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

AUG 29 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of Teratology Study in Rabbits with Alachlor
Registration #524-316, Accession #252570, CASWELL #11

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

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/23/84
Pharmacologist, Section V
Toxicology Branch /HED (TS-769)

THRU: Laurence D. Chitlik, DABT
Section Head, Section V
Toxicology Branch/HED (TS-769)

LDC 8/28/84 8/29/84

Recommendation:

The teratology study in rabbits with Technical Alachlor is classified as Core-Supplementary Data based on the following points:

1. The deviation from the protocol of the earlier study in terms of the vehicle used for Technical Alachlor is of concern to this reviewer. The earlier study, which this present study replaced, utilized corn oil as the vehicle. The present study substituted mineral oil for corn oil. Corn oil is routinely used as a pharmaceutic aid, a solvent for many drugs that are injected in oily solution or suspension (Merck Index, 1976; Goodman and Gilman's The Pharmacologic Basis of Therapeutics, Macmillan, 1980). Mineral oil is used as a laxative-cathartic (Merck Index, 1976; Goodman and Gilman's The Pharmacologic Basis of Therapeutics, Macmillan, 1980), it is indigestible, absorbed only to a limited extent and acts as a lipid solvent where it may interfere with the absorption of fat-soluble substances (Goodman and Gilman's The Pharmacologic Basis of Therapeutics, Macmillan, 1980). These known actions of mineral oil may affect adequate absorption of the chemical from the gut and therefore reduce potential response.

2. There were high levels of preimplantation loss noted in this study. These levels were in excess of levels found in other studies from IRDC noted especially in the control (31.8%) and high dose (49.1%) groups. The high levels are likely an indication of dosing prior to the completion of implantation. This effect may be stress related in the control group. The high level of preimplantation loss may have obscured fetotoxic and teratogenic effects. Also, these levels may have affected postimplantation loss especially at the high dose level (0.2 per dam versus 1.6 per dam in the mid dose group) where preimplantation loss was the greatest since significantly fewer embryos per dam were implanted at this level.

3. The historical control data provided were inadequate (See page 14 of this review) and should be made available by individual study and dated for a period comprising at least 2 years prior to the study for all assayed parameters. This would aid in comparison of test groups in the study.

4. An explanation should be provided by the registrant relative to the nature of the major vessel variations which were noted to slightly increase in the high dose fetuses.

5. The registrant must explain the statements in their report Appendix D, Table 1 and Section VI: "Initial preparations did not meet specifications", "The initial dosing day 1 mixtures did not meet specification and was rejected".

6. The registrant must explain discrepancies found in the table of group mean maternal body weight and body weight changes (see Page 8 of this review). It is recommended by this reviewer that the Director of Quality Assurance of IRDC reinspect all of the data provided in the submitted study (number IR-83-045, IRDC project number 401-208) and submit findings to the Agency.

7. There was an unacceptably large variation in the weight of the animals (2.5 to 4.3 kg), and 38 animals were above the maximum body weight (3.5 kg) specified in the protocol at initiation of this study.

8. Individual fetal weight and variation data were not included in the report and are required to be submitted.

9. Although not required by the Guidelines, the study did not supply food consumption data and fetal crown-rump data which are very useful determinations for evaluation of the study.

Background Information:

This study is a replacement for IRDC Study number IR-79-022, IRDC #401-060 (November 24, 1980), submitted January 15, 1981 by Monsanto. That study was reviewed by the Agency and classified as invalid based on the fact that the study did not contain valid control data.

Data Review:

Study Identification:

Study Title: Teratology Study in Rabbits with Alachlor (IR-83-045).

EPA Identification Numbers: EPA Reg. #524-316, Accession #252570

Sponsor: Monsanto Agricultural Products Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167

Testing Laboratory: International Research and Development Corp.
Mattawan, Michigan 49071

Study Number: IR-83-045

Project Number: IRDC#401-208

Date: December 29, 1983

Study Director: James L. Schardein

Test Substance: Technical Alachlor (2-chloro-N-(methoxy-methyl)-2', 6'-diethyl acetanilide)
Purity: 94%
Lot number: MDLT 0330B

Vehicle: Mineral Oil, light, U.S.P.

