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

005214

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Developmental Toxicity Study in Rabbits on Iprodione

TO: Henry Jacoby, PM 21
Registration Division (TS-767)

FROM: Margaret L. Jones
Review Section III
Toxicology Branch (TS-769)

THROUGH: Marcia Van Gemert, Head
Reveiw Section III
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Toxicology Branch (TS-769)

Compound: Iprodione (Rovral) Tox. Chem No: 470A

Registration No. (Record No.): 167549 Registrant: Rhone Poulenc

Accession No: 260863 Tox. Project No: 1461

Action Requested: Review the Teratology study in rabbits which was received under the Data Call-In program.

Background: A previously submitted study in rabbits (EPA Accession Nos: 232712 and 253443) was classified core Supplementary due to deficiencies in the study and failure to demonstrate a NOEL for maternal and fetal toxicity.

Conclusions: Iprodione Technical was administered by gavage to pregnant New Zealand White rabbits (18 females/group) at 0, 20, 60, and 200 mg/kg/day from day 6 to day 18 of gestation. Maternal toxicity was demonstrated by increased numbers of abortions, body weight loss, lowered food consumption, and by decreased defecation and urination in aborting females and in the survivors at the high dose. Lower body weight gain at the mid dose was a further demonstration of maternal toxicity. The no observed effect level for maternal toxicity was 20 mg/kg/day; the lowest effect level was 60 mg/kg/day.

Developmental toxicity was demonstrated by skeletal variations observed at 200 mg/kg/day. The skeletal variations were 13th full rib, sternebra(e) malaligned, and 27 presacral vertebrae, and these occurred either alone or in combination with each other or accompanied by delayed ossification. The no observed effect level for developmental toxicity was 60 mg/kg/day.

Classification: core- Minimum

showed high background levels in controls.

Tables 8 and 9 from Test Report WIL 21028 show the incidence of malformations in this study. No malformations were reported at the highest dose tested. Malformations occurred in 5 control litters, in 2 low dose litters, and in 3 mid dose litters. [The apparent failure of the test compound to produce malformations at the high dose was most likely due to its effect on the survival of the fetuses. Due to high maternal toxicity (7/18 abortions, 2 total resorptions, and a few nongravid females), only 8 high dose litters survived to final examination, too few to make statistical analysis meaningful, and these 8 would contain the most hardy individuals.] Examination of the mid and low dose groups revealed a dose-related increase in umbilical hernias as compared to controls. This malformation was observed in 2/13 litters (15%) and 2/87 fetuses (2%) at the mid dose and in 1/12 litters (8%) and 1/91 fetuses (1%) at the low dose. The malformation was not observed in 13 control litters with 86 fetuses examined. The highest incidence observed in historical controls was 7% of litters and 1% of fetuses. Although apparently significant at first glance, the incidence of this malformation was not statistically significant when analysed using Fisher's Exact Test at the 0.05 level of significance.

Conclusion: Developmental toxicity (as defined by the Toxicology Branch SEP for Teratology Studies, June, 1985) was demonstrated at the highest dose tested by increased percentage of visceral variations: retrocaval ureter and major blood vessel variation, and by increased number and percentage of skeletal variations: 13th full rib, sternebra(e) malaligned (slight or moderate), and 27 presacral vertebrae. The skeletal effects were apparently related to delayed ossification, according to individual animal data. The NOEL for developmental toxicity for this study was 60 mg/kg/day. No statistically significant dose or compound related increases in malformations over controls were reported.

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DATA EVALUATION REPORT

Study Type: Developmental Toxicity Tox. Chem No.: 470A

Accession Number: 260863 MRID No.: none

Test Material: Iprodione

Synonyms: Rovral®

Study Number: WIL-21028

Sponsor: Rhone-Poulenc, Inc.

Testing Facility: WIL Research Laboratories, Inc.

Title of Report: A Teratology Study in Rabbits with Iprodione

Authors: Dean E. Rodwell, M.S., Study Director

Report Issued: December 12, 1985

Conclusions: Iprodione Technical was administered by gavage to pregnant New Zealand White rabbits (18 females/group) at 0, 20, 60, and 200 mg/kg/day from day 6 to day 18 of gestation. Maternal toxicity was demonstrated by increased numbers of abortions, body weight loss, lowered food consumption, and by decreased defecation and urination in aborting females and in the survivors at the high dose. Lower body weight gain at the mid dose was a further demonstration of maternal toxicity. The no observed effect level for maternal toxicity was 20 mg/kg/day; the lowest effect level was 60 mg/kg/day.

Developmental toxicity was demonstrated by skeletal variations observed at 200 mg/kg/day. The skeletal variations were 13th full rib, sternebra(e) malaligned, and 27 presacral vertebrae, and these occurred either alone or in combination with each other or accompanied by delayed ossification. The no observed effect level for developmental toxicity was 60 mg/kg/day.

