7591/linuron SR 11-29-85

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of linuron reproductive study addendum: cross-mating study in rats; report no. 413-85; August 8, 1985; Accession #259393; Caswell# 528

TO: Ingrid Sunzenauer, Review Manager
Special Review Branch (TS-767C)

and

Robert Taylor, PM #25
Registration Division (TS-767C)

FROM: James N. Rowe, Ph.D.
Section V, Toxicology Branch
Hazard Evaluation Division/HED (TS-769C)

James N. Rowe 11/27/85

THRU: Laurence D. Chitlik, D.A.B.T. Quang Bui for LDC 11/27/85
Section Head, Section V
Toxicology Branch/HED (TS-769C)

11/29/85

and

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch/HED (TS-769C)

ACTION REQUESTED: Review of duPont linuron rat cross-mating study under Accession # 259393.

RECOMMENDATIONS: This addendum study does not provide any additional data which would allow the original reproduction study(I.D. #352-326; Accession #255829) to be upgraded from the classification of Core supplementary, i. e., histological data on the parental animals which are necessary to interpret the marked infertility in the high-dose group (625 ppm). Therefore, it is recommended that a repeat of the reproduction study be performed using the 1982 EPA Testing Guidelines. The results of the ancillary study, however, suggest that there are effects on the male and there is some evidence to indicate an effect on the females, as well.

Excerpts of data submitted by duPont on linuron were included in this review. (11 pages). These pages may be requested by writing Freedom of Information (A-101), EPA, Washington, D.C. 20460. Requesters will be asked to sign an Affirmation of Non-multinational Status.

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See D-7592 B for data excerpts

DATA EVALUATION RECORD

CHEMICAL: Linuron
3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea(Lorox®, INZ-326)
Caswell # 528.

TEST MATERIAL: Linuron, DPX-Z326; Haskell no. 14,703; purity stated as 94.5%; material submitted by John C. Summers.

STUDY IDENTIFICATION: Cross-mating study in rats with INZ-326(linuron)

Medical Research Project No. 4580-001
Haskell Laboratory Report No. 413-85
Date issued: August 8, 1985
Study Director: Timothy P. Pastoor, Ph.D.
Sponsor: E. I. du Pont de Nemours and Company
EPA Accession # 259393

CONCLUSIONS:

The control and treated groups for the study are defined as follows:

	F_{3B}		F_{3C}		F_{2B}†
	Group A¶	Group B¶	Group C¶	Group D¶	"Control"
Control:	female	male	female	male	male+female
Treated:	male	female	male	female	

[¶Group A= maternal grp II (0 ppm), paternal grp VII (625 ppm); Group B= maternal grp VIII (625 ppm), paternal grp I (0 ppm); Group C: maternal grp II (0 ppm), paternal grp VII (625 ppm); Group D= maternal grp VIII (625 ppm), paternal grp I (0 ppm);† F_{2B} "control" group (0 ppm) from original reproductive study(F_{3A} pups)]

Comparison of the counterpart Groups(A-D) against the "control" from the original study indicates that the birth index (defined by the investigators as fertility) is significantly reduced in all the cross-mating groups. It was noted in the original study that fertility progressively declined in the high dose group (625ppm) in the F_1, F_2, F_3 litters (p. 13 of report) and it was suggested at that time that there may have been a generation-to-generation decrease in the general health of rats of the high-dose group. If this is true also in the cross-mating study, it would mean that both the male and female rats are equally susceptible to the effects of linuron on their general health (i.e., in terms of their fertility). While this is unlikely, since the females are generally more sensitive to poor health conditions (in terms of ability to conceive and carry to term the fetus) than the male (in terms of sperm count, ability to successfully fertilize the ovum, etc.), there is a suggestion in the results of the study of both apparent paternal- and maternal-mediated effects.

