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THIRAM

Developmental Toxicity §83-3(b)

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DATA EVALUATION RECORD

STUDY TYPE: Range-Finding Developmental Toxicity/Rabbit GUIDELINE: 83-3(b)

DP BARCODE: D217886 SUBMISSION CODE: S491177

P.C. CODE: 079801 TOX. CHEM. No.: 856

TEST MATERIAL: Thiram Technical

CHEMICAL NAME: *Tetramethylthiuram disulphide*

CITATION: Tesh, JM, Ross, FW, Crisp VC (1987). *"THIRAM: EFFECTS OF ORAL ADMINISTRATION UPON PREGNANCY IN THE RABBIT - PRELIMINARY TERATOLOGY STUDY"*. Life Science Research. Study No. 87/TRK003/112. August 20, 1987. MRID No. 40444702. Unpublished.

REGISTRANT: Thiram Task Force II

EXECUTIVE SUMMARY: In a range-finding study (MRID # 40444702), inseminated New Zealand White rabbits were given oral administration of thiram (99.1%) in 0.5% w/v aqueous carboxymethylcellulose mucilage + 0.5% w/v Tween at 0 (vehicle), 1, 3, 5, 7.5, 10, 20, 40 or 80 mg/kg/day during days 6 through 19 of gestation. Thiram at 80 and 40 mg/kg/day, thiram was excessively toxic (all rabbits died). At 20 mg/kg/day one dam died, and two of the three dams that survived exhibited transitional decreases in body weight gain, decreased food intake, and increased fecal retention and water intake. At 10 mg/kg/day, two females lost weight during the early part of the treatment period. At 7.5 mg/kg/day, dams exhibited impaired body weight gain during the majority of the treatment period (Days 0-6 and 6-10). At 5 mg/kg/day dams showed only a slight impairment of maternal body weight gain between days 8 and 10 of gestation. However, due to lack of statistical analysis, the significance of the of the bodyweight data is not clear. Cesarean section showed no treatment-related effects were seen at 1, 3 or 5 mg/kg/day groups. At 7.5 mg/kg/day, one female had a total litter loss, but in the two surviving females post-implantation loss (15.8%) was similar to the controls (20.8%) At 10 mg/kg/day, all females carried their litters to term. There was a slight increase in post-implantation loss (20%) when compared to the concurrent control (4.3%), but the value was similar to the second control (20.8%) and the historical control range (1.0-20.5%; mean,10.3%). At 20 mg/kg/day, two females had total litter loss and there was a marked increase in post-implantation loss (45.5%) when compared to the control (4.3%). No females survived to term at the 40 and 80 mg/kg/day groups. Other litter parameters were unaffected by treatment. Based on these findings, dose levels of selected for the main study were 1, 2.5 or 5 mg/kg/day. This range-finding study is Core classified as Supplementary since it is not designed to fulfill a Subdivision F Guideline requirement.

I. OBJECTIVE

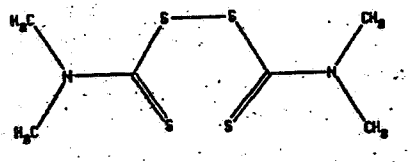
The objective of this range-findings study was to assess the effects of thiram on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis and to establish suitable dose levels for use in the full study.

II. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Thiram, Technical

Description: White powder
Lot/Batch No.: 860410/L
Purity: 99.1%
CAS No.: 137-26-8



2. Vehicle: 0.5% w/v aqueous carboxymethylcellulose mucilage containing 0.5% w/v Tween 80.

3. Test Animals: Species: Rabbits

Strain:	New Zealand White, Sussex, England.
Age at Initiation:	21-27 weeks
Weight at Initiation:	3.36 to 4.49 kg
Identification:	Ear tags.
Acclimation Period:	3 weeks.
Housing:	Individually in stainless steel cages.
Food:	S.O.C Standard Rabbit Diet ad libitum.
Water:	Tap water ad libitum.
Environment:	Temperature, 15-23°C; Humidity, 40-70%; Light cycle, 14 hr. light/10 hr. dark; Air changes, 17-20/hr.

B. PROCEDURES AND STUDY DESIGNS

1. In Life Dates - Start: 7/24/86; End: 12/9/86

2. Mating: Females were artificially inseminated with semen from New Zealand White bucks of established fertility. Following insemination, each female was administered an intravenous injection of 25 i.u of luteinizing hormone ((Profasi, SErnono), to ensure successful ovulation. The day of copulation was considered Day 0 of gestation.

