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

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MAY 2 1 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide (Acetochlor): Review of Toxicology Data Submitted by the Registrant.

Caswell No: 003B
HED Project No: 1-2068
MRID No: 419633-09

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5/20/93

Registrant: ICI Agricultural Products

Action Requested: Review of the following Toxicology study with Acetochlor:

§ 84-2 Mutagenicity- Structural Chromosome Aberration

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Conclusions:

Acetochlor at doses of 1000 and 2000 mg/kg resulted in reduced fertility during weeks 2, 3, and 4 of this study, as shown by reduced pregnancy incidence, decreased implants per pregnancy, increased pre-implantation loss, and decreased live implants per pregnancy. Early and late intra-uterine deaths were not significantly affected in this study. Under the conditions of this study, positive evidence of mutagenicity was observed for acetochlor at 1000 and 2000 mg/kg. Doses tested in Sprague-Dawley rats: 0, 200, 1000, and 2000 mg/kg.

Classification:

acceptable

This study satisfies the requirements (§84-2) for a mutagenicity study (structural chromosome aberrations) in rats.

Reviewed by: Timothy F. McMahon, Ph.D. 5/12/93
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Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 5/12/93
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Data Evaluation Report

Study type: Mutagenicity-Structural Chromosome Aberration (84-2)

Tox. Chem. No.: 003B

MRID number: 419633-09

Test material: Acetochlor Technical

Synonyms: 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide

Study number: RRO527

Testing Facility: ICI Central Toxicology Laboratory
Cheshire, UK

Sponsor: ICI Agricultural Products

Title of report: Acetochlor: Dominant Lethal Study in the Rat

Author(s): M.C.E. Hodge

Study completed: May 10, 1991

Conclusions:
Acetochlor at doses of 1000 and 2000 mg/kg resulted in reduced fertility during weeks 2, 3, and 4 of this study, as shown by reduced pregnancy incidence, decreased implants per pregnancy, increased pre-implantation loss, and decreased live implants per pregnancy. Early and late intra-uterine deaths were not significantly affected in this study. Under the conditions of this study, positive evidence of mutagenicity was observed for acetochlor at 1000 and 2000 mg/kg.

Core Classification: acceptable

This study satisfies the requirements (§84-2) for a mutagenicity study (structural chromosome aberrations) in rats.

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I. MATERIALS

A. Test Materials:

1) Acetochlor Technical; description: brown liquid; purity: 90.4%; batch no: P2

2) Positive Controls: Cyclophosphamide (CTL reference # Y01259/007)

Triethylenemelamine (CTL reference # Y06641/001)

3) Vehicle Control: Corn oil (Kraft-Wesson), sterilized water (for cyclophosphamide dosing solutions), deionized water (for triethylenemelamine dosing solutions).

B. Animals and Husbandry

One-hundred sixty-five unproven male rats and 330 virgin female rats (Alpk:APfSD, 8-9 weeks old) were obtained for use in a pre-study fertility test. Following this phase of the study, 248 virgin female rats of the same strain were used from the same supplier at weekly intervals for 10 consecutive weeks.

Rats were housed in 6 animal rooms during the study. Males were housed individually and females 4 per cage upon arrival. For mating, one male was paired with 2 females and then returned to its individual cage after a 7 day mating period. Rats received CT1 powdered diet (Special Diet Services Ltd, Essex, UK) and filter-sterilized tap water via an automatic watering system. Temperature of animal rooms was between 18-24°C and 51-95% humidity. A 12 hour light/dark cycle was used.

C. Experimental

1)Preliminary fertility testing

One-hundred sixty five male rats were selected and mated with 2 females per male rat for 7 consecutive nights. Females were killed following the 7 day period by halothane anesthesia and cervical dislocation. The total number of corpora lutea, live fetuses, and early and late intra-uterine deaths were recorded. From these data, male rats were graded according to fertility and background dominant lethal frequency and then randomly allocated to six experimental groups.
Males not selected from this study were humanely killed and discarded

2) Main Study

Twenty male rats in six dose groups were used for the main study. Rats received a

single oral dose of 0, 200, 1000, or 2000 mg/kg acetochlor , 100 mg/kg cyclophosphamide, or 0.5 mg/kg triethylenemelamine by gavage. Dose volume was 10ml/kg. Detailed clinical observations were made daily, and body weights were recorded prior to dosing, daily until the first mating, and then at weekly intervals thereafter.