The observations of comparative decreased fertility (groups A, C) and fecundity (only Group A), in treatment groups where males were dosed with linuron in the diet, would suggest that the abnormal response was mediated through the paternal side. However, comparison of Group C and D (F_{3C}) parameters suggest a maternal-mediated effect of linuron on pup viability as evidenced by a decrease in the 0-4 day % viability/ litter, 1-4 day % viability/litter and litter survival as well as decreased number of pups/litter. Similar trends are noted in mean pup

weights at 24 hours (both sexes) and day 4 (both sexes) as well as diminished male and female pup weights at weaning. Direct linuron effects on the dams are suggested from the reduction in Group D mean body weights (285 gms; statistically significant at $p<0.05$) when compared with Group C dam body weights (347 gms) where the females were not administered linuron. This reduction is also noted in Group B mean body weights (nss) where the dams were fed linuron.

While of scientific interest, this ancillary study does not provide the essential histological data on the parental animals that are necessary to properly interpret the results of the original reproduction study or those of the present study.

BACKGROUND: This is an ancillary study derived from a multigeneration reproduction study which was previously reviewed by Dr. Charles Aldous in a memorandum dated April 23, 1985. Several addenda were made in the protocol over the course of that study. The initial protocol, dated Nov. 19, 1982, called for a two generation study. The study was to have involved 90-day feeding periods for the F_0 and F_{1B} parents, with two litters from each generation. Histopathology of selected F_{2B} weanlings was anticipated at that time. An amendment dated May 8, 1984 called for 20 male and 20 female weanlings from each group to be selected to generate F_{3A} and F_{3B} litters, with the provision that perhaps the F_{2B} parents would be cross-mated (i.e., controls with high-dose parents) at breeding time for the F_{3B}, if warranted by results from the F_{3A} litters. Finally, an amendment dated October 1, 1984 stated that the objectives of the study were met, and that the study would be terminated with the F_{3A} generation. The plans for the present study under review were stated on p. 138 of the original report: "The F_{2B} parents will be retained, given their respective diets, and cross mated (control groups with high-dose groups) to produce "F_{3B}" litters. However, data from the cross-mating will not be used in the final report since the constraints of the experimental design (e.g., no true control group) do not meet the objectives of this study. The purpose of cross-mating these F_{2B} rats will be for preliminary investigation only. The fate of F_{2B} rats will be for investigations other than those specifically stated in the protocol or protocol amemdments".

In his review, Aldous noted (p. 2) that a major problem with the original reproduction study was the lack of histological information on parental animals, and that the data generated by the cross-mating study could possibly upgrade the original study if histology data were included. Such information is necessary to properly interpret the marked infertility observed in all three generations (F_0, F_{1B}, F_{2B} parents) in the high-dose group (625 ppm) of the original study.

METHODS: A photocopy of the methods section for this ancillary study is appended. The following comments are noted:

a. As stated in the methods (ancillary study), the protocol was amended to allow for the remating of the F_{2B} rats with different partners to produce F_{3B} and F_{3C} litters. The median age of the F_{2B} rats at mating was 291 days (p. 12 of report). The maturity of the animals might have had an effect on their fertility but since no control was used in the study it is not possible to determine this. The "fertility index" for these animals was 41 to 42 % (F_{3C}/ Groups C,D/see summary table, p.6) for the two test groups--an effect which may relate only to the treatment regimen since the F_{3B} dams (Groups A, B/summary table) also had significantly decreased indices of 12 and 47% when compared with the F_{2B} control in the original reproductive study.

b. Although statistically significant differences (e.g. % fertility) were calculated in Table 1 (of the study report) for the two F_{3B} groups, the fact that only two dams (out of twenty females total) (p. 59) in one group had pups (Group A/ maternal: 0ppm, paternal: 625ppm) as opposed to nine dams (twenty females total) with pups in the second group (Group B/ maternal: 625ppm; paternal: 0 ppm) indicates that any comparisons beyond fertility, mating, or fecundity are uncertain, if not invalid strictly due to the limited number of litters available.

c. There are no histological data presented on the parents in either the F_{3B} or F_{3C} groups. Therefore, effects observed on reproductive performance can not be completely interpreted.

d. The fertility index, as given by the experimenter, (i.e.,total # litters delivered divided by the total # females mated x 100) may have been better reported as the birth index. According to the NAS report (p. 107) on Principles and Procedures for Evaluating the Toxicity of Household Substances (1977), the fertility indices are defined as follows:

$$\text{Male fertility index} = \frac{\text{\# males impregnating females}}{\text{\# males exposed to fertile nonpregnant females}} \times [100]$$

$$\text{Female fertility index} = \frac{\text{\# females conceiving}}{\text{\# females exposed to fertile males}} \times [100]$$