3. Animal Assignment: Animals were assigned, randomly, to dose groups as shown in Table 1.

Table 1. Study Design

Group	Dose [mg/kg/day]	No.of Animals
1	0	4
2	5	4
3	10	4
4	20	4
5	40	4
6	80	4
7[a]	0	4
8[a]	1	4
9[a]	3	4
10[a]	7.5	4

a = Following a high incidence of mortality and/or total litter loss in Groups 4, 5, and 6, four additional groups were added.

4. Dose Selection Rationale: None

5. Dosage Preparation and Analysis of Dosing Solutions: The test material was formulated fresh each day in 0.5% w/v aqueous carboxymethylcellulose mucilage containing 0.5% w/v Tween 80. Concentration analysis was performed on samples taken during the first and last weeks of dosing.

6. Dosage Administration: Thiram was administered once daily orally via gavage from Day 6 to Day 19 inclusive of gestation at a volume-dosage of 5 ml/kg. The control group received the vehicle 0.5% w/v aqueous carboxymethylcellulose mucilage containing 0.5% w/v Tween 80 at the same volume-dosage during the same treatment period. Individual dosages were based on body weights obtained on the day of ddsing.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: All animals were observed daily for mortality and clinical signs of toxicity. Body weights were taken daily. Water intake was recorded for each animal during days 1-5; 6-12; 13-19; 20-23; and 24-28 inclusive. All surviving dams were sacrificed on gestation day 29. The abdominal and thoracic cavities were examined and the fetuses delivered by C-section. The numbers of viable and nonviable fetuses, early and late resorptions, total implantations, corpora lutea, and the fetal body weights were recorded. Females not surviving until the scheduled sacrifice were necropsied in an attempt to determine the cause of death.

2. Fetal Examinations: Weights of individual placentae and the fetuses were recorded.

D. DATA ANALYSIS

The small samples size precluded meaningful statistical evaluation. The significance of intergroup differences was assessed by reference to control data previously recorded in these laboratories.

E. Regulatory Compliances

Signed and dated Data Confidentiality, GLP, and Quality Assurance were provided.

III. RESULTS

A. Maternal Toxicity

1. Mortality: Treatment-related mortality (deaths or sacrificed *in extremis*) resulted in 1 dam at 20 mg/kg/day, 3 dams at 40 mg/kg/day and in all 4 dams at 80 mg/kg/day. These dams showed severe weight loss during treatment. A further two deaths, one in each of control and 40 mg/kg/day were not attributed to treatment.

2. Body Weight: No statistical analysis of the body weight data were performed. Body weight gain data are presented in Table 2. Dams at 1, 3, 5, and 7.5 mg/kg/day exhibited slight reduction in their rates of bodyweight gain during the treatment period when compared to controls. Dams at 10 mg/kg/day showed a marked loss of body weight gain during the first four days of treatment; however, they gained weight similar to that of the controls during the reminder of the study. Of the 2 surviving dams at 20 mg/kg/day, one lost weight rapidly throughout the treatment, while the other showed only a slight depression in its rate of bodyweight gain.

Table 2. Maternal Body Weight Gain[a]

Treatment (mg/kg/day)	# of Dams	Days of Gestation						
		0-6	6-8	6-10	6-14	6-18	6-20	6-28
Control	3	0.16	0.03	0.10	0.15	0.27	0.31	0.34
Control	3	0.03	0.13	0.16	0.15	0.12	0.09	0.17
1	3	0.02	0.07	0.07	0.08	0.04	0.05	0.27
3	3	0.11	0.03	0.05	0.05	0.12	0.15	0.17
5	4	0.15	0.03	0.05	0.08	0.16	0.23	0.36
7.5	2	-0.04	0.02	-0.02	0.02	0.04	0.18	0.07
10	2	0.10	-0.12	-0.25	-0.14	-0.05	0.01	0.23
20	1	0.15	0.00	0.00	0.08	0.11	0.05	0.24
40	No females survived to term							
80	No females survived to term							

a= Data extracted from Study Report Page # 24

3. Clinical Signs: No treatment-related clinical signs of toxicity were seen..

4. Water Consumption: Water consumption was markedly reduced in females at 10 mg/kg/day during the first half of the treatment period. No clear effects were seen at 20 mg/kg/day. At 40 and 80 mg/kg/day, water consumption was greatly reduced following commencement of treatment. No meaningful conclusions could be drawn from the other groups due to considerable inter- and intra-group variations.

