CASWELL

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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID No.: 128901. Chlorimuron-ethyl: Review of a non-guideline multi-generation reproduction study in rats.

Tox Chem No.: 193B
PC Code: 128901
DP Barcode: D232958
Submission No.: Not provided.

FROM: John Doherty 2/26/98
Toxicology Branch II
Health Effects Division 7509C

TO: Jim Tompkins
Product Manager #25
Registration Division 7505C

THROUGH: Steven Dapson, Ph.D. 2/24/98
Branch Senior Scientist
Toxicology Branch II
Health Effects Division 7509C

I. Conclusions:

TB-II classified this special multi generation reproduction study (MRID No.: 44163301) UNACCEPTABLE and determined that the study *cannot* be upgraded to an acceptable series 8304 multi generation reproduction study because of the special design of this study and because the data were submitted in summary form only and not supported by individual animal data.

II. Action Requested:

Toxicology Branch II (TB-II) has been requested to provide a review of a non-guideline multi-generation reproduction study conducted with the chemical chlorimuron-ethyl. The study was submitted as 6(a)2 data and is identified in Part IV below.

III. Toxicology Branch II Comments:

TB-II concluded that this study should be classified as UNACCEPTABLE because review of the study indicated major deficiencies in the design and reporting of data. TB-II also assessed the "one liner" file for other multi generation reproduction studies and noted that there are three such studies already classified as CORE MINIMUM. The study deficiencies are listed in item 1 below and comments on the comparison of this study with existing multi generation reproduction studies are presented in item 2 below.

These deficiencies are listed as follows.

1. Design of the study.

-Only 10 male and 20 female rats were used per dose group. Guideline studies recommend 30 rats per sex.

-Dosing was by gavage for 70 days for males (two sperm cycles) and only 14 days for females (two estrus cycles). This dosing was stated as being for the first parental generation but there was no definite evidence that the dosing was made for the second generation.

-It is unclear if the dams were dosed continuously during lactation and weaning.

-Most of the data are presented in summary tables only and there is no supporting individual animal data,

-There is no analytical report to verify the composition of the actual dosing material for either concentration or stability.

2. Comparison with other multi generation reproduction studies in the "one liners" file.

There are three multi generation reproduction studies with DPX-6025 (code name for chlorimuron-ethyl) and these have NOEL and LOELs as follows.

Study	Systemic NOEL/LOEL	Developmental NOEL/LOEL
Special Study. 2-generation Shriram Institute for Industrial Research, Report No.: 160913, 1/16/95. MRID No.: 44168301, this document. Doses tested: 0, 10, 50 or 200 mg/kg/day (by gavage). Classification: UNACCEPTABLE.	10/50 mg/kg/day. At 50 mg/kg/day: Deaths in males and females. Lower fertility index.	50/200 mg/kg/day. At 200 mg/kg/day: lower fetal body weight.
1-generation reproduction. Haskell Laboratory, # 306-83, September 1, 1983. Acc. No.: 072017, Doc. No.: 003786. Doses tested: 0, 100, 2500 or 7500 ppm. Classification: MINIMUM	2500/7500 ppm (estimated 125 and 375 mg/kg/day): Reduced body weight.	100/2500 ppm (estimated 5 and 125 mg/kg/day): Reduced litter weights on day 4. .
2-generation reproduction. E.I. Dupont, HLR 357-84, 10/25/84. ACC. No.: 07311, HED Doc No.: 004626. Doses tested 0, 25, 250 or 2500 ppm. Classification: MINIMUM	First generation interim report: 250/2500 ppm (estimated 12.5 and 125 mg/kg/day). Response at 2500 ppm not described in one liner.	> 2500 ppm.
2-generation reproduction. Haskell Lab, HLR 422-85, 8/26/85. ACC. No.: 073803 and 073804, HED Doc. No.: 004968. Doses tested 0, 25, 250 or 2500 ppm. Classification: MINIMUM	250/2500 ppm (estimated 12.5 and 125 mg/kg/day). Body weight reduction.	25/250 ppm (estimated 1.25 and 12.5 mg/kg/day). Body weight and histopathological finding.

