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

005936

JUN 15 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review a teratology study with Thiram in rats
EPA ID 457281 Tox Proj No. 1008
EPA Accession No. 259810 Caswell No. 856

FROM: Quang Q. Bui, Ph.D. Quang Q Bui 6/12/87
Acting Head, Review Section V
Toxicology Branch/HED (TS-769C)

TO: Ms. Lois Rossi, PM# 21
Registration Division (TS-767C)

THRU: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Action Requested: Review of a teratology study with Thiam in rats
UCB Pharmaceutical Division, Belgium
Report # LE 78L241, dated December 4, 1978

Registrant: UCB Chemicals Corportion
Portsmouth, VA 23703

Recommendation

Under the conditions of this study, it is recommended that this study be classified supplementary data.

A maternal NOEL was established at 12.5 mg/kg (LDT) with decreased food consumption and body weight gain observed at the 25 mg/kg (LEL). A developmental toxicity NOEL could not be established from the submitted data. Delayed skeletal development (reduced skull ossification, absent sternebral ossification center, and increased rudimentary 13th rib) were noted at all dose levels tested (LEL = 12.5 mg/kg). Further, compound - related increases craniofacial defects (hydrocephaly, anophthalmia, microphthalmia) were observed at 25, 50, and 100 mg/kg.

Study UCB # LE78L241, dated 12/4/1978, cannot be upgraded to satisfy the Agency regulatory requirements for a rat teratology study. It is recommended that a new teratology study with thiram in rats be conducted with dose levels lower than 12.5 mg/kg/day. Comments listed on page 3 of the attached DER should also be considered in the design of the new study.

EPA: 68-02-4225
DYNAMAC No. 259-A
June 9, 1987

DATA EVALUATION RECORD

THIRAM

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Giurgea, M. TMTD rat teratology. (Unpublished report No. LE78L241 by the Laboratory of Toxicology of UCB-Pharmaceutical Division, Braine L'Alleud, Belgium, for UCB Chemicals Corp., Portsmouth, VA; dated ~~October 11, 1985.~~) Accession No. 259810.
December 4, 1978 (ORui 6/10/87)

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 6-9-87

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1. CHEMICAL: Thiram; TMTD; DTMT; tetramethylthiuram disulfide; bis-(dimethylthio-carbamoyl)disulfide.

2. TEST MATERIAL: TMTD from batch No. 2333 was reported to contain 99.5% TMTD, [redacted] The material was described as a fine white-grayish powder.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Giurgea, M. TMTD rat teratology. (Unpublished report No. LE78L241 by the Laboratory of Toxicology of UCB-Pharmaceutical Division, Braine L'Alleud, Belgium, for UCB Chemicals Corp., Portsmouth, VA; dated ~~October 11, 1985.~~) Accession No. 259810.
December 4, 1978 (Q Bui 6/10/87)

5. REVIEWED BY:

Michael Narotsky, B.A.
Principal Reviewer
Dynamac Corporation

Signature: M. Narotsky
Date: 6-9-87

Guillermo Millicovsky, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: G Millicovsky
Date: 6-9-87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 6-9-87

Quang Bui, Ph.D.
EPA Reviewer and Section Head

Signature: Quang Bui
Date: 6-10-87

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7. CONCLUSIONS:

A. The NOEL and LOEL for maternal toxicity of TMTD in rats were 12.5 and 25 mg/kg, respectively, based on reduced body weight gains and food consumption at 25, 50, and 100 mg/kg and sedation, nasal bleeding, ocular encrustation, and rough hair coat at 50 and 100 mg/kg.

The NOEL for developmental toxicity could not be established due to increased incidences of skeletal variations, predominantly reduced ossification, at all dose levels tested (i.e., 12.5, 25, 50, and 100 mg/kg) and anophthalmia, microphthalmia, hydrocephaly, postimplantation losses, reduced litter sizes, and reduced fetal weights at 25, 50, and 100 mg/kg. The developmental LOEL for this study was 12.5 mg/kg.

