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

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SEP 30 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a Teratology Study in Rabbits with Alachlor. EPA MRID# 405794-01 and 405794-02, HED Project No. 8-1024, Caswell# 11.

TO: Robert Taylor/Vickie Walters, PM-25
Herbicide-Fungicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 9/27/88
Pharmacologist, Review Section I
Toxicology Branch-Herbicide, Fungicide,
Antimicrobial Support/HED (TS-769C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q Bui 9/28/88
Section Head, Review Section I
TB-HFAS/HED (TS-769C)
and
William L. Bernam, Acting Chief
Toxicology Branch-Herbicide, Fungicide,
Antimicrobial Support
Health Effects Division (TS-769C)

Registrant: Monsanto Agricultural Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63167

Action Requested: Review rabbit teratology study with alachlor.

RECOMMENDATIONS:

Maternal toxicity was noted at the high dose tested in the form of reduced body weight gain during the dosing period with a rebound increase in body weight gain in the period following dosing. No other maternal toxicity was noted. No biologically relevant fetal external, visceral or skeletal anomalies were noted. This study is acceptable and classified as minimum data. Monsanto has fulfilled the requirement for a teratology study in rabbits.

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Background:

Two teratology studies have been submitted in support of the registration of alachlor:

1. Rabbit, oral teratology, International Research and Development Corporation (IR-79-022; IRDC-401-060), November 24, 1980; submitted by Monsanto on January 15, 1981. This study used technical alachlor (92.19% a.i.) administered by gavage in corn oil to artificially inseminated female Dutch Belted Rabbits from Gestation Days 6 through 27, inclusive. This study was classified as invalid for the following reasons:

a. The study did not have valid control data due to the following observations:

i. There was a decrease in body weight (-59 gm) during the dosing period (Gestation Days 6-28).

ii. There was a high incidence of heart anomalies in 8/66 (12.1%) of the fetuses and 2/10 (20%) of the litters as compared to the histroical control incidence of 2/741 (1.2%) of the fetuses and 2/118 (7.7%) of the litters. The incidence of scoliosis in both the test and control groups was considered to be considerably higher than the historical control incidence.

iii. The fetuses were found to be lower in body weight (27.7 gm) when compared to the treated groups (37.5, 28.5 and 29.5 gm for the low, mid and high dose groups, respectively) and 31.1 gm for the historical control.

iv. At autopsy, congested lungs with red foci (indicating the possibility of gavage error in more than the two animals that were reported dead due to gavage error).

b. The registrant did not submit data on food consumption, fetal length, and individual necropsies of animals that died or aborted during the study.

c. The heart and major vessel "variations" were not described in this study.

d. The available data in the report indicated that many of the animals, in all groups, had congested lungs and red foci. These findings may be reflective of a faulty gavage procedure resulting in additional stress during gestation.

2. Rabbit, oral teratology, International Research and Development Corporation (IR-83-045; IRDC#401-208), December 29, 1983. This study used technical alachlor (94% a.i.) administered by gavage in mineral oil to artificially inseminated female Dutch Belted Rabbits from Gestation Days 7 through 19, inclusive. This study was classified as **supplementary data** for the following reasons:

a. There was deviation from the protocol of the earlier study in terms of the vehicle used to administer technical alachlor. The investigators substituted mineral oil for corn oil used in the earlier study. Corn oil is routinely used a a pharmaceutic aid (as a solvent for many drugs that are injected in oily solution or suspension) whereas mineral oil is used as a **laxative-cathartic**, it is **indigestable**, absorbed only to a limited extent and acta as a lipid solvent where it **may interfere with the absorption of fat soluble substances.** These known actions of mineral oil may affect adequate absorption of the chemical from the gut and therefore reduce potential response.

b. There were high levels of preimplantation loss noted in the control (31.8%) and high dose (49.1%) groups. These levels were in excess of those found in IRDC historical control data and possibly are indicative of dosing prior to the completion of implantation. The preimplantation loss in the control group may be due to maternal stress. The preimplantation loss in the high dose group may have obscured potential developmental toxicity.

c. Several minor deficiencies.