Dosage: 10,30,60 mg/kg/day

Animals: Dutch Belted Rabbits (Longshaw Farms, Augusta, Michigan) 7 to 8 months old at the initiation of the study.

Methods and Procedures: A copy of the methods and procedures section from the investigators report is appended.

Two shipments of rabbits were received for use: 76 animals, 4 1/2 to 5 1/2 month old received on 2/28/83 and 5 rabbits, 5 to 6 months old received on 3/14/83. However, the animals were 7 to 8 months old at the initiation of the study.

Thirty-eight of the animals (9/18 for each of the control, mid and high dose groups and 11/18 for the low dose group) exceeded the maximum body weight specified in the protocol (3.5 kg) on gestation day 0, and the animal weights varied from 2.5 to 4.3 kg. This range of initial body weights is unacceptably large.

In response to the use of artifically inseminated rabbits, this reviewer is aware of a joint study by the Middle Atlantic Reproduction and Teratology Association compiled by D.C. Woo of Hoffman LaRoche, Nutley, New Jersey, which found that artificially inseminated rabbits have high preimplantation loss and consequently smaller litter size.

The investigators stated that the ejaculate was diluted with 3.0 ml of 0.9% sodium chloride but there is no indication as to how much ejaculate was diluted or what the final concentration actually was.

One animal died on the first day of insemination, no cause of death was given. An animal was found to have a ventral thoracic mass and was removed but no details on the mass were given. These animals were replaced with those animals eliminated during the randomization procedure used by the contracting lab (described in the procedures section appended).

Time of day of the dosing of the animals was not given. Submission of this item would allow this reviewer to determine if the dosing time would have affected the feeding behavior.

There was no mention of measurement of food consumption. Present technology in terms of equipment and prepared feed allows accurate measurement of food consumption.

Humidity in the animal rooms varied in a 26% range (32% to 58%). This variation is rather excessive and may have caused some stress in these amimals.

There was no indication of measurement of fetal crown-rump length, an important fetal morphological determination.

Although the test article was prepared daily in mineral oil, the homogeneity of this preparation was only checked on the first and 14th (last) day of dosing and only for the low and high dose, where it was found that those doses appear to be within 92 to 114% of the target dose. Also, the registrant should explain the following statements in Appendix D, Table 1, for day 1 analysis "Initial preparations did not meet specifications", and in Section VI of the report "The initial dosing day 1 mixtures did not meet specifications and was rejected." In light of the above difficulties, this reviewer questions why more frequent analyses were not performed for all dose preparations.

The positive control data provided with this study was from 1978 (11/16/78). The study had combined routes of administration, oral for controls, IP for treated animals. These are not considered useful positive control data for the submitted study.

Results:

Maternal Mortality:

Four animals died during the study.

One animal died during the first day of insemination, as mentioned earlier, and was replaced, no cause of death given.

One animal died in the control group on day 28, no cause of death given, clinical observations involved hair loss on forelimbs, necropsy observations found a pale liver involving all lobes.

One animal died in the mid dose group on day 25, no cause of death given; upon necropsy, the animal was found to have a compound fracture of the left hindlimb, brown colored matting of the anogenital haircoat and a moderately reddened tracheal lining. This animal had the broken hindlimb on day 15 and should have been removed from the study and sacrificed since this injury stressed the animal.

One animal died in the high dose group on day 12, no cause of death given, at necropsy the tracheal lining was found to be severely reddened and all lobes of the lungs moderately congested.

The tracheal and lung lesions observed at necropsy in both the above mentioned mid and high dose animals may be due to gavage error.

In addition to the above findings one animal in the mid dose group aborted on day 26. This animal had a white nasal discharge from day 8 to day 26 of gestation, a wet and matted anogenital haircoat on days 18 to 26 and no stool observed on days 23 to 26. At necropsy all the lobes of the lung were moderately congested and the intestines were moderately distented.

