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

JUN 23 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Casuell # 11

DATE: June 2, 1981

SUBJECT: EPA Reg.#524-316; -314, -285, -296; Lasso (Alachlor); Accession#244369

FROM: Amal Mahfouz, Toxicologist
Toxicology Branch, HED (TS-769)

Amal Mahfouz 6/5/81

PTC 6/5/81

TO: Robert Taylor (25)
Registration Division (TS-767)

Registrant: Monsanto

Action Requested: Review of teratology study in rabbits in support of registration of Lasso (Alachlor) herbicides.

Related Actions: PP#2F2144, 9F2156, 0F2313, 0F2338, 0F2348, 1F2447

Recommendations and Conclusions

This study is classified as invalid:

1. The study does not have valid control data. The following points are noted:

   a. Decrease in weight (-59g) during dosing period (day 6-28 of gestation)

   b. High incidence of heart anomalies in 8/66 (12.1%) fetuses and 2/10 (20%) litters as compared to the historical control 2/741 (1.2%) fetuses and 2/118 (7.7%) litters. The incidence of scoliosis both in the test and control groups is considerably higher than the historical control incidence.

   c. Small weight of fetuses (27.7g) as compared to treatment groups (35.7g in low-dose, 28.5g in mid-dose and 29.5g in high-dose) and historical control (31.1g).

d. Congested lungs with red foci at necropsy (indicating the possibility of gavage error in more than the two animals that were reported dead due to gavage error).

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

Heart and major vessels "variations" were not described in this study. The registrant should be asked to define these "variations".

The available data in this report indicate that many of the animals (in all groups) had congested lungs and red foci. These findings may be reflecting a faulty gavage procedure resulting in additional stress during gestation. Therefore no valid conclusion could be drawn from this study due to the invalidity of the control group.

Review:

Rabbit, Oral Teratology, International Research and Development Corporation (IR-79-022; IRDC-401-060), November 24, 1980; Submitted by Monsanto on January 15, 1981.

Test Substance: Alachlor Technical, 92.19% a.i. (Lot MHK-6).

Sixty-four, sexually mature female Dutch belted rabbits (Langshaw Farms, Augusta, Michigan) were used in this study. These rabbits were approximately 6 months old at the time of mating and had been acclimated in the testing laboratory for at least 30 days before experimentation.

Prior to insemination, the females were randomly assigned to a vehicle control group and three treatment groups of 10, 30 and 60 mg/kg/day with each group consisting of 16 animals. The test material was administered by gavage from days 6 through 27 of gestation to pregnant females. The control group received only 1 ml/kg/day corn oil on a comparable regimen. A pilot study - IR-79-021, IRDC-401-059a - using 0, 50, 100, 200 and 400 mg/kg/day established the dosage levels of Alachlor used in this teratology study.

Sperm from five proven male rabbits with 50% motility or greater, were used for artificial insemination of the females. Immediately following insemination, ovulation was induced with 100 units of A.P.L. (chorionic gonadotropin for injection, U.S.P.; a product of Ayerst Laboratories, Inc., N.Y., N.Y.) injected into the ear vein. The day of insemination was designated day 0 of gestation.

Dosages were determined from the animals individual body weights recorded on gestation day 6. Technical Alachlor was liquified daily (in 50°C water bath), and the appropriate amount (using a base factor of 1.08 to adjust for purity) was then admixed with Mazola corn oil at concentrations to permit administration of the dosages at a constant volume of 1 ml/kg/day corn oil.

All animals were individually housed in wire cages and maintained in a temperature-, humidity- and light-controlled environment. Purina rabbit chow #5322 and tap water were available ad libitum.

The dams were observed daily for mortality and toxic signs. Individual maternal body weights were recorded on gestation days 0, 6, 12, 18, 24 and 28.

All animals found dead were necropsied and on gestation day 28 all surviving dams were sacrificed by injection of an overdose of sodium pentobarbital into a marginal ear vein. All fetuses were delivered by cesarean section, the uterus was excised and weighed prior to removal of the fetuses. The number of total implantations, early and late resorptions, corpora lutea, viable and nonviable fetuses in each uterine born were recorded. The abdominal and thoracic cavities and organs of the dams were grossly examined.

Uteri from females that appeared nongravid were examined for confirmation of pregnancy status. Females showing signs of abortion were sacrificed and examined. Maternal tissues were preserved in 10% neutral buffered formalin only as deemed necessary by gross findings.

All fetuses were individually weighed and examined for external malformations and variations including the palate and eyes. Each fetus was dissected, internally sexed and examined for visceral malformations and variations, including the brain by a mid-coronal slice and the heart by a modified Staples' technique. The eviscerated, skinned fetuses were individually tagged, and treated by a method similar to that described by Dawson for subsequent skeletal examination.

