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

009701

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

AUG 27 1992

MEMORANDUM

SUBJECT: Propachlor - Developmental Toxicity Studies
Labeled 6(a)(2)

TO: Susan Cerrelli
Product Manager (71)
Reregistration Branch, SRRD (H7508C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 8/20/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 8/20/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 8/23/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: Monsanto Company
Chemical: 2-chloro-N-isopropylacetanilide
Synonym: Propachlor
DP Barcode: D180366
Submission No.: S421236
Caswell No.: 194
Case: 818640
Identifying No.: 019101
Shaughnessy No.: 019101
MRID No.: 423480-02
Action Requested: Please review MRID 42190401 (sic), a rabbit teratology study.

Comment: The Registrant has submitted a repeat rabbit developmental toxicity study (and a range-finding study) in response to the Agency's request. The previous rabbit study had not established a NOEL for developmental effects. These two studies, which have the same MRID #, have been reviewed, and the DER's are appended.

1) Range-finding study - Under the conditions of the study, dose levels of 25, 75, 125, 175, and 225 mg test material/kg of body weight/day administered to artificially inseminated rabbits during Days 7 through 19 of gestation resulted in (1) death and gross

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pathological lesions of the stomach and liver at dose levels of 125 mg/kg and greater, (2) reduced defecation and soft stool at doses of 125 mg/kg and above, and (3) decreased body-weight gain, corrected body weight at 175 mg/kg and above. Pregnancy rate was 57.1% at 175 mg/kg and above. Among the groups with gravid does at scheduled sacrifice, there were no differences noted in any of the Cesarean section parameters monitored. No external malformation or developmental variation was observed in any of the fetuses. Maternal toxicity was observed at 125 mg/kg and above, expressed mainly by deaths. Developmental toxicity was not observed at any of the dose levels with available animals (25, 75, and 125 mg/kg). This study was a range-finding study used to determine the dose levels to be used in the definitive developmental toxicity study. The dose levels chosen were 5, 50, and 100 mg/kg.

This range-finding study is classified Acceptable; it does not satisfy the guideline requirement (83-3) for a developmental toxicity study in the rabbit nor was it intended to do so.

2) Rabbit developmental toxicity study - Under the conditions of the study, dose levels of 5, 50, and 100 mg test material/kg of body weight/day administered to artificially inseminated rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach, (2) salivation and reduced defecation, and (3) decreased body-weight gain, corrected body weight, and food consumption at the high dose (100 mg/kg). Although there were no statistically significant differences in the number of corpora lutea/doe, implantations/doe, live fetuses/doe, resorptions/ doe, dead fetuses/doe, litter weight, or fetal body weight (combined and per sex) among the groups, the high-dose litters contained slightly fewer fetuses and fetal body weight tended to be slightly lower than the control and other groups. Additionally, there was a dose-related increase in the number of resorptions, # resorptions/doe, and the % of does with resorptions at the mid- and high-dose levels, with a comparable increase in post-implantation loss at these two dose levels compared to the control. There were no statistically significant differences in the incidence of any fetal malformation (external, visceral or skeletal) that can be attributed to treatment. There was a statistically significant increase in the number of high-dose litters with fetuses with bent hyoid arch(es). Before a final determination can be made with respect to a maternal or developmental NOEL, additional historical control data regarding the incidence of resorptions and bent hyoid arched is required.

This study is classified Core Supplementary, pending the submission of additional historical control data. This study does not satisfy the guideline requirement (83-3) for a developmental toxicity study in the rabbit, but it may be upgraded.

NOTE: The Registrant (correctly) did not label these studies as 6(a)(2) data. This Data Package was received in TB II on July 8,

1992. As stated on the "bean sheet", this submission is a follow-up to an earlier 6(a)(2) submission. It was determined by B. Burnam that (1) these data do not cause undue concern for HED staff and (2) the data would be examined in a non-expedited review cycle (90-120 days). However, the "bean sheet lists 8/1/92 as the due date. Additionally, the review instructions list the MRID # of the study to be reviewed as 42190401, which is in error.

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 8/20/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 8/20/92
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

009701

STUDY TYPE: Developmental Toxicity rabbit (dose range-finding)

TOX. CHEM. NO.: 194 SHAUGHNESSY NO.: 019101

MRID NO.: 423480-02

TEST MATERIAL: Propachlor technical

SYNONYMS: 2-chloro-N-isopropylacetanilide

STUDY NUMBER: WI-91-1 (WIL-50196); WIL Project #: WIL-50196

SPONSOR: Monsanto Company

TESTING FACILITY: WIL Research Laboratories, INC. Ashland, OH

TITLE OF REPORT: A Dose Range-Finding Developmental Toxicity Study of Propachlor Technical in Rabbits

AUTHOR: JF Holson

REPORT ISSUED: May 9, 1992

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, dose levels of 25, 75, 125, 175, and 225 mg test material/kg of body weight/day administered to artificially inseminated rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach and liver at dose levels of 125 mg/kg and greater, (2) reduced defecation and soft stool at doses of 125 mg/kg and above, and (3) decreased body-weight gain, corrected body weight at 175 mg/kg and above. Pregnancy rate was 57.1% at 175 mg/kg and above. Among the groups with gravid does at scheduled sacrifice, there were no differences noted in any of the Cesarean section parameters monitored. No external malformation or developmental variation was observed in any of the fetuses. Maternal toxicity was observed at 125 mg/kg and above, expressed mainly by deaths. Developmental toxicity was not observed at any of the dose levels with available animals (25, 75, and 125 mg/kg). This study was a range-finding study used to determine the dose levels to be used in the definitive developmental toxicity study. The dose levels chosen were 5, 50, and 100 mg/kg.