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Primary Review by: Stephen C. Dapson, Ph.D.
Pharmacologist, Review Section I, TB-HFAS/HED (TS-769C)
Secondary Review by: Quang Q. Bui, Ph.D., D.A.B.T.
Section Head, Review Section I, TB-HFAS/HED (TS-769C)

DATA EVALUATION RECORD

Study Type: Developmental Toxicity - Rabbit
Guideline: 83-3

EPA Identification Numbers: EPA ID No.: 524-316
EPA Record No.: 227850
EPA MRID Nos.: 405794-01
405794-02
Caswell No.: 11
Shaughnessy No.: 090501-8
SACB Project No.: 8-1024

Test Material: Alachlor

Synonyms:

Study No.: Laboratory Project No. 87-3169 (BD-87-83)
RD No. 863

Sponsor: Monsanto Agricultural Company
800 North Lindbergh Boulevard
St. Louis, MO 63167

Testing Facility: Bio/Dynamics, Inc.
East Millstone, NJ 08819

Title of Report: A Teratogenicity Study in Rabbits with Alachlor

Author: Raymond E. Schroeder, M.S., D.A.B.T.

Report Issued: March 28, 1988 (study completion date)

Conclusions:

Maternal toxicity was noted at the high dose tested in the form of reduced body weight gain during the dosing period with a rebound increase in body weight gain in the period following dosing. No other maternal toxicity was noted. No biologically relevant fetal external, visceral or skeletal anomalies were noted.

Classification: Core-Minimum Data

Maternal NOEL = 100 mg/kg/day
Maternal LOEL = 150 mg/kg/day
Developmental Toxicity NOEL > 150 mg/kg/day

A copy of the "Materials and Methods" section from the investigator's report is appended.

A. Materials

1. Test Compound

Purity: 94.7% ai
Description: wine red colored solid
Lot No.: 51486-C
Contaminants: not provided.

2. Vehicle(s)

Mazola corn oil
Lot No. Jun 23 88B.

3. Test Animals

Species: Rabbit
Strain: New Zealand White
Source: Hazleton-Dutchland, Inc.
Denver, PA 17517
Age at receipt: Males: 7 to 9 months
Females: 4 to 5 months
Weight: Females 3876 g (3081-4902 g) at day 0.

B. Study Design

This study was designed to assess the developmental toxicity potential of Alachlor, when administered by gastric intubation from gestational days 7 through 19, inclusive.

Mating - According to the investigator's report:

> "Natural mating was used. Each female selected for mating was placed into the male's cage. When coitus was observed, the female was removed and returned to her original cage. Following an interval of one to two hours, the female was placed into the cage of a different male. When coitus was observed with the second male, the female was considered mated and returned to her cage. The day on which coitus was observed with both males was designated Day 0 of gestation."

Group Arrangement

Test Group	Dose Level (mg/kg)	Number Mated Females Assigned
Control	0	20
Low Dose	50	18
Mid Dose	100	18
High Dose	150	18

Dosing

All doses were in a volume of 1 ml/kg of body weight/day prepared three times during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was initially based on gestational day 7 body weight and adjusted during the treatment period to most recent body weight.

Observations

The animals were checked for mortality or abnormal condition twice daily. Dams were sacrificed on day 30 of gestation. Examinations at sacrifice consisted of: gross postmortem examinations with emphasis on the reproductive systems. All abnormal tissues along with liver, kidneys, adrenals and spleen were weighed and preserved. Intact uterus was removed, weighed, and the location and number of live fetuses, dead fetuses, late resorptions, early resorptions, and implantation sites were recorded. Ovaries were dissected out and corpora lutea were counted. Uteruses with no implants were stained with ammonium sulfate.

The fetuses were examined in the following manner: all fetuses were examined grossly for malformations of the "external form" including palatal defects. Further, all fetuses were examined for soft tissue malformations and variations (and were sexed) by a microdissection procedure similar to that of "Staples". **The fetuses were then skinned, the eyes examined grossly, and the brain evaluated by making a transverse cut parallel and posterior to the frontal parietal suture through the cerebral hemispheres (gross examination was conducted).** The eviscerated and skinned carcasses were then processed by a modified method of Crary (using Alizarin Red S). References and procedures were provided.

Statistical Analysis

The following statistical analysis methods were used:

Bartlett's Test
ANOVA
Dunnett's
Kruskal-Wallis
Summed Rank Test (Dunn)
Regression Analysis
Trend
Lack of Fit
Jonckheere's Statistic
Arc Sine
Chi-square
Fisher Exact Test
Bonferroni Inequality
Armitage's Test

Further detail is available in the attached "Materials and Methods."

Compliance

A signed "Statement of No Data Confidentiality Claims" was provided.