Data on Historical control and positive control (6-aminonicotineamide) were available, however the positive control study was not concurrent since it was conducted 3 months before the present study was initiated.

All statistical analysis compared the treatment groups to the control group with the level of significance at $p < 0.05$. The mean number of viable fetuses, total implantations, corpora lutea, and mean fetal body weights, were compared by analysis of variance, Bartlett's test for homogenicity of variances and the appropriate t-test. The number of early and late resorptions, nonviable fetuses and post-implantation losses were compared by the Mann-Whitney U-test. Fetal sex distribution, and the number of litters with malformations were compared using the Chi-square test.

Results:

Mortality and Observations

Four females in the high-dose group, two in the mid-dose, none in the low-dose, and two in the control group were found dead before the end of gestation period. The reason for mortalities in the treatment groups was unknown; however the mortalities in the control group was due to a gavage error.

One female at the high-dose, two at the mid-dose, one at the low-dose and two in the control group were sacrificed before term because of abortion.

Hair loss, primarily of the forelimbs and thorax, nasal discharge and soft stool were noted to be increased in treated animals as compared to the control group.

The following table represents the total loss in the number of animals before the end of the gestation period:

	Control 0 mg/kg/day	Low-Dose 10 mg/kg/day	Mid-Dose 30 mg/kg/day	High-Dose 60 mg/kg/day
Initial No. of animals	16	16	16	16
No. of animals lost by:				
Death	2	0	2	4
Abortion	2	1	2	1
Total animals loss (%)	4 (25)	1 (6.3)	4 (25)	5 (31.3)

This table reflects an unusually high percentage (25%) of animals loss in the control group as compared to the historical control value of 6% loss. Although maternal loss appears to be dose-related at 30 and 60 mg/kg/day, death and abortion may be due to other factors, i.e., gavage errors. Necropsy data indicate that several animals in the control and treated groups had congested lungs with red foci, and two animals had thoracic abcesses; these findings may be gavage related.

Maternal Body Weight

The initial body weight range was 2469 to 3317 grams. The table below reflects the body weight changes calculated from this study (excluding all non-gravid females and gravid females that died or aborted before gestation day 28).

	Control	Low-Dose	Mid-Dose	High Dose
Mean total Weight Change (Day 0-6)	70	34	40	108
Mean total Weight Change (day 6-28)	-59	96	-213	-218
Mean total Weight Change (day 0-28)	11	120	-173	-112

Before dosing, all groups gained weight between day 0-6. However the inconsistency in these weight gains is not understood.

Except for the low-dose group that continued to gain weight (96g) during dosing period (day 6-28), all groups lost weight during this period including the control group: -59g (control), -213 (mid-dose) and -218g (high-dose). Although the decrease in body weights appears to be compound-related in the mid- and high-dose groups, the unjustified decrease in weight of the control group indicates that the animals may have been unreasonably stressed by the gavage procedure and hence, there is no usefulness in this control data.

Food consumption was not reported in this study and should be available for review.

Gross Pathology

Hydroceles on oviducts and lung congestion with red foci occurred in all groups. Thoracic abcesses were noted in two animals, one at the low-dose group and another that died before the end of the gestation period in the control group (the death of this control animal was due to gavage error). In the high-dose group, hemorrhagic sublumbar adipose, hemorrhagic abdominal musculature, and hemorrhagic cecum mucosa were noted respectively in three of the four animals that died before the end of the gestation period.

The lesions noted above in the lungs of the control and treatment groups may be due to gavage errors. Gavage errors may also have caused abortions, death and loss of weight. These effects were noted in both the control and treatment groups in this study.

Cesarean Data

At the end of the gestation period all surviving animals were examined. The number of gravid females was 10/16 in the control group and 14/16, 9/16 and 10/16 respectively in the low-, mid-, and high-dose groups.

The mean number of corpora lutea and the number of implantations per dam were similar in the control group and treatment groups as seen in the table below:

	Control	Low-Dose	Mid-Dose	High-Dose
Mean No. Corpora Lutea/dam	9.6	9.3	9.6	9.6
Mean No. Implantations/dam	6.9	5.4	6.3	6.5

The mean number of implantations was relatively low (5.4) in the low-dose group only.

Fetal toxicity as manifested by increased resorption/dam (1.2) and reduced number of live fetuses/dam (5.1) was only significant ($p < 0.05$) in the mid-dose (30 mg/kg/day) group, as seen below:

	Control	Low-Dose	Mid-Dose	High-Dose
Mean Resorption/Dam	0.3	0.3	1.2	0.4
Number of live fetuses/dam	6.6	5.1	5.1	6.1
Fetal weight (g)	27.7	35.7	28.6	29.5

Due to the initially low number of implantations/dam (5.4) in the low-dose group, it is not possible to conclude that the resultant decrease in the number of fetuses/dam (5.1) in this group as compared to the control group (6.6) is associated with the test compound. However the cause of this initial low implantations rate in this low-dose group is not understood.

