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DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity / Rabbits **GUIDELINE:** 83-3(b)

PC Code: 079801 **Caswell No.** 856 **MRID No.** 422236-01

TEST MATERIAL: Thiram, technical

REGISTRANT: Thiram Task Force I **STUDY IDENTIFICATION:** IRDC 399-121

TESTING LABORATORY: International Research and Development Corp, Michigan.

TITLE OF REPORT: *"DEVELOPMENTAL TOXICITY STUDY IN NEW ZEALAND WHITE RABBITS"*

AUTHOR: Raymond G. York **REPORT DATE:** February 18, 1992

SUMMARY: Inseminated New Zealand White rabbits were given oral doses of thiram [98.26%, technical] at 0, 1, 5, or 10 mg/kg/day during days 7 through 19 of gestation. No maternal toxicity was observed at any dose; the two deaths at 0 mg/kg/day and the single death at 10 mg/kg/day were attributed to gavage error. Treatment had no effect on the pregnancy rate, pre implantation loss, post-implantation loss, resorption rate, fetal viability, fetal sex ratio, and fetal weight. No treatment-related external, visceral, or skeletal malformation or variation was seen in any of the fetuses.

Although the doses used in this study were selected based on two range-finding studies, the HDT [10 mg/kg/day] did not elicit any maternal toxicity. Therefore, the Toxicology Branch II does not consider the dosage to be adequate to assess the potential developmental toxicity of thiram in rabbits. Based on the results of this study, the following NOELs and LOELs are established.

MATERNAL TOXICITY: NOEL = 10 mg/kg/day [HDT]; LOEL = Not established

DEVELOPMENTAL TOXICITY: NOEL = 10 mg/kg/day; LOEL = Not established.

CORE CLASSIFICATION: Supplementary; this study does not satisfy the requirements for a developmental toxicity study in rabbits [83-3 (b)] and is not acceptable for regulatory purposes. However, this study may be upgraded after sumbission and review of the results of the two range-finding studies.

I. OBJECTIVE

The objective of this study was to assess the effects of technical thiram on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

a. Test Material

Identity: Thiram, technical
Lot No.: 117
Purity: 98.26%
Description: White powder

b. Test Animals

Species/Sex: Female rabbits
Strain: New Zealand White
Age on Gestation Day 0: ≈6 months
Weight on Gestation Day 0: 3.2 to 4.2 kg
Identification: Ear tags.
Acclimation Period: 43-days.
Housing: Individually in stainless steel cages
Food: Purina Certified Rabbit Chow #5322 ad libitum.
Water: Tap water ad libitum
Environment: Temperature, 65 ± 3°F; Humidity, 65 ± 15%; Light cycle, 12 hrs/day
Group Assignment: 20 inseminated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Adult females were artificially inseminated with the day of insemination considered Day 0 of gestation. Approximately three weeks prior to insemination, the does were superovulated by an injection of 50 U.S.P units of HCG.

d. Preparation of Dosing Solutions

Appropriate amount of the test material was suspended with the vehicle, 0.5% Tween 80 and 0.5% carboxymethylcellulose. Dose solutions were prepared weekly and stored at room temperature.

e. Analysis of the Dosing Solutions

Dosing solutions were analyzed for homogeneity prior to initiation, for stability on Day 10, and concentration on the first day of dosing and during the middle of the dosing period.

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f. Administration of Test Article

It was reported that the dose levels for this study were determined based on two range-finding studies; only summaries were provided. In the first study [Life Sciences Research 87/TRK003/122], oral administration of thiram to groups of four pregnant rabbits each at 1, 3, 5, 7.5, 10, 20, 40 or 80 mg/kg/day resulted in maternal toxicity at 20, 40 and 80 mg/kg/day. Maternal toxicity was manifested by mortality and body weight loss at 20 mg/kg/day, reduced food and water consumption at 40 and 80 mg/kg/day. Thiram at 20 mg/kg/day caused whole litter resorptions and increased post-implantation losses. At 10 mg/kg/day only a slight body weight loss was observed. In the second range-finding study [Life Science Research, 87/TRK004/541], oral administration of thiram at 1, 2.5 or 5 mg/kg/day to groups of 15 rabbits each resulted in significantly reduced body weight at 5 mg/kg/day . Based on these studies, dose levels selected for the main study were 1, 5 and 10 mg/kg/day.