A signed EPA "GLP Compliance Statement" was provided.

A signed "Quality Assurance Statement" was provided.

C. Results:

1. Maternal Toxicity

The investigator reported premature delivery in two females of the low dose group and two females of the mid dose group. They provided historical control data for abortions and premature delivery. The incidences noted in this study were well below those of the historical control data.

Mortality

No treatment related mortality was reported. Two controls died from intubation error.

Clinical Observations

The investigator supplied group summary and individual animal data. There were no apparent treatment related observations. There appeared to be an increase in anogenital staining in treated groups, but this finding was limited to a few animals in each dose group.

Body Weight

The investigator supplied the following data: group mean and individual animal data for daily weights, gravid uterine weights, corrected body weights, and weight gains.

Table I. Body Weight Gains and Corrected Body Weight (grams)[a]

Group	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period	Corrected+ Day 30 Body Weight	Corrected++ Body Weight Gain Days 7-30 of Gestation
Control	89	-33	160	202	3478.3	-441.2
LDT	89	-93	201	199	3510.0	-397.0
MDT	93	-153	249	207	3530.0	-457.8
HDT	119	-196	291	204	3578.7	-392.0

\+ = Corrected gestation day 30 body weights = body weight at day 30 less gravid uterine weight.

++ = Corrected body weight gain for gestation day 7 to 30 body = weight gain for gestation day 7 to 30 using corrected day 30 body weights.

[a] = Data extracted from Laboratory Report No. 87-3169, Appendices C, D and E.

The high dose group showed an apparent biologically relevant decrease in body weight gain during the dosing period with a rebound body weight gain during the period following dosing.

Food Consumption

The investigator supplied the following data: group mean and individual animal data.

Table II. Food Consumption Data (g/kg/day)[a]

Group	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period
Control	53	35	32	37
LDT	53	30	29	34
MDT	55	28	34	34
HDT	59	22	37	35

a = Data extracted from Laboratory Project No. 87-3169, Appendix F.

There was a slight decrease in food consumption during the dosing period in the high dose group, this follows the body weight changes discussed previously. A slight increase in food consumption was noted in the high dose group during the period following dosing. No biologically relevant differences were noted in food consumption for the entire gestation period.

Gross Pathological Observations

The investigator supplied the following data: individual animal gross postmortem examination data and mean and individual organ weight and organ to corrected body weight ratios for liver, kidneys, adrenals and spleen.

No treatment related effects were noted in gross postmortem observations or in organ weight data.

Cesarean Section Observations

Table III. Cesarean Section Observations[a]

Dose	Control	LDT	MDT	HDT
# Animals Assigned	20	18	18	18
# Animals Mated	17	17	17	16
Pregnancy Rate (%)	85.0	94.4	94.4	88.9
Maternal Wastage				
# Died	2	0	0	0
# Died/Pregnant	0	0	0	0
# Nonpregnant	2	1	1	2
# Aborted	0	0	0	1
# Premature Delivery	0	2	2	0
# Litters Examined	16	15	15	15
Total Corpora Lutea	154	158	156	142
Corpora Lutea/Dam	9.6 ± 2.4	10.5 ± 1.5	10.4 ± 2.2	9.5 ± 2.4
Total Implantations	146	140	150	132
Implantations/Dam	9.1 ± 2.7	9.3 ± 2.1	10.0 ± 2.3	8.8 ± 2.7
Total Live Fetuses	142	131	141	123
Live Fetuses/Dam	8.9 ± 2.5	8.7 ± 2.2	9.4 ± 2.2	8.2 ± 2.5
Total Resorptions	4	9	9	9
Resorptions/Dam	0.3 ± 0.4	0.6 ± 0.1	0.6 ± 0.8	0.6 ± 1.1
Mean Fetal Weight (g)	45.89 ± 6.42	43.27 ±7.13	44.13 ± 8.18	43.04 ± 9.47
Preimplantation Loss (%)	0.059 ± 0.116	0.113 ± 0.177	0.041 ± 0.062	0.085 ± 0.155
Postimplantation Loss (%)	2.7	6.4	6.0	6.8
Sex Ratio (# Males/ # Females)	1.2	0.6*	1.1	1.3

* = $p < 0.01$

[a] = Data extracted from Laboratory Project No. 87-3169, Appendix G.