Another index of value in evaluating reproduction studies is the fecundity index (NAS definition) which is defined as:

$$\text{Fecundity index} = \frac{\text{\# pregnancies}}{\text{\# copulations}} \times [100]$$

These indices (male and female fertility, fecundity) have been calculated to include copulatory plugs observed plus matings in which the technician failed to note a sperm plug but which resulted in litters, i.e., for the 1) the number of males impregnating females, 2), number females conceiving and 3) number of copulations.

RESULTS: A summary table (p.6) of reproduction/lactation parameters is presented below for the F_{3B} and F_{3C} groups of the ancillary study in comparison to data from F_{2B} "controls" (F_{3A} pups) reported in the original reproduction study. Refer to the summary table for all subsequent discussion. F_{2B} control data (0 ppm) were chosen for comparison purposes since no control was utilized in the ancillary study and the reviewer felt this most closely approximated the ancillary study conditions.

Comparison against "controls": (see summary table, p.6, for definition of treatment groups)

The "fertility" index(as discussed in the methods, which would have been more properly defined as birth index) is considerably less in all F_{3B} (11.8%, 47.4%, Grp A, B, resp.) and F_{3C} (41.2%, 42.1%, Grp C,D, resp.) as compared to the F_{2B} "controls" (89.5%) . This may relate to a compound effect or to the general health of the animals. Pup counts/litter at birth (F_{3B}, Group A= 4.0, Group B= 5.0; F_{3C}, Group C= 9.2, Group D= 6.5; F_{2B}= 13.1) as well as mean postpartum pup counts/litter at weaning-culled at day 4 to ten pups where possible (F_{3B}, Group A=4.0, Group B=4.3; F_{3C}, Group C=8.1, Group D=4.1, F_{2B}=8.1) were also significantly

reduced in both the F_{3B} and F_{3C} groups, although Group C of the F_{3C} generation was equivalent to the "control" value.

In the cross-mating groups the mean pup weights appear similar to the "control" although Group A weights (8.1 gm, 24 hrs; 13.4 gm, day 4 before litter reduction) appeared higher than the "controls" (6.8 gm, 24 hrs; 9.8 gm at day 4 before litter reduction). However, as noted in the Methods section, Group A consisted of litters from only two dams with a small number of pups/litter at birth (4.0) which makes any comparison to the "controls", with a much larger number pups/litter at birth (13.1), inappropriate.

Mean dam body weights in the ancillary study appear similar to the F_{2B} "controls"(300 gm) of the original reproduction study except for F_{3B}, Groups A and C (324, 347 gms., respectively). However the meaning of these differences is uncertain since the use of the F_{1B} control dam weights(335 gm) from the original study would have given a different interpretation to the findings. Therefore, the weight changes can be properly evaluated only within the context of the crossmating study itself.

Group Counterpart Comparisons: (see summary table p.6 for definition on treatment groups)

The male and female fertility indices(%) were reduced in Groups A and C [(males on linuron diet) 47% and 71%-male indices; 47% and 60%-female indices, respectively] as compared to Groups B and D [(females fed linuron diet) 90% and 86%-male indices; 85% and 85%-female indices, respectively]. The fecundity index(%) (somewhat similar to the birth index, i.e., "fertility", in relative numerical values) is lower in Group A (25%) than in Groups B, C and D (53%, 58%, and 47%, respectively). The observations of comparative decreased fertility (both groups) and fecundity (only Group A) in treatment groups where males were dosed with linuron in the diet suggest that the abnormal response may be mediated through the paternal side (e.g., diminished sperm count, sperm viability,etc.) although this can not be established.

Due to the fact that only 2 dams produced litters in Group A, additional comparison of reproductive/lactation parameters will only be discussed in counterpart Groups C and D.

