DICAMBA

Developmental Study §83-3(b)

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DATA EVALUATION RECORD

STUDY TYPE: 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 et. al. (1988). *"THIRAM: TERATOLOGY STUDY IN THE RABBIT"*. Life Science Research. Study No. 87/TRK004/541. March 28, 1988. MRID No. 40577301. Unpublished.

REGISTRANT: Thiram Task Force II

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID No. 40577301) impregnated New Zealand White rabbits (15-20/group) were given oral administration of thiram (99.5%, technical) in 0.5% methylcellulose + 0.5% Tween at 0 (vehicle), 1, 2.5, or 5 mg/kg/day during days 6 through 19 of gestation. No treatment-related mortalities or clinical signs were observed at any treatment group. Cesarean section parameters and fetal morphology were comparable between the control and treated groups. There was an unusually poor pattern of body weight gain due, in part, to the performance of few females. Therefore, a "supplementary" control group obtained from a study that was run concurrently was used in data evaluation. When compared to this supplementary control group, dams at 5 mg/kg/day exhibited a minimal and transitional decreases in body weight gain from day 10 to the end of treatment. The lack of maternal and/or developmental effects at the HDT indicate that the high dose was not adequate to assess the developmental toxic potential of thiram.

Based on these findings the maternal and developmental NOEL is 5 mg/kg/day (HDT); a LOEL was not established. Since the HDT was not adequate to assess the developmental toxic potential of thiram, this study is classified as Supplementary and not upgradable.

The complete data base for this Guideline requirement includes a range-finding study, this study, and another full study conducted in 1992. In the 1992 study (based on the results of a range-finding study), impregnated rabbits received thiram at 0, 5, or 10 mg/kg/day during days 7 through 19 of gestation. No maternal or developmental toxicity was seen at the high dose. It was evident that the high dose used (10 mg/kg/day) could have been higher but definitely lower than 20 mg/kg/day due to severe toxicity seen at this dose in the range-finding study. Results of these three studies indicate that the maximum tolerate dose is somewhere between 10 and 20 mg/kg/day. Although it would have been more acceptable to have obtained some signs of toxicity in the rabbits, it appears that the dose-response curve must be very steep. These studies individually are not ideal and are classified as Supplementary. However, when data from the 1992 study is combined with the range-finding study, they are sufficient to evaluate the developmental toxic effects in this species. Therefore, collectively, these studies are classified as **Acceptable and they satisfy the Subdivision F Guideline requirement for a developmental toxicity study in rabbits.**

I. OBJECTIVE

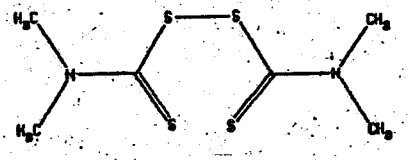
The objective of this study was to assess the effects of thiram on the embryonic and fetal development following oral administration to rabbits during the period of organogenesis.

II. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Thiram, Technical

Description: White powder
Lot/Batch No.: 860410/L
Purity: 99.5%
CAS No.: 137-26-8



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

3. Test Animals: Species: Rabbits

Strain:	New Zealand White, Sussex, England.
Age at Initiation:	19-27 weeks
Weight at Initiation:	3.74 to 4.84 kg
Identification:	Ear tags.
Acclimation Period:	3 weeks.
Housing:	Individually in stainless steel cages.
Food:	S.Q.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: 1/6/87; End: 6/29/87

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
Control	0	18
Low	1	15
Mid	2.5	18
High	5	20
Supplementary Control[a]	0	14

a = This supplementary control group was obtained from a study that was run concurrently. This group was added to assist in interpretation of the results because of poor performance by the concurrent control group (primarily two females).

4. Dose Selection Rationale: Dose levels were selected based on a range-finding study (LSR 87/TRK003/122; MRID # 40444702) in which 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. At 20 mg/kg/day, 1 of 2 dams died and there was transitional decreased body weight gain, decreased food intake, increased fecal retention and water intake, increased early and late resorptions, and increased post implantation loss. The maternal NOEL was 10 mg/kg/day. Based on these findings, the dose levels selected for the main study was 1, 2.5, or 5 mg/kg/day.