Classification: Acceptable. This is a range-finding study, which does not satisfies the guideline requirement (83-3) for a developmental toxicity study in the rabbit nor was it intended to do so.

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A. MATERIALS

1. Test Compound: Propachlor, technical; Description: tan solid; Batch #: Lot # MUS-9012-2657-T; Purity: 96.8% ; Source: supplied by Sponsor; Vehicle: Methylcellulose; Lot #: 50H0209; Source: Sigma Chemical Company, St. Louis, MO.

2. Test Animals: Species: rabbit; Strain: New Zealand White; Age: ≈ 4.5 months old on receipt; Weight: females: 2874-3891 g; Source: Hazleton Research Products, Inc., Denver, PA.

3. Statistics: Analyses were performed by a Digital Microvax 3400 computer. Maternal body weight, weight changes, net body weight changes, gravid uterine weights, and organ weights, fetal body weights, viable fetuses, total implantations, and corpora lutea: one way analysis of variance followed by Dunnett's test. Fetal malformations and variations: Fisher's Exact Test. Early and late resorptions, dead fetuses, postimplantation losses: Mann-Whitney U test.

B. STUDY DESIGN

Fifty-five sexually mature, New Zealand White virgin female rabbits (quarantined ≈ 3 weeks prior to insemination) were used in this study. Semen was collected from 7 resident males from the same strain/ supplier, evaluated for volume, motility, and concentration, diluted with 0.9% physiological saline, and maintained in a water bath at 34-37°C during the insemination procedure. The final concentration obtained was greater than 3 million motile sperm/mL. Semen from one male (0.25-0.5 mL of each diluted semen sample was introduced into each female's vagina) was used to inseminate one female in each group. The artificially-inseminated rabbits were administered human chorionic gonadotropin, via the marginal ear vein, immediately following insemination (considered day 0 of gestation) to ensure ovulation. Each female was housed individually (no nesting material was provided since sacrifice was scheduled prior to parturition). Animals were assigned to the groups according to a printout, which was generated based on body weight stratification in a block design. There were five dose levels (25, 75, 125, 175, and 225 mg/kg/day) of the test material (0.5% aqueous methylcellulose was the vehicle, which control animals received), and each group was composed of 7 females. The test and control materials were administered by oral gavage on Days 7-19 of gestation at a dosing volume of 5 mL/kg of body weight. The total dose administered each day was based on the most recent body weight of each animal. This is a dose range-finding study; it was not stated how the levels were chosen. Throughout the study, Purina Certified Rabbit Chow #5322 (pellet form) and purified tap water were available ad libitum (diet restricted to 150 g/animal/day during acclimation; no reason given).

Dose Preparation: The solutions of test material (dosage levels: 25, 75, 125, 175, and 225 mg/kg) were prepared once during the treatment period and were stored at room temperature. The test material was triturated with the vehicle until a slurry was formed, more vehicle

was added/mixed, and the mixture was transferred to a storage container where it was mixed with more vehicle on a Polytron to reduce particle size. The mixtures were visually inspected for homogeneity prior to dispensing and stirred continuously during dosing to ensure homogeneity.

RESULTS

Information on the stability and homogeneity of the test material in the vehicle was not provided in the report. It was stated that, for the purposes of dose calculation, the test material was considered 100% technical Propachlor. Data regarding the concentrations attained were not provided.

C. Clinical Observations

Each doe was examined twice daily for mortality, moribundity, general appearance, and behavior, and detailed clinical observations were recorded daily from days 0 through 29. Additionally, the rabbits were observed at the time of dosing and ≈ 1, 2, and 4 hours following dosing each day for overt signs of toxicity. Individual body weights were recorded on gestation days 0, 7, 10, 13, 19, 24, and 29, and body-weight changes were calculated for various intervals throughout the study. Gravid uterine weight, net body weight (day 29 body weight minus the weight of the uterus and contents), and net body-weight change were calculated for each doe at the scheduled Cesarean section. Feed consumption was not measured.

D. Terminal Procedures

On Day 29 of gestation, all does were sacrificed via an i.v. injection of Socumb® Euthanasia solution and thoracic, abdominal, and pelvic cavities were opened and viscera were examined; abnormalities were recorded. The uterus and ovaries of each doe were excised, and the trimmed uterus was weighed and opened for internal examination. The liver, kidneys, and spleen of each doe were weighed. Maternal tissues showing abnormalities were preserved for possible future histopathological examination. Does that aborted, died, or were sacrificed moribund were necropsied, and the number and location of implantation sites and corpora lutea were recorded. Recognizable fetuses from does that delivered prior to day 29 were examined externally and were preserved only if malformations were observed. Maternal tissues were processed as noted above.

E. Uterine/Implantation Data

The uterus of each doe was opened and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal uterine horn noting the position of the cervix and continuing from the proximal to distal right uterine horn. Corpora lutea were counted for each ovary. The uteri with no macroscopic evidence of implantation were opened and stained with 10% aqueous

ammonium sulfide solution to detect early implantation loss (Salewski).

Two methods of calculation were used to summarize intrauterine data: Group Mean Litter Basis and Proportional Litter Basis.