The test article was administered daily orally via gavage at doses of 1, 5, or 10 mg/kg/day during days 7 through 19 of gestation. The control group received the vehicle only on a comparable regimen. All groups received a dosing volume of 3 mL/kg body weight and the dose volumes were adjusted daily based on individual body weights.

g. Observations

All animals were observed twice daily for mortality and overt changes in appearance and behavior and once daily for clinical signs of toxicity. Individual body weights were obtained on day 0, 7, 13, 20, 24 and 29 of gestation. Individual food consumption was measured daily.

h. Termination

All surviving does were sacrificed on gestation day 29 and gravid uteri were weighed.

i. Cesarean Section

The thoracic, abdominal and pelvic cavities were examined for gross lesions, and in the event of gross lesions, the tissues were preserved in neutral buffered 10% formalin. The uterus was removed from the body, examined externally, weighed and then opened for internal examination. Uteri that appeared to be from nonpregnant rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantations, early and late resorptions, and live and dead fetuses were recorded.

j. Fetal Examinations

Each fetus was removed from the uterus and individually weighed, and observed for gross external alterations. Every fetus was examined to determine sex and soft tissue alterations. Fetuses were then eviscerated, stained with Alizarin red-S, and examined for skeletal alterations.

k. Statistical Analysis

Mean maternal body weights and body weight changes, mean food consumption, mean number of corpora lutea, total implantations, live fetuses and gravid uterine weights were compared by ANOVA, Bartlett's test for homogeneity of variance and the appropriate t-test using Dunnett's multiple comparison tables or pairwise comparisons with a Bonferroni correction to determine significance of differences. Male to female sex ratios and the proportions of litters with malformations were compared using the Chi-square test criterion with Yates correction for 2 x 2 contingency tables and/or Fisher's exact test. The proportions of resorbed and dead fetuses and postimplantation losses were compared by the Kruskal-Wallis test.

l. Regulatory Compliance

A signed statement of No Data Confidentiality Claim was provided that was dated February 20, 1992.

A signed statement dated 2/20/92 indicated that this study was conducted in accordance with the principles of EPA's Good Laboratory Practices [40CR.160].

A signed statement for Potential Adverse Effects, signed and dated February 12, 1992 indicated that this study neither meets nor exceeds any of the applicable criteria stipulated in 40 CFR 158.34.

A Quality Assurance Statement was provided that was dated February 18, 1992.

III. RESULTS

Analysis of the Dosing Solutions

The mean concentrations of test article found were 114%, 112% and 115% of the nominal concentrations for the 1, 5, and 10 mg/kg/day doses, respectively, on day 1 [gestation Day 7] of dosing. The corresponding values on Day 7 of dosing [gestation Day 13] were 104%, 101% and 102%. Homogeneity and stability analyses indicated that thiram was homogenous [mean values ranged from 107 to 108% of target] and stable [95 to 99% recovery] in aqueous solution when stored for 10 days at room temperature.

1. Maternal Toxicity

a. Mortality

Except for the two deaths in the control group on gestation Day 14 and the one death at 10 mg/kg/day on gestation Day 21 which were due to dosing error, no other maternal mortality occurred in this study. Necropsy revealed a scar in the oesophagus and skeletal muscle abscess in the treated doe and fluid and/or fibrin in the thoracic and/or abdominal cavities, discolored skeletal muscle and lung consolidation in the control does.

b. Clinical Signs

No treatment-related clinical signs of toxicity were observed at any dose.

c. Body Weight/Food Consumption

Mean body weights and food consumption of treated does were generally comparable to those of the control does. However, statistically significant increases in mean body weight gain and food consumption were seen in the treated groups when compared to the control values. The increased gain in body weight continued through the overall gestation period and correlated with the increase in food consumption.

d. Macroscopical Examination

No treatment-related macroscopical changes were observed in the does sacrificed at termination.

2. Developmental Toxicity

As shown in Table 1, no treatment related effects were observed in any of the maternal and fetal parameters at any dose level. No treatment-related or statistically significant external, visceral, or skeletal malformations or variations were seen in any of the fetuses; 104, 135, 113, or 129 fetuses at 0, 1, 5 and 105 mg/kg/day, respectively. Fetal malformations and variations summarized in Tables 5 and 6 of the study report are appended [pages 37 and 38] to this DER.