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. Homogeneity assay was performed during the range-finding study.

Results: Concentration Analysis (% Target Value)

Dose:	Low	Mid	High
Firs Week:	94%	106%	92%
Last Week:	107%	109%	99%

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 dosing.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: All animals were observed daily for mortality and clinical signs of toxicity. Body weights were taken daily. 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: Each fetus was 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.

D. DATA ANALYSIS

1. Statistical Analysis: Multiple t-test or t-test was used for analysis of body weights, body weight changes, fetal weights, placental weights and litter size. Mann-Whitney U-test was used for corpora lutea, implantation, and resorption counts. X^2-test, Fishers Exact Probability test or Mann-Whitney U-test were used for pre and post implantation losses.

E. Regulatory Compliances

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

III. RESULTS

A. Maternal Toxicity

1. Mortality: No treatment-related deaths were seen. A total of 8 dams died or were sacrificed *in extremis*: 1 from control; 1 from 1 mg/kg/day; 2 from 2.5 mg/kg/day and 4 from 5 mg/kg/day. Necropsy revealed evidence of respiratory or gastro-intestinal tract disorder, or accidental tracheal intubation.

2. Body Weight: Dams in the concurrent control group exhibited poor pattern of bodyweight gain due, in part, to two females. Consequently, supplementary bodyweight data, derived from the Control group of a comparable, concurrent study was added to assist in the interpretation of the results. A table showing individual animal data and a figure were included in the Study Report.

   Body weight gain data are presented in Table 2. The body weight gains of dams at 1 and 2.5 mg/kg/day were superior when compared to concurrent controls but were essentially similar to that of the Supplementary Control group. The body weight gain of dams at 5 mg/kg/day was similar to the concurrent controls but were statistically significantly lower when compared to the Supplementary Controls on Days 6-10, 6-12, 6-14, 6-16 and 6-18.

Table 2. Maternal Body Weight Gain[a]

Treatment	Days of Gestation						
	0-6	6-8	6-10	6-14	6-18	6-20	6-28
Control	0.08	-0.02	-0.03*	0.08*	0.09	0.09*	0.14
1 mg/kg/day	0.11	0.00	0.02	0.14	0.18	0.17	0.19
2.5 mg/kg/day	0.12	0.01	0.01	0.12	0.19	0.23	0.26
5 mg/kg/day	0.11	0.02	-0.03*	0.05**	0.07*	0.11	0.15
Supplementary Control	0.15	0.01	0.05	0.18	0.21	0.23	0.28

a = Data extracted from Study Report Page # 24
* Significantly different from Supplementary Control (* = $p < 0.05$; ** = $p < 0.01$)

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

4. Food Consumption: Not measured.

5. Gross Pathology: No treatment-related gross pathology was seen at necropsy on Day 29.

6. Cesarean Section Data: Cesarean section observations are presented in Table 3. No biologically or statistically significant effects were seen on pregnancy rate, number of corpora lutea, number of implantations, total live fetuses per litter, resorption rates, number and percent of litters with resorptions, fetal sex ratio, or mean fetal body weights.

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

Observations	Dose Level [mg/kg/day]			
[Mean ± S.D]	0	1	2.5	5
# Assigned	18	15	18	20
Pregnancy Rate	12 (72%)	14 (93%)	15 (83%)	17 (85%)
# Nonpregnant	5	1	3	3
Maternal Wastage				
# Died pregnant	0	0	1	2
# Died nonpregnant	0	0	0	0
# Sacrificed pregnant	1	1	1	1
# Sacrificed nonpregnant	0	0	0	1
# Aborted	1	0	0	1
# With total resorptions	0	0	1	1
Total Corpora Lutea	124	154	158	142
Corpora Lutea/Dam	11.3	11.8	12.3	11.4
Total Implantations	108	140	125	124
Implantation/Dam	9.8	10.8	9.7	10.1
Total # of Litters	11	13	12	12
Total Live Fetuses	98	126	99	106
Live Fetuses/Dam	8.9	9.7	8.3	8.8
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam				
Total Resorptions/Dam	0.9	1.1	1.4	1.3
Early	0.1	0.4	0.6	0.1
Late	0.8	0.7	0.8	1.2
Preimplantation Loss (%)	13.6	9.1	21.6	14.2
Postimplantation Loss (%)	9.3	10.0	14.7	12.4
Sex Ratio M/F	50:48	60:66	55:44	62:44
Mean Fetal Weight	38.2	38.2	39.2	37.3

a = Data extracted from Study Report Pg #s: 25; 33-36; 43-46 .