B. This study is classified Core Supplementary.

8. RECOMMENDATIONS:

We recommend that page 3 of the CBI study report, which was missing and included information on materials and methods, be submitted for review. If further work is conducted, we recommend that:

1. The study be conducted at lower dose levels so that a NOEL for developmental toxicity can be determined.

2. Quality assurance measures be reported.

3. Randomization procedures ensuring a homogenous distribution of initial body weights be used.

4. Females be housed one per cage to allow the subsequent exclusion of food consumption data from nonpregnant females.

5. Individual clinical observations be reported.

6. Uteri of all females (including those with gross evidence of implantation) be stained with ammonium sulfide in order to detect sites of very early resorption. Since the results of the present study suggest that preimplantation loss was affected by the compound, this parameter should be carefully evaluated in future studies. Ammonium sulfide staining would better distinguish true preimplantation loss, occurring before treatment, from resorptions occurring during or shortly after implantation.

7. Findings from fetal examinations be reported for individual fetuses.

9. BACKGROUND: The testing laboratory determined that the oral LD_{50} in adult female rats was 998 (663-1264) mg/kg. The slope function was reported to be 1.38 (1.20-1.59). According to the author, published literature indicates that mortality in rats occurs at 200 mg/kg and reduced gestation weight gain occurs at 40 mg/kg. The high dose (100 mg/kg) for the present study represents one-tenth of the LD_{50}.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: TMTD from batch No. 2333 was described as a fine white-grayish powder of 99.5% purity. The test material was mixed in 0.5% aqueous methylcellulose at appropriate concentrations to yield 0 (vehicle control), 12.5, 25, 50, and 100 mg/kg when administered at 2.5 mL/kg body weight.

Additional information regarding test material formulations and analyses was not available in the submitted report; however, page 3 was missing from the section on materials and methods of the CBI study report.

2. Animals and Experimental Design: Twenty-six mated females were assigned to each of seven groups receiving daily doses of 0, 12.5, 25, 50, or 100 mg/kg TMTD, 250 mg/kg aspirin in 0.5% methylcellulose (positive control), or 0.9% sodium chloride in distilled water (saline control). All animals received 2.5 mL/kg of their respective dosing material on gestation days (GD) 6 to 15.

Since the positive- and saline-control groups have relatively little bearing on the assessment of the effects of TMTD, data from these control groups will not be presented in this DER.

Information regarding the animals' age range and source, breeding and randomization procedures, and housing conditions was not present in the submitted report. These details may have been reported on (the missing) page 3 of the CBI study report.

3. Observations and Measurements: Animals were observed daily for mortality and clinical symptoms. Body weights and food consumption were also recorded daily. In addition, total fluid intake was also observed.

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[1]Only items appropriate to this DER have been included.

On GD 20, females were killed and the internal organs were macroscopically examined. The number of corpora lutea was determined for each ovary and the number, type, and location of uterine implantations were recorded. Implantations were recorded as live fetuses, dead fetuses, late resorptions (embryonic tissue visible), or early resorptions (only placenta visible). Live fetuses were weighed and sexed.

Both live and dead fetuses were examined for gross external abnormalities. Approximately one-third of each litter (including dead fetuses) was fixed in Bouin's fluid for free-hand sectioning and visceral examination by Wilson's method. The remaining fetuses were dissected and then fixed in methanol for skeletal staining and examination.

4. Statistical Methods: Each dosage group was compared to the vehicle-control group using two-tailed tests. Body weight gains, food consumption, litter weights, and implantation data were analyzed using the Wilcoxon test. Fetal weights were analyzed using Student's t-test. Fetal examination findings were analyzed using the 2x2 chi-square test.

   All females with evidence of implantation were included in the analyses of body weight gains and food consumption. For implantation data, two sets of means were calculated--one for females with live fetuses on GD 20 and the other for all females with evidence of implantation (including those with fully resorbed litters).

   The study author reported that "mostly the litter was used as the unit for the calculations"; however, the report's summary tables indicate that analyses of fetal weight and fetal examination findings were based on the fetus as the experimental unit.

12. REPORTED RESULTS:

Test Material Analyses: As stated in Section 11 of this DER, information regarding chemical analyses of the test material formulations was not present in the submitted report.

Maternal Data: Three deaths occurred during the study. A pregnant female from the vehicle-control group was euthanized on GD 7; an abscess was present on the left hind foot. A nonpregnant female receiving 25 mg/kg TMTD died on day 9 of presumed pregnancy; antemortem observations were weight loss and dyspnea. At necropsy, the urinary bladder contained sanguinolent fluid. In the 50-mg/kg group, a pregnant female had a "sick appearance" and alopecia; she was euthanized on GD 19.