Table 1. Cesarean Section Observations

Observations [Mean ± S.D]	Dose Level [mg/kg/day]			
	0	1	5	10
No. Assigned	20	20	20	20
Females Gravid	19	19	17	19
Maternal Wastage # Died # Aborted # Non pregnant	 2 0 1	 0 0 1	 0 0 3	 1 0 1
Total Corpora Lutea Corpora Lutea/Dam	230 13.5±3.2	223 13.1±2.7	218 12.8±2.4	216 12.7±3.3
Total Implantations Implantations/Dam	121 7.1 ± 3.3	156 8.2 ± 3.1	126 7.4 ± 3.1	144 8.0 ± 2.6
Total Live Fetuses Live Fetuses/Litter	102 6.0 ± 3.0	133 7.0 ± 3.2	113 6.6 ± 3.0	123 6.8 ± 2.7
Total Resorptions Early Late Resorptions/dam	17 8 9 1.0 ± 2.3	21 17 4 1.1 ± 2.1	13 11 2 0.8 ± 1.2	14 10 4 0.8 ± 1.4
No. and % of Litters with Resorptions	6/17 35.2	8/19 42.1	6/17 35.2	6/17 35.2
Pre Implantation Loss [%]	47.4	41.3	42.2	34.7
Post Implantation Loss [%]	15.7	14.7	10.3	14.6
Gravid Uterus Weight [g]	396	471	418	478
Sex Ratio ♂ / ♀	55/49	59/76	57/56	70/59
Fetal Weight [g]	47 ± 8	45 ± 5	47 ± 9	46 ± 6

IV. DISCUSSION

Oral administration of technical thiram at 0, 1, 5, or 10 mg/kg/day to inseminated rabbits during days 7 through 19 of gestation resulted in no maternal or developmental toxicity at the highest dose tested [10 mg/kg dose]. Mortality in the control [2 does] and high-dose groups [1 doe] was due to dosing error. Does in the treated group showed a body weight gain accompanied by increases in food consumption. No treatment-related clinical signs of toxicity were seen at any dose level. Thiram had no adverse effects on the reproductive parameters.

There was an increase in number of fetuses observed with 27 presacral vertebrae at 10 mg/kg/day [33 fetuses/13 litters] when compared to the controls [14 fetuses/6 litters]; however, there was no dose- response [18 fetuses/10 litters at 1 mg/kg/day and 13 fetuses/7 litters at 5 mg/kg/day] and the incidences were within the historical control range of the testing laboratory [1185 fetuses/429 litters]. Therefore, this increase was considered to be unrelated to treatment. No treatment-related external, visceral, or skeletal malformations or variations were seen in any of the fetuses of treated does.

The dose levels for this study were selected based on two range-finding studies. In one study, thiram, at dose greater than 20 mg/kg/day, produced definite maternal toxicity and at 10 mg/kg/day there was a slight decrease in body weight during the dosing period. In the second study, at 5 mg/kg/day, thiram caused significantly reduced body weight during dosing. However, when these two dose levels were employed in the main study, neither produced any adverse effects on body weight. In contrast, does at these doses exhibited significant increases in body weight gain. Although the reason for this inconsistency is not known, it is clear that a dose of 10 mg/kg/day was not adequate to induce any maternal toxicity. Therefore, the entire data from the two range-finding studies [Life Sciences Research 87/TRK003/122 and 87/TRK004/541] must be submitted for Agency review.

V. CONCLUSION

It is evident that the highest dose tested [10 mg/kg/day] was not adequate to induce maternal toxicity. Therefore this study is classified as Core Supplementary but upgradable. Based on the results of this study, the following NOELs and LOELs are established.

Maternal Toxicity **NOEL: 10 mg/kg/day [HDT];** **LOEL: Not established.**

Developmental Toxicity **NOEL: 10 mg/kg/day [HDT];** **LOEL: Not established.**

VI. CORE CLASSIFICATION

Supplementary; this study does not satisfy the requirement for a developmental toxicity study in rabbits [83-3 (b)] and is not acceptable for regulatory purposes. However, this study may be upgraded upon submission and review of the complete results of the two range-finding studies.

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