Clinical Observations and Necropsy:

Hairloss on limbs and abdomen and occasional wet and matted anogenital haircoat were observed in all groups. Also there were occasional incidences of soft or decreased quantities of stool in all groups.

It is suprising to this reviewer that the 10 day treatment with mineral oil, in light of its known laxative-cathartic properties, did not cause more cases of soft stool and at least some instances of diarrhea.

White or clear nasal discharges were noted in the mid and high dosage groups, see Table 1 below:

Table 1 - Summary of Clinical Observations - Technical Alachlor (mg/kg/day)

	Control	10	30	60
Hairloss	12	8	6	7
Wet and matted anogenital haircoat	2	2	1	2
Stool:				
soft	2	4	-	-
decreased amount	3	3	1	1
Nasal Discharge:				
White	-	-	3*	2**
Clear	-	-	-	2**
Yellow	-	1	-	-
Respiration rales	-	-	1	-
Ocular discharge:				
White	-	-	-	1
Nasal matting:				
Red	-	-	-	1

*Lungs were congested in 2 of these animals.

**One animal (#21934) had one each of these incidences of clear and white nasal discharge.

Data extracted from IRDC Study #401-208 Table 2.

One animal in the low dose group (#21894) had misaligned upper and lower incisors, no weight gain was observed in this animal probably due to poor feeding ability. However, without food consumption data no definite cause can be determined. If this animal had been found to have problems eating, it would have been best to remove it from the study. This animal had preimplantation loss and subsequent high late resorption; this may be related to feeding problems.

Necropsy findings at the end of the study reflected observations of fluid in the thoracic cavity, congested lungs (various degrees), adhesions of lungs to thoracic cavity, abscesses in lungs, foci in lungs, pale lobes of the liver and pitted kidneys in various incidences in all study groups, see Table 2 below:

Table 2 - Summary of Necropsy Observations - Technical Alachlor (mg/kg/day)

	Control	10	30	60
Fluid in thoracic cavity	1	2	-	1
Lungs:				
congested	4	4	5	3
abscessed	2	-	-	-
fibrous adhesions	2	-	-	-
areas of consolidation	1	1	-	-
foci	-	1	-	-
Liver:				
pale	2	1	-	-
enlarged and dark in color	-	1	-	-
fibrous adhesions	1	1	-	-
Kidney:				
pale	1	-	-	-
pitted	1	2	-	2
Intestines:				
distended	-	-	1	-
Uterus:				
fluid in horn	-	-	1	-
Trachea:				
lining reddened	-	-	1	1

Data extracted from IRDC Study #401-208 Table 2.

Maternal Body Weight:

As noted on Table 1 below, the mid dose group gained more weight (61 gm) than the control (41 gm) and low dose (46 gm) groups during days 7 to 19 of gestation (see 7 to 20) while the mean body weight gain for the high dose group (14 gm) during this period was significantly lower than the other 3 study groups. However, both the mid and high dose groups gained more weight over the total gestation period, days 0 to 28 (see Table 1), than the two other study groups:

Table 3 - Group Mean Maternal Body Weight and Body Weight Changes (grams) - Technical Alachlor (mg/kg/day)

Day of Gestation	Control	10	30	60
0	3448(3444)*	3590(3584)*	3453	3456
7	3572(3578)	3702(3701)	3571	3560
14	3661(3623)	3752	3622	3527
20	3617(3619)	3752(3747)	3632	3574
25	3567(3561)	3722(3711)	3577	3622
28	3547(3543)	3699(3685)	3628	3614
Days of Gestation				
0 to 7	124(133)	112(117)	118	104(103)
7 to 20	45(41)	50(45)	61	14(65)
0 to 28	99(128)	109(101)	175(181)	158(210)

*Reported values in parenthesis were found to be incorrect and were recalculated by the reviewer. The registrant must explain these discrepancies.