Group Mean Litter Basis

Postimplantation Loss/Litter = # dead fetuses, resorptions (early/late)/group ÷ # gravid 99/group

Proportional Litter Basis

Summation/group (%) = Postimplantation loss/litter (%)* ÷ # of litters/group

* = [# dead fetuses, resorptions (early/late)/litter ÷ # implantation sites/litter] x 100

F. Fetal Data

All fetuses were weighed, and a detailed external examination of each was conducted, which included the eyes, palate, and external orifices. Findings were recorded as either developmental variations or malformations, and each fetus was euthanized and discarded. Crown-rump measurements were recorded for late resorptions and the tissues were discarded.

RESULTS

1. Clinical Observations and Survival - Maternal

There were deaths at the three highest dose levels; one of the deaths at 125 mg/kg was thought to be from a dosing error.

DEATHS

Dose	125 mg/kg	175 mg/kg	225 mg/kg
# dying	3/7	5/7	7/7
day of death	11, 14, 15	9, 11, 11, 17, 19	8, 8, 8, 9, 9, 9, 10

Numerous clinical signs were observed in the does dying on test, with decreased defecation and soft stool being observed most frequently. Other observations included decreased urination, excessive chewing, diarrhea, apparent red material in the urine, clonic convulsions, and spontaneous vocalizations. At the two highest dose levels, labored breathing, salivation, ataxia, and prostration were reported. Lethargy, erratic jerking of the head, impaired righting reflex and tonic convulsions were observed at the highest dose level. The survivors at the 125 and 175 mg/kg dose levels displayed decreased defecation and urination and soft stool. Excessive chewing was displayed on occassion in these groups at the time of dosing and/or 1-4 hours after dosnig. All clinical findings at the 25 and 75 mg/kg dose levels, which included decreased defecation and urination, were considered incidental since the incidence and time of onset of occurrence were similar to the control.

2. Maternal Body Weight and Body-weight Gain

Body weights were comparable among the groups during the week prior to dose initiation, although the highest dose group displayed the smallest gain during this period (both on a gram and % basis) compared to the control. Dose-related negative body-weight gains were displayed at the 75, 125, and 175 mg/kg dose levels during the first 3 days of dosing [all of the highest dose (225 mg/kg) animals died during this period and no body-weight data were presented]. Following the initial negative gain, the 75 mg/kg dose group showed a comparable/greater gain compared to the control during the remainder of the dosing period. The 125 and 175 mg/kg dose groups displayed negative body-weight gains (dose-related) for the entire dosing period. The 75 mg/kg dose group displayed a negative gain during the 10-day period following dosing.

Body-weight Gains [grams]

Dose (mg/kg) Time Interval	0	25	75	125	175	225
0-7	345	423	386	374	350	336
7-10	64	58	-1	-136**	-360**	-
10-13	104	130	136	-25*	-292**	-
13-19	120	118	140	63	-518	-
19-24	91	58	-1	92	-	-
24-29	-68	-4	-75	-27	-	-
7-19	287	307	275	-88**	-1045**	-
19-29	24	48	-76	65	-	-
0-29	656	775	585	370	-	-
0-29 (corrected)	308.4	404.3	356.0	55.2	NA	NA

* $p<0.05$; ** $p<0.01$; NA = no gravid does survived to scheduled necropsy

Body-Weight Gain (%)♦

Dose (mg/kg) Interval (days)	0	25	75	125	175	225
0-7	10.2	13.0	11.8	11.4	10.2	9.7
7-19	8.5	9.4	8.4	-2.7	-30.4	-
0-29	19.4	23.8	17.8	11.3	-	-
0-29 (net)	9.1	12.4	10.9	1.7	-	-

♦ calculated by TB II, no statistics performed

Gravid uterine weight (see table below) was 66% of the control value in the 75 mg/kg-dose does and 91% of the control value at the 125 mg/kg dose level. The corrected body weight was comparable among the groups with does at scheduled sacrifice, although the 125 mg/kg dose level displayed the lowest value (90% of the control value; see table below).

Uterine and Net Body Weights (g)

Dose (mg/kg)/Parameter	0	25	75	125	175	225
gravid uterus (grams)	347.4	370.7	229.2	315.2	NA	NA
corrected body weight (grams)	3693	3646	3635	3324	NA	NA
net weight change from day 7 (grams)	-37.3	-28.7	-29.6	-327.5	NA	NA

NA = no gravid does survived to scheduled sacrifice

3. Food Consumption

Food consumption was not measured in this study.

4. Gross Pathological Observations

Treatment-related lesions were reported in the does that died during the study, which included reddened gastric mucosa and dark red areas and/or dark red contents in the stomach in 1, 3, and 7 does at the 125, 175, and 225 mg/kg dose levels, respectively. The liver was affected in 1 (125 mg/kg) and 3 (175 mg/kg) does also (accentuation of the lobular markings and/or pale). White foamy contents in the lungs and trachea were observed in the 125 mg/kg doe believed to have died because of an intubation error. In the does that aborted, the 25 mg/kg doe displayed red foci in the lungs and the 175 mg/kg doe had clear fluid contents in the thoracic cavity. Of the does that survived to scheduled necropsy, a 75 mg/kg doe displayed reddened mucosa in the stomach. Other findings (accessory spleens, cystic oviduct, uterine dysplasia) occurred usually in only one doe, and these are not viewed as treatment-related.

No assessment of organ weight data could be performed at the two highest doses due to mortality. Due to the small numbers of animals in the groups, it cannot be determined whether the apparent differences noted (increased liver weight at 75 mg/kg and decreased spleen weight at 125 mg/kg) are due to biological variation within the small sample or to exposure to the test material.