Clinical signs of dose-related severity were noted at 50 and 100 mg/kg; these included sedation, rough hair coat, nasal bleeding,

and periocular encrustation. No clinical symptoms or changes in general behavior were noted at 0, 12.5, or 25 mg/kg.

Prior to treatment, maternal body weights (not statistically analyzed) were lower and maternal weight gains were significantly greater than controls in the 12.5-mg/kg group (Table 1). Overall weight gains were significantly reduced in a dose-related pattern at the 25-, 50-, and 100-mg/kg dose levels. Dams receiving 100 mg/kg lost weight during the dosing period (GD 6-15).

Food consumption data also showed significant dose-related reductions at 25-, 50-, and 100-mg/kg during the dosing period (Table 2). After the dosing period, the 50- and 100-mg/kg group values remained reduced. The food consumption values for the 12.5-mg/kg group were also significantly reduced; however, values for the 25-mg/kg group were comparable to controls.

Developmental Data: Pregnancy rates were comparable in all groups (Table 3) and no abortions or premature deliveries were noted. Six totally resorbed litters occurred at 100 mg/kg; all other cesarean-delivered litters had at least one live fetus.

Pre- and postimplantation losses were significantly increased in the 100-mg/kg dosage group (Table 4). Correspondingly, litter sizes were significantly decreased and the numbers of resorptions were significantly increased at this level when compared to controls. In addition, preimplantation loss was significantly increased at the 25-mg/kg dose level and litter size was slightly, but significantly, decreased at a dosage of 12.5 mg/kg.

Fetal weights were significantly decreased in a dose-related pattern at 25, 50, and 100 mg/kg (Table 4); however, a significant increase was evident at 12.5 mg/kg when compared to controls. The fetal and litter incidences of fetuses weighing less than 2.6 g were also significantly increased at 100 mg/kg.

Gross external examination of the fetuses revealed one 12.5-mg/kg fetus with a protruding tongue, one 50-mg/kg fetus with a cranial hematoma, and one 25-mg/kg fetus and two 100-mg/kg fetuses (different litters) with mild hydrocephaly (Table 5). In addition, the study author indicated a "general impression" that 100-mg/kg fetuses had a "soft consistency" of the head and a "stunted appearance." These findings were associated with reduced ossification (confirmed at skeletal examination) and reduced fetal weights, respectively.

Examination of visceral sections revealed increased renal pelvic cavitation occurring with comparable incidences in all groups. Other visceral findings occurred only in the dosed groups (Table 5) and with significantly increased frequencies in the 50- and 100-mg/kg groups. Major visceral malformations included internal hydrocephaly at 25 and 100 mg/kg, anophthalmia/microphthalmia in all thiram-dosed groups, diaphragmatic hernia at 100 mg/kg, unilateral renal agenesis at 12.5 and 100 mg/kg, cleft palate at 12.5 mg/kg, and atrophy of the left lung at 50 mg/kg.

TABLE 1. Mean Maternal Body Weight Data of Rats Gavaged with TMTD

Dose Level (mg/kg)	Body Weight (g) on Gestation Day					
	0	6	9	12	15	20
0	235	257	263	283	298	366
12.5	202	230	237	253	269	332
25	240	263	262	272	288	354
50	228	254	241	247	259	316
100	226	246	236	230	231	264

Dose Level (mg/kg)	Body Weight Gain (g) on Gestation Days				Litter Weight (g)
	0-6	6-15	15-20	0-20	
0	22	42	68	132	49
12.5	28*	39	63	131	45
25	23	25**	67	114**	42*
50	25	5**	58**	88**	39**
100	21	-15**	33**	39**	25**

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

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TABLE 2. Mean Maternal Food Consumption (g/rat/day) of Rats Gavaged with TMTD

Dose Level (mg/kg)	Gestation Days		
	0-5	6-15	16-20
0	23	24	33
12.5	24	23	29**
25	25	20**	32
50	25	15**	24**
100	24	13**	18**

**Significantly different from control value ($p < 0.01$).

TABLE 3. Summary of Mating Results of Rats Gavaged with TMTD

Dose Level (mg/kg)	No. of Females				
				Pregnant on day 20	
	Mated	Dying	Not pregnant	With only resorptions	With live fetuses
0	26	1	4	0	21
12.5	26	0	0	0	26
25	26	1[a]	4[a]	0	22
50	26	1	4	0	21
100	26	0	4	6	16

[a]A nonpregnant female that died is tabulated as both "dying" and "not pregnant."

