	42.1
4	0.779	6.15	33.0		7.52	46.4
8	0.672	5.34	3.1		7.02	43.9
10	0.620	4.76	30.2		6.67	42.2
0 - 10	0.736	5.83	35.5	0.917	7.33	45.1
			F_1 Generation			
1	1.58	13.3	88.0	1.70	13.7	92.4
4	1.18	9.53	69.0	1.27	10.6	76.8
8	0.815	6.79	47.0	1.02	8.64	55.2
10	0.733	6.01	41.3	0.937	7.86	51.7
15	0.623	5.14	34.2	0.859	7.10	47.8
0 - 15	0.948	7.77	54.0	1.12	9.24	63.0

REPRODUCTIVE PERFORMANCE - F_0

Observation	Dose group Control	Low	Mid	High
ting Index (%) (# copulated/cohoused)	96.7 (29/30)	96.7 (29/30)	100.0[a] (29/29)	100.0 (30/30)
tility Index (%) (# delivered/copulated)	75.9 (22/29)	89.7[b] (26/29)	89.7 (26/29)	93.3 (28/30)
station Length (days)	22.5	22.6	22.3	22.3
ber of litter	22	26	26	28

Excluding one female sacrificed *in extremis* during mating period.
Including one female found dead but pregnant.

REPRODUCTIVE INDICES - F_1

Observation	Dose group Control	Low	Mid	High
Mating Index (%) (# copulated/cohoused)	96.7 (29/30)	96.7 (29/30)	100.0 (30/30)	96.6[a] (28/29)
Fertility Index (%) (# delivered/copulated)	72.4 (21/29)	69.0 (20/29)	93.3 (28/30)	53.6 (15/28)
Gestation Length (days)	22.3	22.3	22.5	22.2
Number of litters (Day 1)	21	20	28	15

[a] Excluding one female sacrificed in extremis during mating period.

5. Necropsy results

a. Organ weights: The only organ weights collected at necropsy were for the testes. Relative testes weights were increased in high dose males from the F_0 generation. Absolute testes weights were significantly decreased in F_1 high dose males; however, when expressed as percent of body weight, testes of high dose males were increased in weight although not to a statistically significant extent.

TESTES WEIGHTS FROM LINURON TREATED RATS

Observation	Dose group Control	Low	Mid	High
	F_0 Generation			
Organ weight (g)	3.595	3.497	3.540	3.575
Relative organ weight (%)	0.5330	0.5253	0.5591	0.6505*
	F_1 Generation			
Organ weight (g)	3.698	3.685	3.525	2.941*
Relative organ weight (%)	0.5426	0.5374	0.5482	0.5815

* Statistically significantly different from control, $p<0.05$.

b. Pathology

i. Macroscopic examination: No treatment related gross pathology was reported in F_0 adult males or females due to treatment with the test material.

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In F_1 adult males, the testes of high dose males reduced in size in a statistically significant nunber of rats (9/30), were abnormally large in 3/30 rats and were abnormally soft in 5/30 rats. In addition, abnormalities of the epididymides were reported in a number of high dose males. Incidences of high dose males with small epididymides (8/30) or having unspecified deformities (5/30) were significantly greater than the control. Abcess (2/30), nodules (1/30) and discoloration (1/30) of the epididymides also occurred in high dose males but not in other treatment groups. The eyes of high dose males only were discolored (3/30) or exhibited enophthalmus (1/30). These lesions of the eye have not been previously reported due to linuron treatment. No other noteworthy gross abnormalities in male rats were reported.

High dose F_1 females had cystic ovaries in 4/30 cases, dilatation in 3/30 cases and fluid filled uterine horns in 2/30 cases. Although not statistically significantly increased relative to the control, none of these lesions were present in females from the control groups. No other treatment related effects were reported.

ii. Microscopic examination: No treatment related microscopic lesions were reported in F_0 adults of either sex.

High dose F_1 males had significantly increased incidences of abnormal microscopic pathology of the testes and epididymides. The specific lesions are detailed below. The eyes of high dose males had nonsignificant increases in a number of lesions not present in the control group. These included unilateral, focal, corneal mineralization (4/30), vacuolization of the corneal epithelium (1/30) and lens degeneration (3/30).

High dose F_1 females exhibited similar lesions of the eye. Focal, unilateral, corneal mineralization (1/29), unilateral degeneration of the lens (2/29) and focal, unilateral atrophy of the outer nuclear membrane (2/29) occurred in the high dose group but not in the untreated control group.

C. Offspring

1. Viability and clinical signs: No treatment related effect on the sex ratio or gestation index was reported for F_1 or F_2 offspring. The mean % born alive and 0-4 day viability were decreased in high dose litters from both generations. High dose F_2 litters also had reduced lactation indices and litter survival.