5. Maternal Observations

Pregnancy rate decreased with increasing dose. Two of the does aborted (one each at the 25 mg/kg-dose and 175 mg/kg-dose). The abortion at the 175 mg/kg-dose may have been due to treatment, since it was related to a considerable weight loss. The abortion at the low dose was considered incidental since none of the 75 or 125 mg/kg dose does aborted (no dose response). There was a dose-related decrease in the number of total implantations sites and live fetuses, but there was no dose-related decreased in the number per doe. There were no dose-related differences noted in the number of resorptions, pre-implantation loss, post-implantation loss, or fetal weights . The Cesarean section observations are listed below.

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Table I: Cesarean Section observations

GROUP (mg/kg)	0	25	75	125	175	225
# Females mated	7	7	7	7	7	7
# Pregnant Females	6	4	5	5	4	4
Pregnancy Rate (%)	85.7	71.4	71.4	71.4	57.1	57.1
Maternal Wastage						
#Died	0	0	0	3	4	6
#Died/pregnant	0	0	0	2	2	3
#Non pregnant	0	0	0	1	2	3
#Aborted	0	1	0	0	1	0
#Premature Delivery	0	0	0	0	0	0
# Females with 100% intrauterine deaths	1	0	0	0	0	0
# Females with live fetuses at necropsy (%)	5	4	5	3	0	0
Total # Corpora Lutea	54	30	53	28	-	-
Corpora Lutea/dam	9.0	7.5	10.6	9.3	-	-
Total # Implantation	35	22	21	16	-	-
Implantations/Dam	5.8	5.5	4.2	5.3	-	-
Total # Live Fetuses	33	20	17	15	-	-
Live Fetuses/Dam	6.6	5.0	3.4	5.0	-	-
% of implantations	94	91	81	94	-	-
Total Resorptions	2	2	4	1	-	-
Early	2	2	4	1	-	-
Late	0	0	0	0	-	-
Resorptions/Dam	0.3	0.5	0.8	0.3	-	-
# Litters w/resorptions (%)♦	1 (17)	2 (50)	2 (40)	1 (33)	-	-
Total # Dead Fetuses	0	0	0	0	-	-
Postimplantation Loss(%)	16.7	10.0	10.7	8.3	-	-
Preimplantation Loss(%)	23.1	23.1	63.1	45.1	-	-
Litter Weight (gm)♦	299	259	154	221	-	-
Mean Fetal Weight (gm)	46.5	53.4	51.1	45.7	-	-
Mean Male Weight♣						
Mean Female Weight♣						
Sex Ratio (% Male)♣						

♦ calculated by TB II; ♣ no data available to determine

6. Fetal Observations

There was a dose-related decrease in the number of fetuses available for morphological examination (33, 20, 17, 15, 0, and 0 at 0, 25, 75, 125, 175, and 225 mg/kg). No external malformations or developmental variations were reported in any of the fetuses in this study.

D. Discussion

At the two highest dose levels, all gravid does died prior to scheduled sacrifice. Two does aborted; the one at the lowest dose is considered incidental since the next 2 higher doses showed no abortions. The abortion at 175 mg/kg may be related to treatment since the doe lost considerable weight prior to the abortion, although it is recognized by TB II that abortions are not uncommon in rabbits. The does dying on test displayed numerous clinical signs indicative of

toxicity, which included reduced defecation and soft stool. No clinical signs of toxicity were observed at the 25 and 75 mg/kg dose levels. Stomach and liver lesions were observed at necropsy in those does dying on test. Of the groups with gravid does at scheduled sacrifice, maternal body-weight gains were decreased at the 125 mg/kg dose level compared to the control values, mainly during the dosing period; the corrected body weight was decreased at this dose level also.

The pregnancy rate was decreased at all dose levels compared to the control value, but the two highest dose levels displayed the lowest (comparable) rate [85.7% in control, 71.4% in the 3 lowest dose levels and 51.7% in the 2 highest dose levels]. There were no significant differences among the groups in any of the Cesarean parameters. Due to the deaths of the does at the 2 highest dose levels, no litters/ fetuses were available at these dose levels for examination. There were no dead fetuses, and no external malformations or developmental variations were observed in this study.

D. CONCLUSION

Under the conditions of the study, dose levels of 25, 75, 125, 175, and 225 mg test material/kg of body weight/day administered to artificially inseminated rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach and liver at dose levels of 125 mg/kg and greater, (2) reduced defecation and soft stool at doses of 125 mg/kg and above, and (3) decreased body-weight gain, corrected body weight at 175 mg/kg and above. Pregnancy rate was 57.1% at 175 mg/kg and above. Among the groups with gravid does at scheduled sacrifice, there were no differences noted in any of the Cesarean section parameters monitored. No external malformation or developmental variation was observed in any of the fetuses. Maternal toxicity was observed at 125 mg/kg and above, expressed mainly by deaths. Developmental toxicity was not observed at any of the dose levels with available animals (25, 75, and 125 mg/kg). This study was a range-finding study used to determine the dose levels to be used in the definitive developmental toxicity study (reviewed elsewhere; MRID # 423480-02, which is the same as this range-finding study). The dose levels chosen were 5, 50, and 100 mg/kg. This study is classified Acceptable; it does not satisfy any guideline requirement, nor was it intended to.