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TABLE 4. Summary Uterine Findings and Fetal Weights of Rats Gavaged with TMTD

Dose Level (mg/kg)	No. of litters	Mean Per Litter						
		Corpora lutea	Implantations	Resorptions and dead fetuses	Live fetuses	Percent loss		Mean fetal weight (g) per group
						Preimplantation	Postimplantation	
0	21	14.6	13.7	0.6	13.1	4.8	4.4	3.72
12.5	26	14.0	12.8	1.0	11.8*	6.7	8.2	3.83***
25	22	16.6	12.7	0.6	12.1	21.0*	4.8	3.46***
50	21	14.3	12.5	0.2	12.3	12.0	2.0	3.21***
100	22	14.1	12.3	6.3**	6.5**	12.8*	46.4**	--
100[a]	16	14.2	12.1	3.8**	8.9**	14.6*	26.4*	2.81***

[a]Second set of means for the 100-mg/kg group includes only litters with live fetuses.

*Signficantly different from control value ($p < 0.05$).

**Signficantly different from control value ($p < 0.01$).

***Signficantly different from control value ($p < 0.001$).

TABLE 5. Summary of Gross External Observations and Visceral Malformations in Fetuses from Rats Gavaged with TMTD

Dose Level (mg/kg)	Litter ID No.	Fetuses Affected/Examined	Observations
0	--	--	--
12.5	174	1/10[a]	Protruding tongue[b]
		1/3	Cleft palate
			Microphthalmia
			Unilateral renal agenesis
25	131	1/3	Microphthalmia
	138	1/14	Hydrocephaly[b]
	139	1/4	Microphthalmia
		1/4	Anophthalmia
			Hydrocephaly
50	107	2/4	Microphthalmia
	108	1/4	Microphthalmia
	112	1/5	Anophthalmia
	116	1/5	Microphthalmia
	120	1/3	Microphthalmia
	122	1/5	Microphthalmia
	129	1/15[a]	Cranial hematoma[b]
		1/5	Left lung atrophy
100	90	1/3	Unilateral renal agenesis
	91	1/1	Internal hydrocephaly
	92	1/4	Microphthalmia
	94	1/1	Hydrocephaly[b]
			Internal hydrocephaly
			Diaphragmatic hernia
	95	1/11[a]	Hydrocephaly[b]
		1/3	Microphthalmia
	96	1/1	Internal hydrocephaly
	100	1/3	Microphthalmia
	101	1/1	Microphthalmia

[a]The study author did not indicate if gross external and visceral findings noted in the same litter were observed in the same fetus.

[b]Observed at gross external examination (all other findings were observed at visceral examination).

Skeletal examinations revealed increased incidences of findings consistent with delayed development at all TMTD dose levels (Table 6). These findings were noted in the skull, sternum, ribs, vertebrae, and metacarpals. In general, fetuses from the 100-mg/kg group were most frequently affected; however, significant increases were also evident at 12.5, 25, and 50 mg/kg for wider cranial suture, incomplete development/reduced ossification of skull, rudimentary 13th rib, and absent sternebral ossification center.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that 12.5 mg/kg TMTD did not affect any maternal or litter parameters. Effects on litter parameters and litter and fetal weights were noted by the author at 25, 50, and 100 mg/kg and were associated with maternal effects at these dose levels.

B. No quality assurance measures were reported.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material Analyses: We were unable to assess the adequacy of the test material formulations for this study since chemical analytical procedures and results were not available for review. It is unclear whether the necessary information was reported on CBI page 3; the page was not submitted with the rest of the report.

Maternal Data: Mortality occurred with low frequency and in a nondose-related pattern. We regard the deaths to be incidental.

We assess that the sedation, rough hair coat, and other clinical observations were indicative of maternal toxicity at 50 and 100 mg/kg. We consider the reductions in body weight gain (or actual weight loss) during the dosing period to reflect maternal toxicity at 25, 50, and 100 mg/kg. Reduced weight gains for the entire gestation period also reflect maternal toxicity at these dose levels; reductions in litter weight (Table 1) did not fully account for these reductions in weight gain.

Food consumption data were generally consistent with the body weight data and support our assessment that the compound was toxic to the dams at 25, 50, and 100 mg/kg. The slight, but significant, reduction after treatment at 12.5 mg/kg did not occur in a dose-related pattern; we regard this finding to be incidental.