B. DEVELOPMENTAL TOXICITY

No treatment-related external, visceral, or skeletal effects were observed in any of the 98, 126, 99, and 106 fetuses examined from the 11, 13, 12, and 12 litters at vehicle control, 1, 2.5, or 5 mg/kg/day dose groups, respectively. Fetal examinations, presented in Tables 4, 5 and 6 of the Study Report are appended to this DER.

IV. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

Thiram did not cause maternal mortality or clinical signs of toxicity. In view of the atypical performance of the study control group, primarily due to two dams, the results were compared to a "supplementary" control group obtained from a comparable, concurrent study. When compared to this "supplementary" control group, dams at the high dose (5 mg/kg/day) showed a significantly lower body weight gain. Additionally, in the range-finding study used to select the dose level for this study, body weight loss was observed in dams at 5.0 mg/kg/day between Days 6-12 *post coitum* and in dams at 7.5 mg/kg/day between Days 6-18 *post coitum*. Consequently, the possibility of a slight adverse effect upon bodyweight gain in dams at the high dose (5 mg/kg/day) cannot be entirely excluded. Thiram had no adverse effect in any of the other parameters at C-section. There were no biologically meaningful differences in the number of litters with malformations between the control and thiram-treated groups. It was concluded that oral administration of thiram to pregnant rabbits during organogenesis at a dosage of 5 mg/kg/day was associated with a statistically significant impairment of maternal bodyweight performance but survival, growth and morphogenesis *in utero* were unaffected by treatment. At dosages of 1 and 2.5 mg/kg/day, no effects were observed that were attributable to treatment with thiram.

B. REVIEWER'S DISCUSSION

1. Maternal Toxicity: the authors compared the bodyweight gain of the treated groups to that of a "supplementary" control group because of "an unusually poor pattern of bodyweight gain of the concurrent control group". This poor performance was attributed, in particular, to 2 females, Dam #18TK078 and 18TK253 which showed an overall body weight gain of 0.07 kg and -0.04 kg, respectively). However, review of the individual animal data show that 3 other females, Dam # 18TK167, 18TK213 and 18TK523, also showed poor body weight gain; 0.06 kg, -0.34 kg and 0.08 kg, respectively. Also, the mean body weight gain for the concurrent control females was 0.21 kg whereas the value was 0.42 kg for the "supplementary" control group. It is apparent that the while "supplementary" control group "accurately" reflected normal control values, the concurrent controls did not. This may be attributed to the reality that rabbits are finicky and fluctuations in bodyweight, along with food consumption are often encountered due to extraneous factors.
The concurrent control group must be used for evaluation of the study. However,

in this case (study), comparison to a "supplementary" control group was necessary because of an unsatisfactory concurrent control group. This reviewer does not agree with the authors statement that the possibility of a slight adverse effect upon bodyweight gain in dams at the high dose (5 mg/kg/day) cannot be entirely excluded. Even when compared to a "supplementary" control group that had normal body weight values for control dam, the "effect" on bodyweight at 5 mg/kg/day were minimal and transitional. Therefore, it is evident that an MTD was not approached in this study and animals probably could have tolerated a higher dose as confirmed by lack of any maternal toxicity at 5 mg/kg/day.

V. CONCLUSION

The highest dose tested in this study is not adequate to assess the developmental toxic potential of thiram since no maternal or developmental toxicity was seen at this dose level. The maternal and developmental NOEL is > 5 mg/kg/day (HDT); a LOEL was not established. Consequently, this study is classified Supplementary and not upgradable. This study does not satisfy the Subdivision F Guideline requirement for a developmental toxicity study in rabbits [§83-3 b].

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