Data extracted from IRDC Study # 401-208 Table 3.

Cesarean Section Observations:

The mid dose group exhibited evidence of increased early resorptions, postimplantation loss and total implantations per dam when compared to the control and low dose groups. However, this effect did not appear to be dose related since similar incidences were not noted at the higher dose level tested. However, the high dose group had a 49.1% preimplantation loss (see Table 4 on the next page). This may be an indication of dosing prior to completion of implantation in these animals which may have obscured any further assessment of the possible fetotoxic effect (increase in post implantation loss) observed at the mid dose level. The high preimplantation loss may be due to maternal stress since the control group also had high preimplantation loss, as opposed to embryotoxicity due to an effect of the chemical.

There was no noticeable difference in the mean fetal body weight between the 4 groups in the study but no individual fetal weight data was provided (litter data was provided).

The incidence of total implantations per dam in the mid dose group (8.8), and the incidence of corpora lutea per dam in the high dose group (13.3) were outside the range given in the historical control data provided with this study (total implantations per dam range 4.4 to 7.8; corpora lutea per dam range 7.0 to 12.2).

Table 4 - Cesarean Section Data Summary - Technical Alachlor (mg/kg/day)

	Control	10	30	60
Animals in study	18	18	18	18
Animals at cesarean section - gravid	16	17	16	17
- non-gravid	1	1	0	0
Resorptions - complete	0	1	0	0
- early	9	4	21	2
- late	5	10*	4	0
Resportions/dam	0.9	0.8	1.6	0.1
Dams with viable fetuses	16	16	16	17
Total viable fetuses	104	113	116	111
Total non-viable fetuses	2	1	0	2
Viable fetus/dam	6.5	6.6	7.3	6.5
Total Postimplantation loss/dam	1.0	0.9	1.6	0.2
Implantations/dam	7.5	7.5	8.8	6.8
Corpora Lutea/dam	11.0	9.6**	11.5	13.3
Group mean preimplantation loss (%)	31.8	18.2**	23.4	49.1
Group mean Postimplantation loss (%)	13.3	11.7	17.7	3.5
Mean fetal body weight (gms)	31.9	31.9	33.3	34.0

* Animal #21894 contributed 1/2 these late resorptions. This animal was previously reported on page 5 of this review as likely to have low food intake due to jaw misalignment problem. Hence, the effect noted above may be an artifact associated with this morphological defect.

**Calculation based on 16 dams due to one of the 17 with "regressing" corpora lutea, this particular animal (#21887) also had complete early resorptions.

Data extracted from IRDC #401-208 Tables 5 and 6.

Fetal Morphological Observations:

Malformations: External fetal malformations included 1 fetus with anasarca in the high dose group, 2 fetuses with dome shaped head, one each in the mid and high dose groups. The incidences of anasarca and dome shaped head occured in the same fetus of the high dose group (this fetus also had hydrocephaly, see Table 4 on the next page). The external fetal malformation data are presented in Table 5:

TABLE 5

Summary of Incidence of Fetal External Malformations - Technical Alachlor (mg/kg/day)

	Control		10		30		60	
Numbers of litters examined	16		16		16		17	
Numbers of fetuses examined	106		114		116		113	
Malformations	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters
Anasarca	—	—	—	—	—	—	1*(0.9%)	1*(5.9%)
Dome Shaped Head	—	—	—	—	1**(0.9%)	1**(6.3%)	1*(0.9%)	1*(5.9%)
Malformed Pinna	1(0.9%)	1(6.3%)	—	—	—	—	—	—
Carpal Flexure	1(0.9%)	1(6.3%)	—	—	—	—	—	—
Omphalocele	1(0.9%)	1(6.3%)	—	—	—	—	1***(0.9%)	1***(5.9%)

* Fetus #4, mother #21926 (see also Table 6).

** Fetus #1, mother #21913 (see also Table 6).

*** Fetus #1, mother #21929 (see also Table 6).

Data extracted from IRDC Study #401-208 Tables 7 and 8.