The above tables allows for the four studies to be compared by the interested party. It should be noted that the MINIMUM classification assigned to the other three studies may be downgraded pending the review of this chemical in the RED process.

IV. Studies reviewed:

Study Identification	Executive Summary
Non-Guideline multi-generation reproduction in rats. Shriram (India) Institute for Industrial Research, Project No.: TOX/2, , January 16, 1995.	In specially designed multi generation reproduction study (MRID No.: 44163301) with Wistar strain rats, four groups of 10 males and 20 females were dosed with classic technical (95% purity) at dose levels of 0, 10, 50 or 200 mg/kg/day by gavage (vehicle not identified). Males were dosed for 70 days and females were dosed for 14 days prior to mating so that two spermatogenic periods and two consecutive estrus cycles were included in the scheduled dosing period. The P1 parental group was bred to produce two sets of litters (F1a and F1b) and the P2 parental groups were selected from the F1b generation. Thus, four sets of pups were generated. At 50 mg/kg/day there was a single incidence of **death** in males, males also had reduced **body weight** and there were degenerative lesions in the **liver** and **kidney** in both sexes. At 200 mg/kg/day, there were additional deaths in both sexes and a decrease in fertility, there was also some evidence of an unspecified lesion in the male gonads. The parental systemic toxicity LOEL is 50 mg/kg/day based on deaths, body weight decrease and histopathology of the liver and kidneys. The systemic toxicity NOEL is 10 mg/kg/day. The reproductive toxicity LOEL is 200 mg/kg/day based on decreased fertility. The reproductive toxicity NOEL is 50 mg/kg/day. Classification: This study is classified as UNACCEPTABLE (GUIDELINE) There are numerous deficiencies in the design and reporting of the data. This study *cannot* be upgraded to a series 83-4 guideline study.

[Classic technical/1995] [Special Study: Multi generation repro]

EPA Reviewer: John Doherty John Doherty 2/20/98 _____, Date ___
Toxicology Branch II (7509C)
EPA Secondary Reviewer: Stephen Dapson Stephen C. Dapson 2/24/98, Date ___
Toxicology Branch II (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: Non-Guideline Multi generation Reproduction Study - rat

DP BARCODE: D232958
P.C. CODE: 128901

SUBMISSION CODE: Not provided
TOX. CHEM. NO.: 193B

TEST MATERIAL (PURITY): Classic Technical (95% w/w, purity)

CITATION: Aggarwal, M.L. et al, 1995. "Two Generation (4 litters) Reproduction Study in Albino Rats" Shriram Institute for Industrial Research. Study No.: "Tox/2". Also Report No.: 160913, January 16, 1995. MRID No.: 441633-01.

SPONSOR: Du-Pont Far East, Inc. (New Delhi).

EXECUTIVE SUMMARY:

In specially designed multi generation reproduction study (MRID No.: 44163301) with Wistar strain rats, four groups of 10 males and 20 females were dosed with classic technical (95% purity) at dose levels of 0, 10, 50 or 200 mg/kg/day by gavage (vehicle not identified). Males were dosed for 70 days and females were dosed for 14 days prior to mating so that two spermatogenic periods and two consecutive estrus cycles were included in the scheduled dosing period. The P1 parental group was bred to produce two sets of litters (F1a and F1b) and the P2 parental groups were selected from the F1b generation. Thus, four sets of pups were generated.

At 50 mg/kg/day there was a single incidence of **death** in males, males also had reduced **body weight** and there were degenerative lesions in the **liver** and **kidney** in both sexes. At 200 mg/kg/day, there were additional deaths in both sexes and a decrease in fertility, there was also some evidence of an unspecified lesion in the male gonads. The parental systemic toxicity LOEL is 50 mg/kg/day based on deaths, body weight decrease and histopathology of the liver and kidneys. The systemic toxicity NOEL is 10 mg/kg/day. The reproductive toxicity LOEL is 200 mg/kg/day based on decreased fertility. The reproductive toxicity NOEL is 50 mg/kg/day.