Four days after dosing, dosed males were paired with 2 females per male and left for 7 consecutive days. This procedure was repeated each week with females of similar age until 10 test matings had been conducted. Nineteen days after being housed for mating, female rats were humanely killed and subject to analysis of the reproductive tract as described above in the preliminary test phase.

The following parameters were calculated:

a) proportion of females pregnant

b) proportion of males successfully mating

c) mean number of corpora lutea

d) number of implantations per female

e) percentage pre-implantation loss

f) number of early deaths per pregnancy

g) percentage of implantations which were early deaths

D. Compliance

Signed statements of No Data Confidentiality Claims, GLP Compliance, Quality Assurance and EPA Flagging Criteria were provided. This study does not meet or exceed any of the applicable EPA flagging criteria.

II. RESULTS

A. Dosing solutions

Stability and homogeneity analyses were performed on dosing solutions of acetochlor used in this study. Results (Tables 2 and 3, paged 31-32 of the report) showed that achieved concentration and homogeneity of acetochlor dosing solutions were within acceptable limits.

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B. Clinical Observations

Significant clinical toxicity was observed in dosed male rats primarily at the 2000 mg/kg dose level of acetochlor. These included 3 rats with diarrhea on days 4-7 of the study, 2 rats with hunched posture on days 4-6 of the study, 16 rats with piloerection from days 3-47 of the study, 6 rats with stains around the mouth from days 3-8 of the study, 5 rats with stains around the nose from days 3-75 of the study, and 5 rats with signs of urinary incontinence from days 3-6 of the study. Four rats from the cyclophosphamide group were sacrificed in extremis on days 8-9 of the study. Similar clinical signs were observed at lower doses of acetochlor in this study, but were found at very low incidence and were not dose-related.

Three rats from the 2000 mg/kg acetochlor dose group were found dead on days 3-4 of the study. Aside from the deaths occurring in the positive control group, no other mortality was reported.

C. Body weight

Body weight gain data for male rats used in this study are summarized in the following table (Table 1)

Table 1

Body Weight Gain in Male Rats Dosed with Acetochlor[a]

	Dose Groups (mg/kg)			
	0	200	1000	2000
days				
1-7	10.4	9.4	-4.6**	-35.4**
7-26	43.4	46.9	51.6	84.9
1-26	53.3	57.4	47.1	43.7*

[a]data taken from Table 5, pages 38-39 of the report or calculated from Appendix 2, pages 150-157 of the report

*$p < 0.05$ vs. 0 mg/kg; **$p < 0.01$ vs. 0 mg/kg.

An apparent dose-related decrease in body weight gain was evident in dosed male rats beginning at the 1000 mg/kg dose level from days 1-7 of the study. According to data provided, this decrease in body weight gain was significant for male rats at the 1000 mg/kg dose level through day 19 of the study. For rats in the 2000 mg/kg dose group, significantly decreased body weight gain was apparent through day 26 of the study in comparison to negative controls. Weight gain was also significantly decreased in the groups receiving cyclophosphamide and triethylenemelamine from study days 2-75.

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D. Reproductive parameters

A summary of reproductive measurements taken from female rats which were mated to dosed male rats is listed in the following table:

TABLE 2a

Reproductive Parameters in Female Rats Mated with Dosed Male Rats[a]

% pregnancy incidence	Dose groups (mg/kg) 0	200	1000	2000	cyc	TEM
week 0	72	75	75	72	71	75
week 1	85	88	85	85	71	20*
week 2	92	80	72	52*	82	13*
week 3	88	85	80	22**	76	5*
week 4	82	90	95	87	71	42**

Table 2b

implants per pregnancy	Dose groups (mg/kg) 0	200	1000	2000	cyc	TEM
week 0	11.5	11.6	10.6	10.6	11.6	11.0
week 1	11.7	11.3	11.0	12.3	8.2**	5.1**
week 2	11.4	11.3	10.2	10.8	11.7	4.3**
week 3	12.0	11.0	7.7**	2.2**	9.2**	1.0**
week 4	11.5	12.3	12.1	9.5*	9.9	7.6**
week 5	12.3	11.4	12.2	10.6*	11.9	11.0

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Table 2c

corpora lutea	Dose groups (mg/kg)					
	0	200	1000	2000	cyc	TEM
week 0	13.8	12.7	13.2	13.3	13.8	13.1
week 1	13.6	13.9	13.3	13.8	12.4	17.3
week 2	14.6	13.5	14.4	14.4	13.6	20.6
week 3	13.4	13.4	12.1	10.6	12.4	16.5
week 4	13.0	13.5	14.0	11.8	12.1	11.1