NOTE: In Table 9, page 276 of the submission (page 40 of the study report), the initial body weight of the 25 mg/kg dose level is in error (3242 should read 3252, according to the data listed on page 299 (page 63 of the study report).

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 8/20/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 8/24/92
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

009701

STUDY TYPE: Developmental Toxicity rabbit TOX. CHEM. NO.: 194

MRID NO.: 423480-02 SHAUGHNESSY NO.: 019101

TEST MATERIAL: Propachlor technical

SYNONYMS: 2-chloro-N-isopropylacetanilide

STUDY NUMBER: SB-91-86 (SLS#3044.182); Pilot study WI-91-1 (WIL-50196)

SPONSOR: Monsanto Company

TESTING FACILITY: Springborn Laboratories, Inc., Spencerville, OH;
Pilot: WIL Research Laboratories, INC. Ashland, OH

TITLE OF REPORT: Rabbit Teratology Study with Propachlor

AUTHOR: GP Adam; Pilot study: JF Holson

REPORT ISSUED: February 7, 1992; Pilot study: May 9, 1992

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, dose levels of 5, 50, and 100 mg test material/kg of body weight/day administered to artificially inseminated rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach, (2) salivation and reduced defecation, and (3) decreased body-weight gain, corrected body weight, and food consumption at the high dose (100 mg/kg). Although there were no statistically significant differences in the number of corpora lutea/doe, implantations/doe, live fetuses/doe, resorptions/ doe, dead fetuses/doe, litter weight, or fetal body weight (combined and per sex) among the groups, the high-dose litters contained slightly fewer fetuses and fetal body weight tended to be slightly lower than the control and other groups. Additionally, there was a dose-related increase in the number of resorptions, # resorptions/doe, and the % of does with resorptions at the mid- and high-dose levels, with a comparable increase in post-implantation loss at these two dose levels compared to the control. There were no statistically significant differences in the incidence of any fetal malformation (external, visceral or skeletal) that can be attributed to treatment. There was a statistically significant increase in the number of high-dose litters with fetuses with bent hyoid arch(es). Before a final determination can be made with respect to a maternal or developmental NOFL, additional historical control data regarding the incidence of resorptions and bent hyoid arched is required.

Classification: Core Supplementary, pending the submission of additional

historical control data. This study does not satisfy the guideline requirement (83-3) for a developmental toxicity study in the rabbit, but it may be upgraded.

A. MATERIALS

1. Test Compound: Propachlor, technical; Description: tan solid; Batch #: Lot # MUS-9012-2657-T; Purity: 96.8% ; Source: supplied by Sponsor; Vehicle: Methylcellulose; Lot #: 901215; Source: Fisher Scientific, Cincinnati, OH.

2. Test Animals: Species: rabbit; Strain: New Zealand White; Age: ≈ 6 months old; Weight: females: 2.9-3.9 kg; Source: Hazleton Research Products, Inc., Denver, PA.

3. Statistics: Analyses were performed by a Digital Vax 11/730 computer. Maternal and fetal data, including body weights, food consumption, # viable fetuses, implantation sites, and corpora lutea: one way analysis of variance followed by Dunnett's test. Incidence and number of fetal malformations and variations utilizing the litter as the experimental unit: Fisher's Exact Test. # resorptions: Mann-Whitney U test.

B. STUDY DESIGN

Eighty artificially inseminated rabbits (quarantined for 27 days prior to insemination) were used in this study. Semen was collected from male New Zealand White rabbits from the same supplier (volume, motility, and concentration were evaluated), diluted with 0.9% physiological saline, and maintained in a water bath at 35-36°C during the insemination procedure. Semen from one male (≈ 0.5 mL of diluted semen was introduced into each female's vagina) was used to inseminate an equal number of females in each group. The females were administered human chorionic gonadotropin, via the marginal ear vein, immediately following insemination (considered day 0 of gestation). Each female was housed individually (there was no statement regarding nesting material). Animals were assigned to the groups according to a randomization table in a block design, which was generated based on body weight stratification. There were three dose levels (5, 50, and 100 mg/kg) of the test material (0.5% aqueous methylcellulose was the vehicle, which control animals received), and each group was composed of 20 females. The test and control materials were administered by oral gavage on Days 7-19 of gestation at a dosing volume of 5 mL/kg of body weight. The total dose administered each day was based on the most recent body weight of each animal. The dose levels were chosen based on the results of a pilot (dose range-finding developmental toxicity; WIL Project No.: WIL-50196; same MRID # as current study, reviewed separately) study in pregnant New Zealand rabbits. Throughout the study, Purina Certified Rabbit Chow #5322 and purified tap water were available ad libitum.

Dose Preparation: The solutions of test material (intended dosage: 5, 50, and 100 mg/kg) were prepared once in sufficient quantities to last for the treatment period. The test material was ground and passed through a 40 mesh sieve prior to weighing. Total amounts of 7, 70, and 140 grams (not adjusted for purity) were weighed out and the vehicle (homogeneous suspension of distilled water and methylcellulose) was added; the mixtures were stirred for ≈ 30 minutes, stored in a

refrigerator, and were stirred for 30 minutes prior to dispensing and continuously during dosing to ensure homogeneity. Stability and homogeneity of the test material (low and high dose, prepared as above) in the vehicle were determined prior to study initiation. Samples were taken from the bottom, middle, and top of these solutions and analyzed for homogeneity and stability at 3, 8, and 15 days following preparation. The concentration of the dosing solutions was measured twice (at dosing initiation and completion) to verify target concentrations.