Comparison of Group C (control dams) and D (treated dams)[F_{3C}] parameters suggest a maternally-mediated effect of linuron on pup viability as evidenced by a decrease in the 0-4 day % viability/ litter (98.5 vs 77.5, respectively; not statistically significant=nss), 1-4 day % viability/litter (98.7 vs 88.6 %, respectively , nss) and litter survival (100 vs 75 %, respectively, nss) as well as decreased number of pups/litter (statistically significant ,$p<0.05$, at day 12 and day 21; 8.1 vs 4.4 and 8.1 vs 4.1, respectively). Similar trends are noted in mean pup weights at 24 hours (both sexes; 7.5 vs 6.4 gms, respectively, nss) and day 4 (both sexes; 11.6 vs 8.2 gms, respectively, statistically significant, $p< 0.05$) as well as diminished male (55 vs 44 gms, respectively, statistically significant, $p<0.05$) and female (49.5 vs 41.9 gms, respectively, nss) body weights.

Group D mean dam body weights (285) are also statistically significantly less ($p<0.05$) than Group C dam body weights (347 gms). This effect is also noted in Group B mean dam body weights (nss) where the females were fed linuron.

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SUMMARY TABLE OF REPRODUCTION/LACTATION PARAMETERS

	F_{3B} Group A¶	F_{3B} Group B¶	F_{3C} Group C¶	F_{3C} Group D¶	F_{2B}† "Control"
Control:	female	male	female	male	male+female
Treated:	male	female	male	female	
"Fertility(%)"	11.8	47.4*	41.2	42.1	89.5
Sperm plugs obsd.: #(%)	8/17 (47)	16/19 (80)	11/17 (65)	14/19 (74)	----
Male Fertility(%)	47.1	84.2(89.5)[c]	58.8(70.6)	73.7(85.5)	----
Female Fertility(%)	47.1	80.0(85.0)[c]	55.0(60.0)	70.0(85.0)	----
Fecundity Index(%)	25.0	56.2(52.9)[c]	63.6(58.3)	57.1(47.1)	----
Lactation Index % per litter	100.0	100.0	100.0	78.1	86.3
% born alive/litter	100.0	96.4	98.2	100.0	92.1
0-4 day % viability/litter	100.0	95.0	98.5	77.5	92.1
1-4 day % viability/litter	100.0	91.1	98.7	88.6	98.8
Litter survival (% at weaning)	100.0	100.0	100.0	75.0	82.4
Pup counts/ltr(birth)	4.0(0.0)[b]	5.0(2.1)	9.2(3.9)	6.5(4.0)	13.1(2.7)

Mean postpartum pup counts/litter

Day 4 after reduction	4.0(0.0)[b]	4.3(1.9)	8.1(2.3)	5.3(3.4)	9.4(2.4)
Day 12	4.0(0.0)	4.3(1.9)	8.1(2.3)	4.4**(3.5)	9.4(2.4)
Day 21 (weaning)	4.0(0.0)	4.3(1.9)	8.1(2.3)	4.1**(3.5)	8.1(3.9)

Mean pup weights(gm)

24 hrs (both sexes)	8.1(0.4)	6.5*(0.6)	7.5(1.0)	6.4(0.9)	6.8(0.5)
Day 4 (both sexes) before litter reduction	13.4(1.3)	8.5**(1.1)	11.6(2.1)	8.2**(2.1)	9.8(1.0)
Males at weaning	59.3(7.9)	41.8(7.8)	55.0(8.9)	43.7**(9.3)	43.2(6.0)
Females at weaning	55.6(6.6)	39.8(6.0)	49.5(4.4)	41.9(10.6)	41.8(4.6)
Mean dam bdy wt[a](gm)	324.1	291.5	346.9	285.1**	299.6

¶ Group A= maternal grp II (0 ppm), paternal grp VII (625 ppm); Group B= maternal grp VIII (625 ppm), paternal grp I (0 ppm); Group C: maternal grp II (0 ppm), paternal grp VII (625 ppm); Group D= maternal grp VIII (625 ppm), paternal grp I (0 ppm)

† F_{2B} control group (0 ppm) from original reproductive study(F_{3A} pups)

* Significantly different($p<0.05$) from counterpart groups by Fisher's Exact test