Classification: This study is classified as UNACCEPTABLE (GUIDELINE) There are numerous deficiencies in the design and reporting of the data. This study *cannot* be upgraded to a series 83-4 guideline study.

COMPLIANCE: Only a signed and dated Quality Assurance statement was provided. The study was conducted in India circa 1995.

Experimental Constants

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Classic Technical
   Description: White powder
   Lot/Batch #:: Not provided.
   Purity: 95 % a.i. w/w
   CAS #: 90982-32-4.

2. Vehicle: No information. Since the route of administration was oral intubation (gavage), there must have been some vehicle used to aid in administration of the test material.

3. Test animals: Species: rat
   Strain: Wistar
   Age at start of dosing: Not stated.
   Weight at start of dosing: Males ~180-220, females ~160-200.
   Source: Not specified.
   Housing: groups of 5 according to sex.
   Diet: Not specified.
   Water: Not specified.
   Environmental conditions:
   Temperature: 24 ± 2 degrees C.
   Humidity: 40-70%
   Air changes: 15/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): Not stated.

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: Basically one male was placed with two females. The males were rotated among the females in the same group. Mating was confirmed by the presence of a vaginal smear. The following mating schedule was reportedly followed.

Premating period (P1):
Males dosed 70 days prior to mating.
Females dosed 14 days prior to mating.

Apparently both males and females remained on the dosing program through gestation and lactation and until the second mating. Apparently only a 10 day interval between weaning the F1a generation and breeding for the F1b generation was allowed. The F1a generation pups were discarded after weaning. The P1 group was sacrificed 21 days after the birth of the F1b generation and their organs saved for evaluation. The F1b pups were culled to be the parental groups for the F2a and F2b generation. Apparently the F1b pups were raised to maturity to be

the P2 group in the same way the P1 group was apparently meaning the males were dosed for 70 days and the females for 14 days prior to mating. F2a pups were delivered, examined and discarded after weaning and an F2b generation was also bred and after weaning their organs saved for evaluation.

2. Study schedule: In this study, males were dosed by gavage for 70 days prior to mating in order to cover the spermatogenic cycle. Females were dosed for 14 days prior to mating in order to cover two estrus cycles. The report states that the "dosing is continued till F2 generation to check any effects on overall reproduction." It is not entirely clear as to whether or not the female rats were dosed contiguously through lactation and until their second mating.

3. Animal assignment: P animals were randomly (method of randomization was not provided) assigned to test groups as seen in Table 1.

Table 1. Study design.

Dose Group	Dose Level mg/kg/day	Animals/group for P1 and P2 Males	Females
Control	--	10	20
Low	10	10	20
Mid	50	10	20
High	200	10	20

4. Dose selection rationale: No rationale for dose selection was provided in this report.

5. Dosage preparation and analysis. No information was provided on the preparation of the test diets and there was no analytical report verifying the concentration, homogeneity or stability of the test material in the diets.

D. DATA ANALYSIS

Mean and standard deviations were calculated. The only statistical test referenced was the student's t test for determining the significance in the pups on weight gains on days 0, 4, 14 and 21.

E. Historical control data: None provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: There were no clinical signs which the author attributed to

treatment.

In the P1 group there was one mortality in the mid dose group males and two in the high dose group males occurring during the treatment period. Two pregnant females in the 200 mg/kg/day dose group died during pregnancy and/or delivery.

There was no mortality reported in the P2 group.

The study author determined that the deaths in the mid and high dose males and high dose females were the result of treatment.

2. Body weight and food consumption: Body weight was determined at weeks 0, 1, 2, 3, 4, 5, 6, 7, 8 for males and weeks 0, 1 and 2 for females presumably for the treatment periods for the P1 group. Food consumption was not determined. Table 2 below summarizes the body weight and gain data.