Developmental Data: Pregnancy rates did not suggest a compound-related effect; however, the increased preimplantation losses, reaching significance at 25 and 100 mg/kg, suggested a compound effect during implantation or shortly thereafter at 25, 50, and 100 mg/kg. We consider the increased postimplantation losses, total-litter resorptions, and reduced litter sizes to indicate toxicity at 100 mg/kg.

TABLE 6. Percent of Fetuses (Litters[a]) Affected with Selected Skeletal Observation of Rats Gavaged with TMTD

Dose Level (mg/kg)	Incomplete development, reduced skull ossification	Absent sternebral ossification center	Rudimentary 13th rib	Bipartite vertebra
0	6.5 (42.9)	6.5 (28.6)	10.2 (33.3)	1.1 (9.5)
12.5	23.7***(65.4)	18.6***(57.7)	18.6* (65.4)	4.2 (26.9)
25	21.0***(72.7)	29.7***(86.4)	18.4* (54.5)	2.2 (18.2)
50	21.9***(76.2)	34.3***(85.7)	16.3 (52.4)	6.2*[b]
100	30.0***(60.0)	66.0***(93.3)	28.0***(60.0)	19.0***(80.0)

[a]Calculated by the reviewers.

[b]No. of litters affected was not reported by the study author.

*Signficantly different from control value ($p < 0.05$).

***Signficantly different from control value ($p < 0.001$).

We attribute the slight, but significant, reduction in the litter sizes of the 12.5-mg/kg group to slight increases in both pre- and postimplantation losses. Since litter sizes at 25 and 50 mg/kg were comparable to controls, we regard the reduction at 12.5 mg/kg to be incidental.

We consider the reduced fetal weights at 25, 50, and 100 mg/kg to be compound related. We attribute the increased fetal weights at 12.5 mg/kg to the reduced litter sizes in this group.

We regard the significantly increased incidences of skeletal variations to indicate delayed skeletal development at 12.5, 25, 50, and 100 mg/kg. In addition, the increased incidences of craniofacial defects (particularly anophthalmia/microphthalmia and hydrocephaly) indicate compound effects at 25, 50, and 100 mg/kg. The combined litter incidences (compiled by reviewers) of craniofacial defects were 0/21, 1/26 (3.8%), 3/22 (13.6%) 7/21 (33.3%), and 7/16 (43.8%) for the control and 12.5-, 25-, 50-, and 100-mg/kg groups, respectively. The fetal and litter incidences of major visceral malformations reached significance at 50- and 100-mg/kg. We consider the single 12.5-mg/kg litter with malformations to be incidental.

B. We concur with the study author that maternal toxicity was evident at 25, 50, and 100 mg/kg; however, we do not concur with the study author's interpretation regarding the developmental data. Whereas the author cited no effects at 12.5 mg/kg, we assess that the significantly increased incidences of reduced ossification demonstrate developmental toxicity at this level.

C. The reviewers noted the following deficiencies in the procedures and reporting of this study:

1. Page 3 was missing from the submitted report. It cannot be determined whether this page includes all the needed information regarding the animals, housing conditions, and the formulations and administration of the test material.

2. No quality assurance measures were documented in the report.

3. Individual food consumption values were generally reported in identical pairs, suggesting that the animals were housed in pairs (housing conditions were not described in the submitted report; see Section C.1, above.) Although we assess that this did not substantially alter the results, the data on nonpregnant females could not be accurately deleted from the analyses of food consumption.

4. Individual data for clinical observations were not reported.

5. The fetal examination data were not itemized according to positive findings for each fetus. This precluded the pooling of findings into different classifications or the correlating of gross findings with skeletal or visceral findings.

6. The body weights of females receiving 12.5 mg/kg were notably less than controls on GD 0. In view of the developmental effects observed at this dose level, we do not believe that this deficiency affected the overall interpretation of this study; however, rerandomization or a weight-stratified randomization procedure would have avoided the initial disparity observed between groups.

7. Numerous data discrepancies were present on Tables VII and IX of the study report. In particular, one fetus with protruding tongue was reported in the 12.5-mg/kg group; however, the proportions of fetuses and litters affected were both tabulated as 0%.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 4-7.

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APPENDIX A

Materials and Methods

THIRAM

Page_____ is not included in this copy.

Pages 19 through 22 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.