Although the data in the above table appear to reflect no fetotoxic effects in the high dose group (# resorption/dam = 0.4 and # live fetuses/dam = 6.1) as compared to the control group (# resorption/dam = 0.3 and # live fetuses/dam = 6.6), evaluation of fetotoxicity in this high-dose group may be hindered by the high level of maternal mortality (25%) and other inconsistencies. (See gross pathology review p. 6, last paragraph).

Finally the control group mean fetal weight (27.7g) is low when compared to the historical control (31.1g) or to any one of the treatment groups. Consequently fetal weight data cannot be properly evaluated.

No data were reported on fetal length.

Fetal Examinations

Skeletal

Scoliosis was reported in one fetus in the control group, one in the low-dose, two in mid-dose and two in the high-dose group.

The tail was also absent in one of the mid-dose fetuses with scoliosis.

Bent clavicle was noted in one fetus in the low-dose and one fetus in the high-dose.

Also noted in the low-dose group forked rib in one fetus and fused sternebrae in another fetus, both fetuses belong to different litters.

The total of skeletal anomalies reported affected 1 of 10 (10%) litters in the control group, 4 of 14 (28.6%) litters in the low-dose group, 2 of 9 (22.9%) litters in the mid-dose group and 3 of 10 (30%) litters in the high-dose group.

The following table reflects the incidence of scoliosis in this study:

	Historical Control		Control (Vehicle)		Low-Dose		Mid-Dose		High-Dose	
	(F)*	(L)*	(F)	(L)	(F)	(L)	(F)	(L)	(F)	(L)
Total #examined	741	118	66	10	71	14	46	9	61	10
Incidence of Scoliosis (%)	0.54	3.39	1.5	10	1.4	7.1	4.3	22.2	3.2	20
No. affected	1	1	1	1	1	1	2	2	2	2

*F= Fetuses, L = litter

Despite that the higher incidence of scoliosis in the vehicle control group (F: 1.5%, L: 10%) as compared to the historical control (F: 0.54%, L: 3.39%) the incidence of scoliosis increased in the mid-dose (F: 4.3%, L: 22.2%) and high-dose (F: 3.2%, L: 20.0%) groups. However, the incidence of scoliosis in the low-dose group increased (F: 1.4%, L: 7.1%) only in comparison with the historical control.

Due to the overall invalidity of the control data, potential skeletal teratogenic effects of Alachlor could not be properly assessed in this study. The incidence of scoliosis in this study (treatment and control groups) is of concern since it is considerably increased as compound to the historical incidence.

Other skeletal variations were reported:

Increased number of litters with 27 presacral vertebrae was noted in all treatment groups as well as unossified hyoid body or/and variations in hyoid body arches; the number of litters with unossified sternebrae (including sternebrae #5) doubled in the mid- and high-dose groups as compared to the control group. These variations appear to reflect a fetotoxic effect at all dose levels.

The following table presents these variations:

Incidence of Skeletal Variations

Total No. of (F) & (L) examined	Historical Control		Control (Vehicle)		Low-Dose		Mid-Dose		High-Dose	
	F	L	F	L	F	L	F	L	F	L
	741	118	66	10	71	14	46	9	61	10
Variations										
27 presacral vertebrae										
%	7.8	26	10	20	13	43	8.7	33	13	50
#affected	58	31	7	2	9	6	4	3	8	5
25 presacral vertebrae										
%	0.14	0.85	0	0	0	0	0	0	1.6	10
#affected	1	1	0	0	0	0	0	0	1	1
Hyoid body unossified										
%	1.08	3.4	0	0	0	0	2.2	11	8.2	10
#affected	8	4	0	0	0	0	1	1	5	1
Hyoid body arches										
%	1.6	7.6	0	0	2.8	7.1	2.2	11	1.6	10
#affected	12	9	0	0	2	1	1	1	1	1
Sternebrae #5 unossified										
%	5.7	25	3	20	4.2	14	2.2	11	9	50
#affected	42	29	2	2	3	2	1	1	6	5
Other sternebrae unossified										
%	0.14	0.85	0	0	0	0	4.3	22	0	0
#affected	1	1	0	0	0	0	2	2	0	0

Visceral

Incidence of heart anomalies(interventricular septal defect)was high in the control group (8/66 fetuses from a total of 2/10 litters, 6 of these fetuses were the total size of one litter) as compared to the historical control (2/741 fetuses from a total of 2/118 litters affected). Only 2/71 fetuses from 2/14 litters in the low-dose group had the same heart anomalies as the control group. Although no heart anomalies were noted in the high-dose group heart developmental variations (not described in the study) were noted in this group in 4/61 fetuses from 1/10 litters.