INCIDENCE OF MICROSCOPIC OBSERVATIONS
IN F_1 ADULT MALE RATS

Observation	Dose Group Control	Low	Mid	High
Testes (N =	30	1	30	30)
Atrophy				
- All Types	1	1	2	14*
- Bilateral	1	0	1	6
- Unilateral	0	1	1	8*
Granular/Fibrosis, Intratubular				
- All types	0	1	2	8*
- Bilateral	0	0	0	2
- Unilateral	0	1	2	6*
Hyperplasia, Interstitial, Focal	0	1	1	7*
Epididymides (N =	30	1	30	30)
Arteritis	0	0	0	6*
Inflammation/Tubular Degeneration, Focal	0	0	2	5*
Lymphoid Foci, Interstitial/Perivascular	11	0	13	20*
Oligospermia				
- All types	1	1	2	12*
- Bilateral	1	0	0	6
- Unilateral	0	1	2	6*
Sperm Granuloma				
- All types	0	0	0	3
- Bilateral	0	0	0	1
- Unilateral	0	0	0	2
Eyes (N =	30	0	28	30)
Cornea				
- Focal, Unilateral Mineralization	1	0	0	4
- Vacuolation, Epithelium	0	0	0	1
Lens, Degeneration	0	0	0	1
Lens, Degeneration, Unilateral	0	0	0	2

*Statistically significant difference ($p<0.05$)

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INCIDENCE OF MICROSCOPIC OBSERVATIONS
IN F_1 ADULT FEMALE RATS

Observation	Dose Group Control	Low	Mid	High
Eyes (N =	30	0	0	29)
Cornea, Mineralization, Focal, Unilateral	0	0	0	1
Lens, Degeneration, Unilateral	0	0	0	2
Outer Nuclear Membrane Atrophy, Focal, Unilateral	0	0	0	2

* Statistically significantly different from control, $p<0.05$.

LITTER VIABILITY - LACTATION

Observation and study time	Dose group Control	Low	Mid	High
F1 Generation				
Sex Ratio (males)[a]	0.52	0.49	0.49	0.50
Gestation Index[b]	100.0	96.0	100.0	96.4
Mean % Born Alive	97.8	95.2	99.2	91.1
0-4 Day Viability	99.4	98.0	99.8	91.7*
Lactation Index[c]	100.0	100.0	100.0	100.0
Litter Survival[d]	100.0	100.0	100.0	96.3
F_2 Generation				
Sex Ratio (males)	0.49	0.50	0.46	0.55
Gestation Index	100.0	100.0	100.0	100.0
Mean % Born Alive	97.1	95.4*	95.9	88.3*
0-4 Day Viability	96.8	92.7	99.5	76.2*
Lactation Index	100.0	100.0	99.5	89.8
Litter Survival	100.0	95.0	100.0[e]	85.7[f]

*Statistically significantly different from control, $p<0.05$.
[a]Ratio of males to total number of sexable pups born.
[b]Percent litters delivered having at least one live pup.
[c]Mean percent survival from day 4 postculling to day 21.
[d]Mean viable litters born with at least one pup alive on day 21.
[e]Excluding one litter sacrificed due to death of dam.
[f]Excluding one litter accidentally killed.

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Litter sizes were decreased in the high dose group of both generations, although a greater reduction was observed in F_2 than F_1 litters. At the Day 4 culling, less than eight pups survived in the high dose F_2 litters. Litter sizes in all other treatment/generation groups were stable following the Day 4 culling. However, the high dose F_2 litters continued to decrease in size until the second lactational week.

Changes in mean litter sizes were summarized in the report as follows:

MEAN PUP NUMBERS AND SURVIVAL

Observation and study time	Dose group: Control	Low	Mid	High
	F_1 Generation			
Born	12.4	12.6[a]	13.5	11.9
Born Alive	12.1	12.5[a]	13.4	11.0
Day 4 Preculling	12.0	12.8	13.4	10.5
Day 4 Postculling	7.7	7.8	8.0	7.8
Day 7	7.7	7.8	8.0	7.8
Day 14	7.7	7.8	8.0	7.8
Day 21	7.7	7.8	8.0	7.8
	F2 Generation			
Born	11.8	13.8	12.4	8.5*
Born Alive	11.4	13.2	12.0	7.3*
Day 4 Preculling	11.2	12.4	12.0	5.6*
Day 4 Postculling	7.2	8.0*	7.4	5.8*
Day 7	7.2	8.0*	7.4	5.6*
Day 14	7.2	8.0*	7.4	5.5*
Day 21	7.2	8.0*	7.4	5.5*

[a]The mean number of pups born and born alive is lower than the number alive on day 4 because the day 0 data include a litter of one pup born dead, 0 alive. Since this was not a viable litter, survival data on day 4 does not include this litter and thus, the mean is higher.
*Statistically significantly different from control, $p<0.05$.