RESULTS

A technical error (detected on study day 17) occurred during the preparation of the dosing solutions and the beakers were calibrated to 6 liters instead to 7, as specified in the calculation and dosage preparation sheets. Therefore, the final concentrations of the dosing solutions were 1.16, 11.66, and 23.33 mg/mL for the low-, mid-, and high-dose groups, respectively (intended concentrations: 1.0, 10.0, and 20.0 mg/mL, respectively). The concentrations attained were within ± 10% of the targeted concentrations for samples taken at study initiation and completion. NOTE: The values listed on page 13 of the study report and those in Table 3 of Appendix B for the low dose are in error (see table below). Also, the average value for the high dose should read 21.81 instead of 21.80 mg/mL.

Target concentration (mg/mL)	Nominal concentration (mg/mL)	Actual concentration (mg/mL)	Recovery of Nominal concentration (%)	Recovery of Targeted concentration (%)	Average Recovery of Nominal concentration (%)	Average Recovery of Targeted concentration (%)
1	1.16	1.03 1.07	88.8 100.9 [92.2]•	103.0 117.0 [107.0]•	94.9 [90.5]•	110.0 [105.0]•

• [correct #]

The solutions were found to be homogeneous and stable for at least 15 days at both the low- and high-dose concentrations. The actual dosage levels exceeded the intended levels by ≈16-17% (5.8, 58.3, 116.7 mg/kg, respectively).

C. Clinical Observations

Each doe was examined daily for clinical signs of toxicity, including physical and behavioral abnormalities, and mortality checks were conducted twice a day (am and pm). Additionally, the rabbits were observed for 0.5-2 hours following dosing each day for overt signs of toxicity. Individual body weights were recorded on gestation days 0, 7, 10, 13, 16, 19, 24, and 29, and body-weight changes were calculated for various intervals throughout the study. During gestation, individual feed consumption was measured daily. Additionally, feed consumption was calculated for various intervals throughout the study as grams/rabbit/day and grams/kg/day.

D. Terminal Procedures

On Day 29 of gestation, all does were sacrificed via an i.v. injection of T-61® and subjected to a morphological examination (thoracic, abdominal, and pelvic cavities opened and viscera examined; abnormalities were recorded and representative tissue samples with the lesions were preserved for possible histological examination). The uterus of each doe was weighed, and opened for internal examination.

E. Uterine/Implantation Data

The uterus of each doe was removed, examined externally, weighed, opened and (starting with the left distal horn, noting the position of the cervix, and continuing up the right uterine horn), the number and position of viable fetuses and early and late resorptions were recorded. Corpora lutea were counted for each ovary. The uteri with no macroscopic evidence of implantation were opened and stained with 10% aqueous ammonium sulfide solution to detect early embryolethality (Salewski).

F. Fetal Data

All fetuses were weighed, individually identified (tagged), and examined for external, internal (visceral), and skeletal abnormalities. The findings were classified based on the severity of the anatomical change(s) and the extent of their potential interference with organ and/or body function.

(1) **External examination** - Each fetus was examined for external abnormalities. The crown-rump length of late resorptions was measured and the tissues discarded.

(2) **Visceral examination** - Each fetus was dissected and examined (low power dissection scope) using a technique similar to the Staples technique. The sex of each fetus was determined.

(3) **Skeletal examination** - Each fetus was eviscerated, skinned, and fixed in 95% isopropyl alcohol, macerated in a 1-2% aqueous potassium hydroxide solution, stained with Alizarin Red S, and then cleared in glycerin. Skeletal examinations were performed using substage lighting.

RESULTS

1. Clinical Observations and Survival - Maternal

Two does at the high-dose level (100 mg/kg) died on gestation day 10, and these deaths were considered to be related to treatment. One control doe also died following dosing on day 12, and this was attributed to dosing error. There were no deaths at either of the two lower dose levels. Salivation was displayed in several high-dose does immediately after dosing, and reduced defecation occurred in the majority of these does during the treatment period. Thrashing, vocalization, prostration, labored breathing, and convulsions were displayed prior to death by the two does that died. All other clinical findings were considered incidental since they occurred in only a few

does in all groups.

2. Maternal Body Weight and Body-weight Gain

Body weights, body-weight gain, and net body weight of the low- and mid-dose groups were comparable to the control group during the study. Body-weight loss occurred in the high-dose group throughout the dosing period, although a statistically significant decrease in body weight was displayed only on days 16, 19, and 24 of gestation, compared to the control value. Following the dosing period, the mean body-weight gain was significantly greater in the high-dose does compared to the controls, but the net weight gain at the high-dose level was reduced compared to the control value (see table below). All does gained weight during the 7 days prior to dosing. Four control does lost weight during the first 3 days of dosing (one subsequently died); one low-, 2 mid-dose and 16 high-dose does lost weight during this interval (one of the latter does subsequently died).

Body-weight Gains [grams]

Dose (mg/kg) Time Interval	0	5	50	100
0-7	313	370	322	299
7-10	30	62	55	-108**
10-13	51	61	58	-12*
13-16	94	97	116	-82**
16-19	13	68	39	-75**
19-24	144	105	91	248**
24-29	79	79	62	162**
7-19	194	288	268	-284**
19-29	223	184	152	396**
0-29	733	838	742	443**
0-29 (corrected)	319	396	297	85**

* $p<0.05$; ** $p<0.01$

Body-Weight Gain (%)

Dose (mg/kg) Interval (days)	0	5	50	100
0-7	9.5±3.9	11.1±2.7	9.4±3.2	8.8±3.1
7-19	5.8±2.6	8.6±2.9	7.9±2.3	-7.0±9.4
0-29	21.8±5.5	25.1±4.0	21.9±6.6	13.3±7.1

• calculated by TB II, no statistics performed

Gravid uterine weight was decreased (86% of control) in the high-dose does, and the corrected body weight was lowest for this group also.