Table 2. Body weight and gain data for the P1 generation.

Interval (Weeks)	Dose Group			
	Control	10 mg/kg	50 mg/kg	200 mg/kg
P1 Parental Premating - males				
Body Weight (week 0) P1	202.9±24.6	193.2±0.6 (-5%)	191.3±11.3 (-6%)	195.8±12.3 (-4%)
Mean weight gain (g) P1 week 0 to week 4	31.9	45.5	24.9	(-8.4)
Mean weight gain (g) P1 week 4 to week 8	55.4	53.9	36.5	43.4
Body weight at week 8 (%gain for group) (% ↓ relative to control)	290.2±23.5 43%	292.3±25.1 51%	252.7±27.3 32% (-13%)	230.8±33.2 18% (-21%)
P1 and P2 Parental Premating -females				
Mean Weight Week o (g) P1	181.7±8.16	190.4±8.88	193.1±9.9	202.4±16.3
Mean weight gain P1 weeks 0 to 2	4.9	5.5	6.0	5.4

Data extracted from Tables 6 and 7, of the study report (page number is not readable).

Table 1 above indicates that in males there is a decrease in body weight gain at the mid

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dose level of 50 mg/kg/day. There were no data on the weight gain for the P2 parental group to compare with the P1 groups. The study author did not include a decrease in body weight among the effects of treatment.

Table 13a presents a limited amount of data on the body weight of males in the P2 parental group to indicate that there was no similar decrease in weight due to treatment. For example, the high dose group body weight is given as 298.10 ± 18.8 gm and the control as 269.8 ± 42.7 gm. The mid and low dose groups are close to the control.

4. Reproductive function:

The reproductive performance for the four matings was summarized in Tables 1, 2, 3 and 4 of the study report. These tables are attached. The fertility index (number of pregnancies/number of matings) was the lowest in the 200 mg/kg/day dose group for all matings. Subsequently, there were fewer number of litters, pups born and other associated parameters. Table 3 illustrates the reproductive parameters of the P1 and P2 parental groups in producing the F1a, F1b, F2a and F2b generations.

Table 3. Reproductive Performance.

Parameter		Dose Group Control mg/kg	10 mg/kg	50 mg/kg	200
Fertility Index	P1/F1a	80%	80%	75%	75%
	P1/F1b	75%	75%	70%	60%
	P2/F2a	95%	90%	85%	75%
	P2/F2b	70%	65%	60%	50%
Lactation/weaning Index	P1/F1a	80.3%	74.1%	72.3%	70.6%
	P1/F1b	91%	88.4%	77.08%	76.3%
	P2/F2a	96.6%	96.4%	96%	90.8%
	P2/F2b	94.9%	93.3%	92.1%	91%
Litter Survival	P1/F1a	87.5%	87.5%	100%	80%
	P1/F1b	100%	100%	85%	75%
	P2/F2a	94.7%	100%	94.1%	100%
	P2/F2b	100%	100%	100%	100%

Data are from Tables 1, 2, 3 and 4 of the study report.

Table 3 shows that the high dose is associated with decreased fertility index in all generations. The lactation/weaning index and litter survival index were also lower in some but not all generations.

5. Parental postmortem results

a) Organ weights: Organ weight data for males and females (liver, kidney and testis or ovaries and uterus) were presented for the P1 and P2 groups only and only summary tables (no individual animal data) were presented. The study author asserts that there was no effect on any organ weight (absolute or relative). Inspection of Tables 12 to 15 of the study report which present the organ weight data indicate some differences in one parental group but not in the other. These are itemized as follows.

Males:

Liver: P1 relative weight *increased* 19% in both mid and high dose and the absolute weights were increased ~5% and 2.4% in the mid and high dose groups respectively. .

P2 relative weight *decreased* 18% in the mid but only 12% in the high dose while the absolute weights was *decreased* 21% in the mid dose group but was *increased* 11% in the high dose group. .