Classification: core- Minimum

A. Materials

1. Test compound Iprodione; 3(3,5-dichlorophenyl)-N-

(1-methylethyl) 2,4-dioxo-1-imidazolidinecarboxamide, in two lots:
Lot No. CA 8425101, 95.0% pure, white granular solid, used for dosing;
Lot No. GD 7578, 99.3% pure, white powder, used as a standard for analysis

Vehicle control: Methocel® methylcellulose water soluble gum, Lot No. 820-7112-A, fine white powder

Test material was prepared daily by forming a slurry with 0.5% aqueous Methocel®. Vehicle control (0.5% aqueous methylcellulose) was prepared weekly.

2. Test animals: Species: rabbits; Strain: New Zealand White virgin females; Age: 5 1/2 months at study initiation(7/9/85) Weight: 3.204 - 4.540 kg on day 0 of gestation (day 0 of gestation = study initiation) Source: Hazleton-Dutchland, Inc., Denver, Pa.

B. Study Design

1. Animal Assignment- Animals were assigned in a computer randomized scheme to the following test groups, after 42 days quarantine:

Test Group	Dose by gavage: mg/kg/day in 1 ml/kg volume of 0.5% Methocel®	Main Study period	No. of females
1-vehicle control	0	gestation days 6-18 (13 days total)	18
2-Low	20	"	18
3-Mid	60	"	18
4-High	200	"	18

Test compound was administered by gastric intubation using a 12 gauge stainless steel gavage cannula from day 6 through 18 of gestation (total 13 administrations). Dosages were based on most recent body weight.

2. Animals were fed Purina Certified Rabbit Chow # 5322, allowed approximately 150 g/animal/day during quarantine. Diet and drinking water were allowed ad libitum during the study period.

3. Statistics: Data was analysed for significance using two-tailed tests at the 5% level, as compared to vehicle

controls. Fetal sex ratios-compared using Chi-square test. Numbers of fetuses and litters with malformations and developmental variations-compared using Fisher's Exact Test. Numbers of early and late resorptions, dead fetuses and postimplantation losses-compared using the Mann-Whitney U-test. Mean numbers of corpora lutea, total implantations, viable fetuses, fetal body weights, maternal body weights at each interval, maternal body weight gains, food consumption (g/animal/day, g/kg/day) and gravid uterine weights-compared using a one-way analysis of variance and Dunnett's test.

4. Insemination Procedure and Dosing Schedule: Females were artificially inseminated on three separate days with 0.25 to 0.50 ml. of diluted semen which was previously collected from 5 males of the same strain. Following insemination each female received 100 U.S.P. Units of human chorionic gonadotropin i.v. to ensure ovulation. Diluted semen from each male was used to inseminate an equal number of females in each group. Animals were dosed from day 6-18 of gestation, according to the report.

5. Quality assurance inspections and 8 reports were made between July 9, 1985 through December 9, 1985.

C. Methods and Results:

1. Observations: Table 1 from Test Report WIL 21028 shows the survival and pregnancy status of females in all dose groups. Data on abortions are discussed below, and the remainder are discussed under the section on necropsy and final examinations. Animals were inspected daily from day 0 through 29 of gestation for signs of toxicity and mortality. Females which aborted during the test period were necropsied on the same day and number and location of implantation sites were recorded. Fetuses recovered prior to gestation day 29 were examined and preserved in 10% neutral buffered formalin. Data on females which aborted were not combined with data from females delivered by caesarean section on gestation day 29.

   Results: Seven high dose (200 mg/kg/day) females aborted between gestation days 17 and 23. Prior to aborting all had decreased urination and defecation. One mid dose female (60 mg/kg/day) aborted on gestation day 28. One control group female aborted on day 20. All other females survived to scheduled sacrifice.

Nine high dose females which did not abort, showed decreased urination and/or defecation during treatment. Incidence of these symptoms and others in the mid and low dose groups was comparable to controls.

Conclusion: Maternal toxicity was demonstrated at the high dose in 7/18 abortions.

2. Body Weight: All females were weighed on gestation days 0, 6, 12, 19, 24, and 29. Mean body weights were calculated and mean body weight changes were determined for each interval between weighings and for days 6-19, 19-29, and 0-29. Mean gravid uterine weight, net body weight (day 29 weight minus uterus and contents) and net body weight change (day 0-29 weight change minus uterus and contents) were also measured.

Results: Females at 60 mg/kg/day gained less weight than controls and females at 200 mg/kg/day lost weight during the 6-19 day dosing interval. The loss was significant at 200 mg/kg/day ($p<0.01$) for days 6-19, and overall for days 0-29 ($p<0.05$), as compared to controls. At 60 mg/kg/day, weight gain was significantly less than controls ($p<0.05$) for the predosing interval, days 0-6.