Major vessel anomalies (bullus aortic arch) was noted in the low-dose (1/71 fetuses from 1/14 litters) and mid-dose groups (1/46 fetuses from 1/9 litters). Undescribed major vessels variations were also reported in these two groups (1/71 fetuses in the low-dose and 2/46 fetuses from 1/9 litters in the mid-dose).

Urinary tract anomalies were noted in two fetuses from one litter in the low-dose group. Bulging eyes were reported in two fetuses from one litter in the mid-dose group and intestinal anomalies were noted in one fetus in this group.

Other visceral variations were noted only in the high-dose and control groups. Variations in the high-dose group included major vessels in 3 fetuses each one from a different litter, the heart in 4 fetuses from one litter, the gall bladder (bilobed) in one fetus, renal papillaes (not developed) and/or ureter (distended) in 2 fetuses, each one from a different litter. In the control group one fetus and undeveloped renal papillaes and 8 fetuses from 4/10 litters and major vessels variations (not described).

The report should have described the heart and major vessels "variations" in order for us to concur that these are true variations.

The table below represents the incidence of visceral anomalies and variations noted in this study.

Total No. of (F) and (L) examined	Historical Control		Control (Vehicle)		Low-Dose		Mid-Dose		High-Dose	
	F	L	F	L	F	L	F	L	F	L
	741	118	66	10	71	14	46	9	61	10
Anomalies										
Heart										
%	0.27	1.7	12	20	4.2	14	0	0	0	0
#affected	2	2	8	2	2	2	0	0	0	0
Major vessels										
%	0	0	0	0	1.4	7.1	2.2	11	0	0
#affected	0	0	0	0	1	1	1	1	0	0
Eyes										
%	0	0	0	0	0	0	4.3	11	0	0
#affected	0	0	0	0	0	0	2	1	0	0
Intestinal										
%	0	0	0	0	0	0	2.2	11.1	0	0
#affected	0	0	0	0	0	0	1	1	0	0
Urinary tract										
%	0	0	0	0	2.8	7.1	0	0	0	0
#affected	0	0	0	0	2	1	0	0	0	0
Kidney/ureter										
%	0.14	0.85	0	0	0	0	0	0	0	0
#affected	1	1	0	0	0	0	0	0	0	0
Variations										
Heart										
%	0	0	0	0	0	0	0	0	6.5	10
#affected	0	0	0	0	0	0	0	0	4	1
Major vessels										
%	13	42	12	40	1.4	7.1	4.3	11	4.9	30
#affected	97	49	8	4	1	1	2	1	3	3
Renal/ureter										
%	0	0	1.5	10	0	0	0	0	3.2	20
#affected	0	0	1	1	0	0	0	0	2	2
Gall bladder										
%	0.41	2.5	0	0	0	0	0	0	1.6	10
#affected	3	3	0	0	0	0	0	0	1	1

The total of visceral anomalies reported in this study were found in 2 litters of 10 (20%) in control group, 3 litters of 14 (21.4%) in the low-dose group, 1 litters of 9 (11.1%) in the mid-dose, and none in the high-dose group.

The high incidence of heart anomalies in the control group (F: 12%, L: 20%) as compared to the historical control (F: 1.2%, L: 7.7%) is indicative of unreliable and invalid control data.

Conclusions

This study is classified as invalid because control data are considered invalid and hence the entire conduct of this study comes into question.

The control group lost weight (59g) during the dosing period (day 6-28 of gestation) and two animals in this group died due to gavage errors.

The incidence of heart anomalies in this control group is high (8/66 fetuses and 2/10 litters) as compared to the historical control (2/741 fetuses and 2/118 litters). Also the incidence of scoliosis in this study is significantly higher than the historical control.

The fetuses in this control group are smaller (27.7g) than the treatment groups (35.7g in low-dose, 28.5g in mid-dose and 29.5g in high-dose) and the historical control (31.1g).

In addition to the above erratic findings in the control group, low level of implantations/dam (5.4) is noted in the low dose group as compared to the control group (6.9) and the other treatment groups (6.3 in mid-dose and 6.5 in high-dose). A large weight increase (108g) is also noted in the high-dose group during pre-dosing (day 0-6 of gestation) as compared to the control group (70g), low-dose (34g) and mid-dose (40g) groups; no explanation is provided for this unusually large increase.

The registrant should be informed as to the above findings and that data on food consumption, fetal length, statistical analysis, and individual necropsy data on animals that died or aborted should have been submitted with the report. The available data in this report indicate that most of the animals had congested lungs with red foci and these findings may be reflecting a faulty gavage procedure or poor animal husbandry.

The registrant should be requested to define the heart and major vessels "variations" reported.

TS-769:th:TOX/HED:AMahfouz:6-2-81:#2