No one lesion was consistently reported in both generations. The high dose F_1 pups had an increased incidence of hernias relative to the control group. "Small whole body" was also indicated to be increased in the high dose group. Increases were evident on both individual pup basis and on a litter basis. No outstanding lesion was apparent in the F_2 generation. Total clinical signs were increased in high dose F_1 pups but not in the F_2 pups. The number of litters affected was increased at the high dose in both generations. The results below are excerpted from the report.

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Observation	Dose group Control	Low	Mid	High
	F_1 Generation			
Hernia	0	0	0	18 (5)
Small Whole Body	1 (1)	2 (2)	0	20 (8)
Total No. of Signs	1	11	5	46
Number of Litter Affected*	1	8*	4	15*
Total Number of Litters	22	24	26	27
	F_2 Generation			
Total No. of Signs	10	31	27	21
Number of Litters Affected*	3	6	10	9*
Total No. of Litters	21	20	28	15

*Statistically significant increase in total litters affected (p = 0.05).
*Statistically significant trend.

2. Body weight: Significant reductions in body weights of offspring from the mid and high dose F_1 litters was evident at delivery and throughout lactation. These reductions correspond to maternal body weight decrements relative to the control during the same period. However, unlike maternal effects, body weight reduction in pups in the F_2 generation was less pronounced than in the F_1 pups, with significant reductions occurring only in the high dose group.

3. Necropsy results - Macroscopic examination: No treatment related abnormal gross pathological lesions were reported in pups or weanlings.

III. DISCUSSION

No evidence of adverse effects on reproductive parameters were reported. However, due to the variable nature of the data, few inferences can be drawn. The number of F_2 litters produced was only 53.6% of matings in the high dose group compared to 72.4% for the control group. Although this is an 18.8% decrease, it can not be separated from the background noise to permit comment as to whether it reflects a compound related effect. Litter viability was reduce in F_2 high dose offspring as were the individual pup weights.

The major systemic effects reported were reduced body weights and body weight gains in both parental generations at doses of 100 and 625 ppm. No clinical signs of overt toxicity were reported other than the body weight decrements. Body weight decrements persisted through gestation and lactation, but when gestational body weight gains were normalized to body weights at the end of

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gestation, the percentage increases were almost identical. Mid and high dose females gained significantly more weight during lactation than the control group which experienced actual weight reductions during lactation.

The treatment related pathological lesions of importance in this study were testicular and epididymal effects in high dose F_1 males only and ocular effects in high dose F_1 adult males and females. The effects in the male reproductive organs have been noted in earlier studies with linuron. However, the ocular effects have not been previously noted. Ocular effects occurred in six males and two females. These rats were exposed in utero and during lactation to the test compound at levels that were considered maternally toxic. The ocular effects, consisting of corneal mineralization and lens degeneration, appear to be treatment related.

IV. CONCLUSIONS

Linuron had no effect on fertility or reproductive performance at doses of 12.5, 100 or 625 ppm in diet (= 0.625, 5.0 and 31.25 mg/kg/day). F_1 adult males in the 625 ppm group exhibited testicular and epididymidal abnormalities and ocular abnormalites consisting of mineralization of the cornea and lens degeneration.

Reproductive NOEL > 625 ppm
Systemic NOEL = 12.5 ppm
Systemic LOEL = 100 ppm based on body weight gain decrement

Core Classification: Guideline

This study satisfies the guideline requirements (83-4) for a "Multigeneration Reproduction Study in Rats".

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BODY WEIGHTS OF OFFSPRING

Observation and study time	Dose group			
	Control	Low	Mid	High
F1 Generation				
Males				
Body weight (g) - Day 0	7.1	7.0	6.6*	6.0*
Body weight (g) - Day 21	60.3	59.0	55.7*	41.2*
Females				
Body weight (g) - Day 0	6.6	6.7	6.2*	5.7*
Body weight (g) - Day 21	56.9	57.1	53.0*	39.3*
F_2 Generation				
Males				
Body weight (g) - Day 0	6.7	6.6	6.6	6.0*
Body weight (g) - Day 21	53.6	56.1	54.1	40.5*
Females				
Body weight (g) - Day 0	6.3	6.1	6.3	5.7*
Body weight (g) - Day 21	51.4	52.9	51.3	39.6*

*Statistically significantly different from control, $p<0.05$.