Table A shows the mean weights of interest at term. Mean gravid uterine weight was 10% less than controls at the high dose, most likely due to the 2 total litter resorptions which occurred at this dose. Mean term body weight and mean net body weight were also 10% less than controls at the high dose, indicating that body weights did not catch up to controls between withdrawal of the test compound at 18 days and sacrifice at 29 days.

Table A

Mean Gravid Uterine Weight and Mean Net Maternal Weight in grams*

Dose (mg/kg/day)	Gravid Uterus	Term Body Weight	Net Body Weight	Initial Body Weight	Net Body Weight
0	392	4185	3793	3712	81
20	411	4255	3844	3755	89
60	404	4140	3735	3805	-70
200	353	3772	3419	3716	-297

* All weights are group means.

Conclusion: Maternal toxicity was demonstrated at the high and mid doses in weight loss and lowered weight gain, respectively. The NOEL for this effect is 20 mg/kg/day.

3. Food Consumption: Individual food consumption was recorded daily for days 0-29 of gestation. Food intake was calculated (g/animal/day and g/kg/day).

Results: Food consumption was significantly lower at 200 mg/kg/day during the dosing period (days 6-19). Thereafter, food consumption was 4-6 g/kg/day less than controls. Food consumption at the mid and low doses was similar to controls.

Conclusion: The test compound produced maternal toxicity as reflected in significantly lowered food consumption at the high dose during the dosing period.

4. Necropsy and Final Examinations: After sacrifice with T-61® Euthanasia solution the animals were opened via a ventral mid-line incision. Uterus and ovaries were examined. Uterus and contents were trimmed and weighed and then opened. Numbers and sex of dead and viable fetuses were recorded, along with time of resorption, postimplantation losses, implantation sites, corpora lutea and fetal weights. Each uterus without evidence of implantation was opened and placed in 10% ammonium sulfide to detect early implantation loss (Salewski).

Fetal Examinations: After weighing and external examination, each fetus was sexed internally then examined viscerally (method of Staples) including heart and major vessels. Brains were examined via mid-coronal slice. Skeletons were fixed in 95% isopropyl alcohol then macerated in potassium hydroxide and stained with Alizarin Red S (similar to Dawson technique). Skeletons were examined under low magnification and external, visceral, and skeletal observations were recorded as "developmental variations" or "malformations". Herein, these observations will be referred to as "developmental toxicity".

Results: Table 1 from Test Report WIL 21028 shows the survival and pregnancy status of females in each dose group. Table 7 from Test Report WIL 21028 shows the results of fetal examination at 29 days (Laparotomy). Tables 8, 9, 10, and 11 from Test Report WIL 21028 show the number and percent of fetuses with visceral and skeletal variations and malformations.

Maternal Data: Referring to Table 1, in the high dose group, 2/10 (80%) gravid females who survived to day 29 had total resorption of fetuses, as compared to

1/13 (8%) controls. Since a large number of females in the high dose group aborted prior to scheduled sacrifice, and since post-implantation losses in this group were not significantly different from controls, the high percentage of total resorptions is apparently not compound-related.

Developmental Toxicity: Tables 10 and 11 from Test Report WIL 21028 show the number and percent of fetuses and litters with developmental variations. The following visceral variations occurred in percentages which exceeded the control levels at the high dose:

Retrocaval ureter- percentage of litters: 25% in the high dose compared to 17% in controls

Major blood vessel variation- percentage of litters: 75% in the high dose compared to 67% in controls

The following skeletal variations occurred in numbers and/or percentages which exceeded the control levels at the high dose:

13th full rib- number and percentage of fetuses: 28 (48%) fetuses in 6 (75%) litters at the high dose, compared to 18 (21%) fetuses in 10 (83%) litters in controls; (percentage of fetuses but not litters exceeded controls at the high dose)

Sternebra(e) malaligned (slight or moderate)- number and percentage of of litters and fetuses at all doses, as follows:

Number(%) of Fetuses and Litters With Sternebra(e) Malaligned

	Historical Controls	Controls	Low	Mid	High
Fetuses	64(0-22)	1(1)	7(8)	7(8)	4(7)
Litters	37(0-53)	1(8)	5(42)	4(31)	4(50)

27 presacral vertebrae- number and percentage of litters and fetuses: 13 (22%) fetuses in 5 (63%) litters at the high dose compared to 6 (7%) fetuses in 4 (33%) litters in controls

In 16 fetuses examined at the high dose, the above skeletal variations occurred in combination with each other or with indications of delayed ossification (e.g. sternebrae #5 and/or #6 unossified). This variation