No treatment related effects were noted in the supplied data. It should be noted that the low dose group had a lower sex ratio than control or other treated groups, however, this does not appear to be treatment related.

2. Developmental Toxicity

Table IV. External Examinations

Observations	Control	Low Dose	Mid Dose	High Dose
# Pups (litters) examined	142 (16)	131 (15)	141 (15)	123 (15)

No differences in fetal external abnormalities between control and dosed groups were reported (individual animal data were provided). The investigator examined pups/fetuses delivered prematurely and found no external abnormalities.

Visceral Examinations

Table V. Visceral Examinations+

Observations	Control	Low Dose	Mid Dose	High Dose
# Pups (litters) examined	142 (16)	131 (15)	141 (15)	123 (15)
Diaphragmatic hernia	0	0	0	1 (1)[a]
Ectopic kidney	1 (1)	0	0	0
Distended renal pelvis - papilla absent	0	0	0	1 (1)
Ectopic ovary	1 (1)	0	0	0
Abnormal origin of subclavian artery	1 (1)	0	0	0

[a] = Fetal (litter) incidence.
+ = Data extracted from Laboratory Report No. 87-3169, Appendix M.

No treatment related effects were noted in the presented data. The investigator examined pups/fetuses delivered prematurely and found no visceral abnormalities.

Skeletal Examinations

Table VI. Skeletal Examinations (Selected Observations)[a]

Observations[+]	Control	Low Dose	Mid Dose	High Dose
# Pups (litters) examined	142 (16)	131 (15)	141 (15)	123 (15)
Hyoid arch(es) angulated	1 (1)[++]	5 (4)	2 (2)	3 (3)
Sternebrae-fused	1 (1)	0	0	3 (3)
Presence of supernumerary suture(s)	4 (4)	1 (1)	3 (2)	2 (2)
Hyoid body incompletely/ not ossified	6 (3)	12 (6)	12 (8)	8 (5)
Cervical centra incompletely/ not ossified	2 (1)	2 (2)	3 (1)	4 (4)
5th sternebrae incompletely/ not ossified	6 (5)	11 (7)	23 (12)	4 (3)
6th sternebrae incompletely/ not ossified	13 (9)	3 (2)	6 (6)	9 (6)
Rib(s) - 13th short	34 (11)	24 (10)	25 (12)	21 (9)
13th rib unilateral	19 (11)	10 (9)	21 (13)	16 (8)
13th rib floating	13 (8)	13 (8)	20 (10)	15 (10)
1st Metacarpal not ossified	4 (1)	2 (2)	5 (3)	6 (2)
5th Forelimb mid-phalange - not ossified	4 (4)	3 (3)	8 (3)	11 (3)
5th Hindlimb mid-phalange - not ossified	0	0	1 (1)	1 (1)
Talus - not ossified	0	0	2 (2)	4 (2)
Pubis - incompletely ossified	0	0	1 (1)	2 (1)

[+] = Some observations may be grouped together.
[++] = Fetal (litter) incidence
[a] = Data extracted from Laboratory Project No. 87-3169, Appendix N.

Observations of hyoid arch(es) angulated, hyoid body incompletely/not ossified, 5th sternebrae incompletely/not ossified, 13th floating ribs, non-ossified talus (mid and high dose only) and fused sternebrae (high dose only) appeared slightly higher in treated groups than those of the control. However, there was no dose response present and these incidences were within the historical range provided by the investigators and were not statistically significantly different from control findings. **Thus, these findings, even at the highest dose tested, could not be definitely related to the administration of the test material. The investigator examined pups/fetuses delivered prematurely and found no skeletal abnormalities.**

D. Discussion/Conclusion

a. Maternal Toxicity

Maternal toxicity was evidenced at the high dose as a decrease in body weight gain during the dosing period followed by a rebound in body weight gain in the period following dosing (no statistically significant differences were achieved).

b. Developmental Toxicity

i. Deaths/Resorptions

No biologically relevant differences were noted.

ii. Altered Growth

No biologically relevant differences were noted.

iii. Developmental Anomalies

No biologically relevant differences were noted.

iv. Malformations

No biologically relevant differences were noted.

c. Study Deficiencies

No major deficiencies were noted in this study.

E. Classification: Core-Minimum Data

Maternal NOEL = 100 mg/kg/day
Maternal LOEL = 150 mg/kg/day
Developmental Toxicity NOEL > 150 mg/kg/day

F. Risk Assessment

None needed at this time.

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