5. Gross Pathology: No treatment-related effects were seen at necropsy on Day 29.

6. Cesarean Section Data: Cesarean section findings are presented in Table 3. No treatment-related effects were seen at 1, 3 or 5 mg/kg/day groups. At 7.5 mg/kg/day, one female had a total litter loss, but in the two surviving females post-implantation loss (15.8%) was similar to the controls (20.8%) At 10 mg/kg/day, all females carried their litters to term. There was a slight increase in post-implantation loss (20%) when compared to the concurrent control (4.3%), but the value was similar to the second control (20.8%) and the historical control range (1.0-20.5%; mean,10.3%). At 20 mg/kg/day, two females had total litter loss and there was a marked increase in post-implantation loss (45.5%) when compared to the control (4.3%). No females survived to term at the 40 and 80 mg/kg/day groups. Other litter parameters were unaffected by

treatment.

Table 3. Cesarean Section Findings in Pregnant Rabbits Treated with Thiram[a].

Observations	Dose Level [mg/kg/day]							
[Mean ± S.D]	0	0	1	3	5	7.5	10	20
# Assigned	4	4	4	4	4	4	4	4
Pregnancy Rate	3	3	3	3	4	3	2	3
# Nonpregnant	1	1	1	1	0	1	2	1
Maternal Wastage								
# Died pregnant	0	0	0	0	0	0	0	0
# Died nonpregnant	0	1	0	0	0	0	0	1
# Sacrificed pregnant	0	0	0	0	0	0	0	0
# Sacrificed nonpregnant	0	0	0	0	0	0	2	0
# Aborted	0	0	0	0	0	0	0	0
# With total resorptions	0	0	0	0	0	1	0	2
Total Corpora Lutea	27	28	31	33	45	22	26	11
Corpora Lutea/Dam	9	9.3	10.3	11.0	11.3	11.0	13.0	11.0
Total Implantations	23	24	27	27	35	19	25	28
Implantations/Dam	7.7	8.0	9.0	9.0	8.8	9.5	12.5	11.0
Total # of Litters	3	3	3	3	4	3	2	1
Total Live Fetuses	22	19	25	24	35	16	20	6.0
Live Fetuses/Dam	7.3	6.3	8.3	8.0	8.8	8.0	10.0	6.0
Total Dead Fetuses	0	0	0	0	0	0	0	0
Total Resorptions/Dam	0.3	1.7	0.7	1.0	0.0	1.5	2.5	5.0
Early	0.3	1.3	0.3	0.3	0.0	1.0	2.5	3.0
Late	0.0	0.3	0.3	0.7	0.0	0.5	0.0	2.0
Preimplantation Loss (%)	14.0	14.3	12.9	18.2	22.2	13.6	3.6	0.0
Postimplantation Loss (%)	4.3	20.8	7.4	11.1	0	15.8	20.0	45.5
Sex Ratio M/F	15:7	10:9	15:10	7:17	17:18	9:7	12:8	4:2
Mean Fetal Weight	44.0	42.8	43.5	36.8	41.2	37.6	33.6	39.8

a = Data extracted from Study Report Pg #s: 26; 33-36; 43-46 .

B. DEVELOPMENTAL TOXICITY

Range-finding study; developmental toxicity was not completely evaluated.

IV. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

It was concluded that dose levels of thiram for use in the full teratology study in the rabbit should not exceed 5 mg/kg/day.

B. REVIEWER'S DISCUSSION

Thiram induced unequivocal maternal toxicity at doses above 20 mg/kg/day. At 10 mg/kg/day, there was weight loss, increased post-implantation loss and reduced fetal weights. At 7.5 mg/kg/day, there was a decreased bodyweight gain and one with total resorptions along with a slight increase in post-implantation loss and a marginal fetal weight loss. Except for a slight transitional decrease in maternal weight gain from day 8-10 of gestation (which was not analyzed), no maternal toxicity was seen at 5 mg/kg/day. Therefore, it is evident that animals could have tolerated a dose higher than 5 mg/kg/day.

V. CONCLUSION

The highest dose selected for use in the main study (5 mg/kg/day) is not considered to be appropriate. Due to lack of significant maternal toxicity at this dose and minimal maternal toxicity at the next higher dose (7.5 mg/kg/day) it is concluded that animals could have tolerated a higher dose (i.e., somewhere between 7.5 and 20 mg/kg/day). This study is classified Supplementary since it is a range-finding study and is not designed to fulfill a Subdivision F Guideline requirement.