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Uterine and Net Body Weights (g)

Dose (mg/kg) Parameter	0	5	50	100
gravid uterus (grams)	414.5	441.8	444.9	358.1
corrected body weight (grams)	3649	3740	3672	3445
net weight change from day 7 (grams)	-15	20	-25	-243

3. Food Consumption

The high-dose group (100 mg/kg) displayed a statistically significant decrease in mean daily food consumption compared to the control value throughout the dosing period, as well as during the 5 days following the end of dosing. Additionally, this group displayed a statistically significant increase in food consumption during days 24-29 of gestation compared to the control group.

Food Consumption [grams/animal/day]

Dose (mg/kg) Days	0	5	50	100
0-7	202	214	206	203
7-10	200	216	195	101**
10-13	201	213	194	94**
13-16	204	219	192	72**
16-19	194	229	196	63**
19-24	189	208	182	141**
24-29	151	149	142	205**
7-19	200	219	194	82**
19-29	170	178	162	177
0-29	190	202	186	146**

** $p \leq 0.01$

4. Gross Pathological Observations

Treatment-related lesions were reported in the high-dose does that died during the study, which included reddened gastric mucosa and erosion. The control rat that died following dosing had consolidated lungs with multiple dark red and raised foci, which is consistent with a possible aspiration pneumonia (dosing error). No unusual findings were reported in the does that aborted or delivered prematurely. Other findings (enlarged ovaries, reddened area in uterus, red fluid contents in uterus, enlarged cervical lymph nodes, abdominal subcutaneous edema, sternal nodule and rough liver surface) occurred usually in only one doe. Hair loss and periovarian cyst(s) occurred slightly more frequently in the mid- and high-dose and high-dose does, respectively, but these are not viewed as treatment-related.

5. Maternal Observations

Pregnancy rate was comparable among the groups. Two of the does aborted (one each at the low- and high-dose on day 24) and one high-dose doe delivered prematurely on day 29. The abortion at the high

dose was attributed to treatment, since it was related to a considerable weight loss and reduced food intake during treatment. The abortion at the low dose was considered incidental since none of the mid-dose does aborted (no dose response). The number of corpora lutea were comparable among the groups. The number of implantations sites and live fetuses were decreased at the high-dose level, and the mid- and high-dose does displayed an increased number (dose-related) of resorptions (total) compared to the control, although statistical significance was not attained in either case. There was a dose-related increase in the number of resorptions/doe at the mid- and high-dose levels. Post-implantation loss was increased at the mid- and high-dose levels (dose-related), although a $p<0.05$ was not attained in either case. The Cesarean section observations are listed below.

Table I: Cesarean Section observations

GROUP:	CONTROL	5 mg/kg	50 mg/kg	100 mg/kg
# Females mated	20	20	20	20
# Pregnant Females	19	18	19	19
Pregnancy Rate (%)	95	90	95	95
Maternal Wastage				
#Died	1	0	0	2
#Died/pregnant	1	0	0	2
#Non pregnant	1	2	1	1
#Aborted	0	1	0	1
#Premature Delivery	0	0	0	1
# Females with 100% intrauterine deaths	0	0	1	2
# Females with live fetuses at necropsy (%)	18 (100)	17 (100)	18 (95)	13 (87)
Total # Corpora Lutea	167	183	190	162
Corpora Lutea/doe	9.3	10.8	10.0	10.8
Total # Implantations	123	121	141	106
Implantations/Doe	6.8	7.1	7.4	7.1
Total # Live Fetuses	116	115	122	85
Live Fetuses/Doe	6.4	6.8	6.4	5.7
% of implantations	94	95	87	80
Total # Resorptions	7	6	19	21
Early	7	3	9	12
Late	0	3	10	9
Resorptions/Doe	0.39	0.35	1.0	1.4
# Litters w/ resorptions (%)♦	4 (22)	4 (24)	6 (32)	9 (60)
mean litter % in litters w/R♣	25.7	15.7	34.8	40.1
mean litter % (all litters)	5.7	3.7	13.5	27.8
Total # Dead Fetuses	0	0	0	0
Postimplantation Loss(%)♦	5.7	5.0	13.5	19.8
Preimplantation Loss(%)♦	26.3	33.9	25.8	34.6
Litter Weight (gm)♦	291	307	302	281
Mean Fetal Weight (gm)	47.7	47.2	46.6	43.8
Mean Male Weight♦	48.2	46.5	46.2	43.4
Mean Female Weight♦	46.5	45.9	46.5	43.2
Sex Ratio (% Male)♦	44.8	48.7	40.2	51.8

♦ calculated by TB II; ♣ w/R = with resorptions♦

6. Fetal Observations

The number of fetuses/litter was comparable among the groups, although the high-dose litters were the smallest. Although there were no statistically significant differences among the groups in litter weight or mean fetal body weight (combined and per sex), the high-dose group displayed the lowest weights. The sex ratio varied among the groups, with the high-dose group displaying the highest percentage (52%) of males and the mid-dose group the lowest (40%).