The fetal soft tissue malformations observed involved 3 cases of hydrocephaly: one in the mid dose and 2 in the high dose groups; and 2 instances of cryptorchidism: one each in the low and high dose groups. Some of these malformations occured in fetuses which were already noted with external malformations, for example, one fetus in the mid dose group exhibited both dome-shaped head and hydrocephaly and one fetus of the high dose group exhibited both omphalocele and crytorchidism, and as mentioned on page 11 another fetus, from a different litter, had anasarca, dome shaped head and hydrocephaly (see Table 5). Table 6 below reflects the incidence of soft tissue malformations.

TABLE 6

Summary of Incidence of Fetal Soft Tissue Malformations - Technical Alachlor (mg/kg/day)

	Control		10		30		60	
Numbers of litters examined	16		16		16		17	
Numbers of fetuses examined	106		114		116		113	
Malformations								
	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters
Hydrocephaly	—	—	—	—	1**(0.9)	1**(6.3%)	2*(1.8%)	2*(11.8%)
Cryptorchidism	—	—	1(0.9%)	1(6.3%)	—	—	1***(0.9%)	1***(5.9%)

* One of these fetuses is Fetus #4, mother #21926 (see also Table 5).

** Fetus #1, mother #21913 (see also Table 5).

*** Fetus #1, mother #21929 (see also Table 5).

Data extracted from IRDC Study #401-208 Tables 7 and 8.

Fetal skeletal malformations involved one fetus with fused, misshapen skull bones in the control, one fetus with an atlas-occipital defect in the low dose group, 2 fetuses from 2 different litters with centrum anomalies (involving cervical centrum #4) in the low dose group, 2 fetuses with vertebral anomalies with associated rib anomalies, one each in low and mid dose group, one fetus with an extra site of ossification in the sternum in the control group, one fetus with malformed ribs (thoracic rib #1) in the control group and 3 fetuses with forked scapula, one in the mid dose and 2 in the high dose group (both in the same litter). One of the fetuses in the low dose group had both the atlas-occipital defect and the vertebral anomaly with associated rib anomalies, see Table 7 below:

Table 7

Summary of Incidence of Fetal Skeletal Malformations - Technical Alachlor (mg/kg/day)

	Control		10		30		60	
Numbers of litters examined	16		16		16		17	
Numbers of fetuses examined	106		114		115		113	
Malformations	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters
Fused Skull Bones	1(0.9%)	1(6.3%)	—	—	—	—	—	—
Atlas-occipital Defect	—	—	1*(0.9%)	1*(6.3%)	—	—	—	—
Centrum Anomalies	—	—	2(1.8%)	2(12.5%)	—	—	—	—
Vertebral anomalies with associated rib anomalies	—	—	1*(0.9%)	1*(6.3%)	1(0.9%)	1(6.3%)	—	—
Extra site of ossification in sternum	1(0.9%)	1(6.3%)	—	—	—	—	—	—
Malformed Ribs	1(0.9%)	1(6.3%)	—	—	—	—	—	—
Forked Scapula	—	—	—	—	1(0.9%)	1(6.3%)	2(1.8%)	1(5.9%)

* Fetus #8, Mother #21897.

Data extracted from IRDC Study #401-208 Tables 7 and 8.

Overall, there was an increased incidence of fetuses (2.7%) and litters (17.6%) with soft tissue malformations in the high dose group as compared to no incidence in the control group.

The historical control data did not provide instances of anasarca, malformed pinna, omphalocele, cryptorchidism or atlas-occipital defect. These occured singly and not in any set pattern. The historical control data included one non-viable fetus.