Testis: P1 relative weight for the mid and high dose were about 17% less than the control. Absolute weight was *decreased* 27% for both doses.

P2 relative weight for the high dose was essentially the same as the control. And absolute weight was 19% *increased.*

Females:

All P1 organ weights (absolute and relative) were considered reasonably close to the controls.

For the P2 groups:

Kidney: The control group had a very high reading and large standard deviation (2.82 ± 0.83) and all dosed groups were lower but the high dose group was still disproportionally lower for absolute weight. Relative weight was also disproportionally lower in the high dose group.

Liver: Relative weight was 10% *lower* in the high dose group but was also 39% and 19% *higher* in the mid low and mid dose groups meaning that the values were widely scattered. Absolute weight was also scattered with large standard deviations (i.e. ~20% for the mid dose group). The high dose was 25% lower and the low and mid dose groups were 5% and 15% higher.

Uterus: The control had the highest weight and the high dose group the lowest suggesting as much as a 35% decrease in absolute and 33% decrease in relative weight.

In conclusion, TB-II notes the possible changes as above but since there are no individual animal data to verify the observations, declines from formally interpreting these data.

b.) Pathology The study report asserts that there were no gross necropsy lesions noted. There were no tables presented.

Microscopic examination: The liver and kidney were noted to have degenerative lesions in both the mid and high dose group as indicated in the following table.

Organ and Lesion		Males (dose in mg/kg/day) Control	10	50	200	Females (dose in mg/kg/day) Control	10	50	200
Liver (Degenerative changes and bv congest.	P2	0/5	0/5	5/5 [1]	3/5[2]	0/10	0/10	10/10 [1]	10/10[2]
	F2b2	0/5	0/5	5/5 [1]	5/5[1]	0/5	0/5	5/5[1]	5/5[1]
Kidney (degenerative changes and bv congest.	P2	0/5	0/5	5/5[1]	5/5[2]	0/10	0/10	10/10[1]	10/10[2]
	F2b2	0/5	0/5	5/5[1]	5/5[1]	0/5	0/5	5/5[1]	5/5[1]
Gonads (no specified lesion)	P2	0/5	0/5	0/5	2/5[1]	0/10	0/10	0/10	0/10
	F2b2	0/5	0/5	0/5	2/5[1]	0/5	0/5	0/5	0/5

Data from Tables 16 and 17 of the study report.
[1] Lesion described as mild change. [2] lesion described as moderate change.

On the basis of Table 4 above, the NOEL for pathology is 10 mg/kg/day and the LOEL is 50 mg/kg/day based on liver and kidney lesions. At 200 mg/kg/day, in addition, there is some evidence of unspecified changes in the male gonad.

III. DISCUSSION

A. **Study Author's Conclusions.** The study author asserts a NOEL and LOEL of 10 and 50 mg/kg/day but was unclear as to what effects of treatment were evident at 50 mg/kg/day.

B. **Toxicology Branch II's Conclusions.** This study is classified as UNACCEPTABLE. The following deficiencies were noted.

-There is insufficient information available on the identification of the test material and there is no analytical report to verify the dose levels administered for either the composition or stability in the dosing matrix.

-The vehicle was not identified. For gavage studies, the identification of the vehicle is critical because the vehicle can effect the adsorption of the test material.

-The study *cannot* be ungraded to an acceptable guideline series 83-4 multi generation reproduction study because the animals were not continuously dosed with the test material. In this study, the males were dosed to include two sperm cycle (~70 days) but the females were dosed for only about 10 days to cover two estrus cycles.

-There are also deficiencies related to reporting of data with most of the data being

presented in summary tables only without individual animal data.

Overall, TB-II assigns a parental systemic toxicity NOEL and LOEL of 10 and 50 mg/kg/day based on deaths in males (one), lesions in the liver and kidney and decreases in body weight. The reproductive toxicity LOEL is 200 mg/kg/day based on a decrease in fertility. The reproductive toxicity NOEL is 50 mg/kg/day.