Mean Fetal Body Weight (grams)

GROUP/SEX	MALES♦	FEMALES♦	LITTER♦
CONTROL	48.2	46.5	291.2
LOW DOSE	46.5	45.9	306.8
MID DOSE	46.2	46.5	301.7
HIGH DOSE	43.4	43.2	280.7

♦ data calculated by TB II

There were no statistically significant increases observed in the type or incidence of external, visceral, or skeletal malformations at any dose level, and no malformations were observed at the high-dose level. Those malformations observed were considered random and are commonly seen in rabbit fetuses. Additionally, with the exception of bent hyoid arch(es), there were no statistically significant increases in the type or incidence of variations at any dose level compared to the control (see Tables 11 and 12 of the study report, copies appended). A statistically significant increase in the number of litters with this variation was observed at the high-dose level compared to the concurrent control. The mean litter % for this variation was 1.9, 13.9, 7.8, and 8.6 in the control, low-, mid-, and high-dose groups, respectively. In general, the high-dose group displayed very few alterations compared to the concurrent control and the other treatment groups.

D. Discussion

At the high-dose level, two does died on day 10 of gestation (both pregnant), one doe aborted on gestation day 24, and one delivered prematurely on day 29. The stomach of each doe that died displayed reddened mucosa and erosion. The only clinical signs observed (salivation and reduced defecation) were found at the high-dose level. Maternal body weight gains and food consumption were decreased at this dose level compared to the control values, mainly during the dosing period, and the corrected body weight was decreased also.

The pregnancy rate was comparable among the groups. There were no statistically significant differences among the groups in any of the Cesarean parameters. Due to the deaths and the aborted/premature delivery in the high-dose group, a smaller number of litters (13) were

available for this dose level compared to the control and other treatment groups, although the number available is adequate. The number of corpora lutea/doe and implantations/doe were comparable among the groups, but the number of live fetuses per doe was slightly lower at the high-dose level compared to the control value. There was a dose-related increase in the number of resorptions and the number per doe, with the mid- and high-dose groups displaying a 2.6- to 3.6-fold increase, respectively, compared to the controls. Additionally, the percent of does with resorptions at these 2 dose levels was increased compared to the control value, and a similar increase was observed in the post-implantation loss. The historical control data provide only the # and % of pregnant does with 100% resorptions. No dead fetuses were observed. The total number of fetuses was decreased in the high-dose group, due mainly to the smaller number of litters, although the number of live fetuses/doe was slightly lower than the control and other groups. There were no statistically significant differences in mean litter weight or mean fetal weight (combined or per sex), although the high-dose group displayed the lowest weights.

There was no statistically significant increase in the incidence of any external, visceral or skeletal malformations or variations at any dose level that can be attributed to treatment. The statistically significant increase in the number of litters with fetuses displaying bent hyoid arches was considered a chance occurrence by the author since a comparable number of litters at the low-dose level displayed more fetuses with this variation, there is no dose response, and the incidence at the high-dose is within the historical control range, although at the high end. However, before a final determination can be made, additional information on the historical control with regard to this variation is required [the mean incidence; the incidence in each study of the historical control; # fetuses with hyoid arch(es) bent)/doe; # affected litters; # litters and fetuses examined/study]. Additionally, historical control information on the # resorptions/doe, the mean litter % in litters with resorptions, # does with resorption, and clarification of the units used for post-implantation loss (page 221) are required before a NOEL can be determined for this study.

D. CONCLUSION

Under the conditions of the study, dose levels of 5, 50, and 100 mg test material/kg of body weight/day to artificially inseminated rabbits during Days 7 through 19 of gestation resulted in (1) death and gross pathological lesions of the stomach, (2) salivation and reduced defecation, and (3) decreased body-weight gain, corrected body weight, and food consumption at the high dose (100 mg/kg). Although there were no statistically significant differences in the number of corpora lutea/doe, implantations/doe, live fetuses/doe, resorptions/doe, dead fetuses/doe, litter weight, or fetal body weight (combined and per sex) among the groups, the high-dose litters contained slightly fewer fetuses and fetal body weight tended to be slightly lower than the control and other groups. Additionally, there was a dose-related increase in the number of resorptions, # resorptions/doe, and the % of does with resorptions at the mid- and high-dose levels, with a comparable increase in post-implantation loss at these two dose

levels compared to the control. There were no statistically significant differences in the incidence of any fetal malformation (external, visceral or skeletal) that can be attributed to treatment. There was a statistically significant increase in the number of high-dose litters with fetuses with bent hyoid arch(es). Before a final determination can be made with respect to a maternal or developmental NOEL, additional historical control data regarding the incidence of resorptions and bent hyoid arched is required. This study is classified Core Supplementary, pending the submission of additional historical control data. This study does not satisfy the guideline requirement (83-3) for a developmental toxicity study in the rabbit, but it may be upgraded..

NOTE: There were several discrepancies noted in the report. On page 7, the Monsanto summary indicates that dosing occurred during gestation days 6 through 19, which is probably a "typo", since days 7 through 19 are listed later in the same paragraph and in the conclusions of this summary, and on pages 19, 24, and elsewhere of the report. On page 81, Chemistry Table 3 is in error with regard to the 1 mg/mL dose. Additionally, the values listed on page 13 of the study report and those in Table 3 of Appendix B for the low dose are in error, and the average value for the high dose should read 21.81 instead of 21.80 mg/mL.

Propachlor toxicology review

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