Variations:

The fetal developmental variations which were observed, although only presented in summary form, included increased incidence of fetuses, not litters, with 25 and 27 presacral vertebrae in the high dose group, 13th rudimentary ribs in all 3 treatment groups (not dose related), and an increase in major vessel variations over control in the high dose group. There was no indication of what was meant by major vessel variations (see Table 8). The incidence of 13th full ribs appears to be dose related when considering individual fetal observations, but when litter incidence is taken into account the most significant effect is at the high dose level. When 13th rudimentary rib(s) and 13th full ribs are combined, a dose response increase can be seen (see Table 8).

TABLE 8

Summary of Incidence of Fetal Developmental Variations - Technical Alachlor (mg/kg/day)

	Control		10		30		60	
	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters
27 presacral vertebrae	11(10.4%)	7(43.8%)	10(8.8%)	7(43.8%)	12(10.4%)	6(37.5%)	22(19.5%)	8(47.1%)
25 presacral vertebrae	—	—	—	—	—	—	3(2.7%)	1(5.9%)
13th rudimentary rib(s)	12(11.3%)	8(50.0%)	14(12.3%)	11(68.2%)	20(17.4%)	10(62.5%)	17(15.0%)	11(64.7%)
13th full rib(s)	11(10.4%)	7(43.8%)	15(13.2%)	8(50.0%)	20(17.4%)	7(43.8%)	35(31.0%)	10(58.8%)
Major vessel variations	4(3.8%)	3(18.8%)	5(4.4%)	4(25.0%)	1(0.9%)	1(6.3%)	7(6.2%)	6(35.3%)
Combined 13th rudimentary and full rib(s)	23(21.7%)	—	29(25.4%)	—	40(34.8%)	—	52(46.0%)	—

Data extracted from IRDC Study #401-208, Table 7.

The fetal variation data was not presented by individual fetal incidence.

Conclusions:

Technical Alachlor at the highest dose tested (60 mg/kg/day) caused a lower maternal body weight gain during the treatment period (days 7 to 19), however these animals along with the mid dose group gained more weight over the entire gestation period than the other two study groups (see Page 8 of this review). Thus, there is only minimal evidence of maternal toxicity and only during the dosing period.

Fetotoxicity at the high dose level was reflected by the increased incidences of fetuses with 25 presacral vertebrae and 27 presacral vertebrae, 13th full rib(s), combined 13th rudimentary and full rib(s) and major vessel variations. Also, an increase was noted in the incidence of fetuses and litters with soft tissue malformations (hydrocephaly and cryptorchidism) in the high dose group as compared to the control group (high dose-fetuses 2.7%, litters 17.6%; control no incidence).

There may be fetotoxicity at the mid dose level seen as increased combined 13th rudimentary and full rib(s) and by an increase in the incidence of post implantation loss. Although this effect may have been obscured at the high dose level due to increased preimplantation loss, see discussion at the end of page 9 of this review.

The following defects were noted in the study:

1. The substitution of mineral oil for the corn oil used in the original study may have had an effect on the absorbance of the test compound after gavage.

2. The high incidence of preimplantation loss at the high dose level may have obscured the fetotoxic effect noted at the lower dosage groups.

3. The historical data was inadequate from the standpoint of certain instances of defects not reported (see Page 14 of this review) and the data must comprise studies up to 2 years prior to the present study for <u>all</u> assayed parameters, presented by individual study and <u>dated</u>.

4. Individual fetal body weight data were not provided.

5. Individual fetal variation data were not presented, only provided in summary form and no description was provided for what was meant by major vessel variations.

6. No explanation was provided for the statements "Initial preparation did not meet specifications", "The initial dosing day 1 mixtures did not meet specifications and was rejected" (see page 4 of this review).

7. Unreasonably large variation in weights of animals used in the study.

8. Discrepancies were found in Table 3 which depicts group mean maternal body weight and body weight changes.

Also, the following would be useful (although not required by the Guidelines):

1. Food consumption data.

2. Fetal crown-rump length determinations.

Due to deficiencies 1 to 7 noted above (with emphasis on item 1) this study was not considered adequate to assess the teratogenic potential of Alachlor in the rabbit.

Core Classification:

Core Supplementary Data, based on defects of study presented above.