** Significantly different($p<0.05$) from counterpart grps by Mann-Whitney U test

[a] Body weight after weaning; [b] (standard deviation); [c] includes those with copulatory plugs observed plus matings in which the technician failed to note a sperm plug but which resulted in litters

"Fertility" (the report's definition) = $\frac{\text{total \# litters delivered}}{\text{total \# females mated}} \times [100]$

Male Fertility (NAS definition) = $\frac{\text{\# males impregnating females}}{\text{\# males exposed to fertile nonpregnant females}} \times [100]$

Female Fertility (NAS definition) = $\frac{\text{\# females conceiving}}{\text{\# females exposed to fertile males}} \times [100]$

Fecundity Index (NAS definition) = $\frac{\text{\# pregnancies}}{\text{\# copulations}} \times [100]$

DISCUSSION:

Comparison of all the counterpart Groups(A-D) against the "control" from the original study indicates that the birth index (defined by the investigators as fertility) is significantly reduced in all the cross-mating groups, an effect which could relate to either the compound effects on reproductive capability or to the animals' general health status. It was noted in the original study that fertility progressively declined in the high dose group (625ppm) in the F_1, F_2, F_3 litters (p. 13 of report) and it was suggested by the investigators, at that time, that there may have been a generation-to-generation decrease in the general health status of rats of the high-dose group. If this is true in the cross-mating study it would mean that both the male and female rats are equally susceptible to the effects of linuron on their general health (i.e., in terms of their fertility). While this is unlikely, since the females are generally more sensitive to poor health conditions (in terms of ability to conceive and carry to term the fetus) than the male (in terms of sperm count, ability to successfully fertilize the ovum, etc.), there is a suggestion in the results of the study of both apparent paternal- and maternal-mediated effects.

Pup counts/litter at birth (F_{3B}, Group A= 4.0, Group B= 5.0; F_{3C}, Group C= 9.2, Group D= 6.5; F_{2B}= 13.1) as well as mean postpartum pup counts/litter at weaning-culled at day 4 to ten pups where possible (F_{3B}, Group A=4.0, Group B=4.3; F_{3C}, Group C=8.1, Group D=4.1, F_{2B}=8.1) were also significantly reduced in both the F_{3B} and F_{3C} groups although Group C of the F_{3C} generation was equivalent to the "control" value.

In the cross-mating groups the mean pup weights appear similar to the "control" although Group A weights (8.1 gm, 24 hrs; 13.4 gm, day 4 before litter reduction) appeared higher than the "controls" (6.8 gm, 24 hrs; 9.8 gm at day 4 before litter reduction). However, as noted in the Methods section, Group A consisted of litters from only two dams with a small number of pups/litter at birth (4.0) which makes any comparison to the "controls", with a much larger number pups/litter at birth (13.1), inappropriate.

Regarding counterpart comparisons, the observations of comparative decreased fertility (groups A, C) and fecundity (only Group A), in treatment groups where males were dosed with linuron in the diet, would suggest that the abnormal response was mediated through the paternal side. However, comparison of Group C and D (F_{3C}) parameters suggest a maternally-mediated effect of linuron on pup viability as evidenced by a decrease in the 0-4 day % viability/litter (98.5 vs 77.5, respectively; not statistically significant=nss), 1-4 day % viability/litter (98.7% vs 88.6 %, respectively, nss) and litter survival (100% vs 75 %, respectively, nss) as well as decreased number of pups/litter (statistically significant at day 12 and day 21, $p<0.05$; 8.1 vs 4.4 and 8.1 vs 4.1, respectively). Similar trends are noted in mean pup weights at 24 hours (both sexes; 7.5 vs 6.4 gms, respectively, nss) and day 4 (both sexes; 11.6 vs 8.2 gms, respectively, statistically significant, $p<0.05$) as well as diminished male (55 vs 44 gms, respectively, statistically significant, $p<0.05$) and female (49.5 vs 41.9 gms, respectively, nss) body weights.

Direct linuron effects on the females are evident since Group D mean dam body weights are also statistically significantly less (285 gms; $p<0.05$) than Group C dam body weights (347 gms). This effect is also noted in Group B mean dam body weights (nss) where the females were fed linuron.