Table 2d

pre-implantation loss	Dose groups (mg/kg)					
	0	200	1000	2000	cyc	TEM
week 0	16.1	8.3	20.1	20.2	15.9	16.0
week 1	14.0	15.7	17.0	10.5	31.8**	65.8**
week 2	21.1	17.7	27.0	22.3	14.1	74.3**
week 3	11.7	19.5	35.9**	78.4**	23.5**	92.8**
week 4	13.3	9.7	13.0	20.5	15.8	26.4**

Table 2e

total early deaths	Dose groups (mg/kg)					
	0	200	1000	2000	cyc	TEM
week 1	38(1.1)	31(0.9)	25(0.7)	38(1.0)	127(4.5)	39(4.8)
week 2	33(0.9)	26(0.8)	23(0.8)	10(0.4)	126(4.2)	13(3.0)
week 3	25(0.7)	25(0.7)	22(0.7)	9(0.9)	171(5.9)	2(1.0)
week 4	7(0.2)	24(0.6)	19(0.5)	16(0.5)	114(4.4)	99(6.0)

( )-indicates early deaths as a percentage of total number of pregnant rats.

Table 2f

total late deaths	Dose groups (mg/kg)					
	0	200	1000	2000	cyc	TEM
week 1	17	1	2	1	11	1
week 2	0	0	1	0	1	0
week 3	3	1	3	0	4	0
week 4	1	1	2	2	3	2

For Tables 2a-2f above, cyc = cyclophosphamide; TEM = triethylenemelamine
*$p < 0.05$ vs. 0 mg/kg; **$p < 0.01$ vs. 0 mg/kg for Tables 2a through 2f.

As shown from the above Tables (**Tables 2a-2f**), significant effects on fertility were evident at the 1000 and 2000 mg/kg dose levels. At 1000 mg/kg, a significant decrease in implants per pregnancy was observed during week 3 as was a significant increase in pre-implantation loss. Total early and late intra-uterine deaths (Tables 2e and 2f) were not affected in a significant manner.

At 2000 mg/kg, a significant reduction in pregnancy incidence was observed at weeks 2 and 3 of the study, as was a significant decrease in implants per pregnancy on weeks 3, 4, and 5, and a significant increase in pre-implantation loss on week 3 at this dose level. Early and late intra-uterine deaths were unaffected at this dose level. A marked increase in early deaths per pregnancy was seen at the 2000 mg/kg dose level on week 3 (Table 2e). The registrant stated that this was related to a decrease in the number of implantations during this week (Table 2b), so that the percentage of early deaths as a proportion of implants increased. Examination of the relationship between the number of early deaths and number of implants to determine if early deaths remain the same as the number of implants is reduced showed no statistically significant difference when control rats with low implantations were compared with the 2000 mg/kg group in week 3. This is supported by the observation that the number of early deaths in the positive control groups were increased even when the number of implants was low.

In addition to the above data, calculation of the number of live implants per pregnant dam was made from data provided by the registrant (pages 177-244 of the report). These data are in general agreement with that found in Table 9, page 46 of the report. In this table, the intergroup comparison of implantations per pregnancy is made, and a significant dose-related effect is observed for week 3 of treatment. In fact, the number of implants per pregnancy for the 1000 and 2000 mg/kg dose groups (7.7 and 2.2, respectively vs. 12.0 in control) is lower than that found for the positive control cyclophosphamide (9.2) at this time point. Calculation of live implants per pregnancy shows that at week 3, live implants for the 0, 200, 1000, and 2000 mg/kg dose groups are 11.0, 9.8, 6.7, and 1.4, respectively. Thus, these data also show a significant dose-related decrease in live implants per pregnancy.

CONCLUSIONS

Acetochlor at doses of 1000 and 2000 mg/kg resulted in reduced fertility during weeks 2, 3, and 4 of this study, as shown by reduced pregnancy incidence, decreased implants per pregnancy, increased pre-implantation loss, and decreased live implants per pregnancy. Early and late intra-uterine deaths were not significantly affected in this study. Under the conditions of this study, positive evidence of mutagenicity was observed for acetochlor at 1000 and 2000 mg/kg.

Core Classification: acceptable

This study satisfies the guideline requirement (§84-2) for a mutagenicity (structural chromosome aberration) study.