42313401

PRIMARY REVIEWER: SanYvette Williams, D.V.M. 12-22-93
Section IV, Toxicology Branch II (H7509C)

SECONDARY REVIEWER: Jess Rowland, M.S., Acting Section Head
Section IV, Toxicology Branch II (H7509C) 12/22/93

DATA EVALUATION REPORT

STUDY TYPE: Carcinogenicity Study in Mice. **GUIDELINE: 83-2[b]**

MRID NO.: 423134-01 **PC CODE: 079801**

TEST MATERIAL: Thiram

TITLE: *"ONCOGENICITY STUDY IN MICE WITH THIRAM"*

AUTHOR: Janet A. Trutter, M.S., D.A.B.T

LAB PROJECT ID NO: 798-223

TESTING LABORATORY: Hazleton Washington, Inc.

SPONSOR: Thiram Task Force II, Uniroyal Chemical Company, Inc.

REPORT DATE: 03/13/1992

EXECUTIVE SUMMARY: Thiram was administration in the diet of CD-1 mice at dose levels of 0, 15, 150, or 300 ppm for males [50/sex/dose], and 0, 15, 300 or 600 ppm for females [50/sex/dose] CD-1 for 97 weeks.

Based on the decreases in mean body weight, mean body weight gain, anemia, and non-neoplastic lesions in the eyes, non-glandular stomach, and urinary bladder the LOEL for systemic toxicity is 150 ppm [24 mg/kg/day] in males and 300 ppm [57 mg/kg/day] in females. The NOEL is 15 ppm in both sexes [2.5 mg/kg/day in males and 3.1 mg/kg/day in females]. It is concluded that the highest dose tested in this study [300 ppm in males and 600 ppm in females] was adequate to assess the carcinogenic potential of Thiram. Thiram was non carcinogenic in both sexes of mice at the dose levels tested.

CORE CLASSIFICATION: Minimum; this study satisfies guideline requirements [83-2b] for a carcinogenicity study in mice and is acceptable for regulatory purposes.

I. INTRODUCTION

This Data Evaluation Report summarizes the findings of a study designed to evaluate the carcinogenicity of Thiram following dietary administration to mice. It was originally designed for 78 weeks, but due to high survival rates was extended to 97 weeks.

II. MATERIALS AND METHODS

1. Test Substance

Test Chemical Name: Thiram
Purity: **Technical, 97.6%** at initiation; assumed to be 100% for the purposes of this study.
Lot No.: 117
Description: Off-white, lumpy powder

2. Test Animals

Species: Mice
Strain: Crl:CD-1 (ICR)BR
Source: Charles River Labs, Raleigh, NC
Sex: Males and females
Age at Initiation: 7.5 weeks
Weight at Initiation: 27.8 ± 2.2 g (♂); 21.2 ± 1.7 g (♀)
Identification: Tail tattoo
Acclimation: 24 days
Health Status: Good
Housing: Individually housed in stainless steel cages.
Food: Purina Certified Rodent Laboratory Chow
Water: Tap water ad libitum
Environment: Temperature - 72 ± 6°F; Humidity - 50.% ± 20; Light/dark cycle-12 hrs.

3. Test Substance Formulation and Analyses

Fresh diets were prepared weekly and no adjustment for purity was made, i.e. 100% a.i. was assumed for dosing calculations. Prior to initiation of treatment, diet mixes [at concentrations ranging from 15-300 ppm] were prepared in order to assess the homogeneity and stability of technical Thiram in the basal feed. [Prior assays for stability showed that Thiram binds to the basal diet to a degree that freezing of the formulated diets and daily feeding was done to minimize the potential for binding to occur.]

Routine assays for test diet concentration were performed at Weeks 1, 2, 3, 4, and monthly [either 4- or 5-week intervals] through the in-life phase of the study.

III. STUDY DESIGN

1. Treatment

Mice were fed the Thiram diets 7 days per week for a period of 97 weeks. Control animals received standard laboratory diet on the same schedule.

Table 1. Dietary Levels of Thiram

Group	Dose Level (ppm)		Number of Animals	
	Male	Female	Male	Female
Control	0	0	50	50
Low-Dose	15	15	50	50
Mid-Dose	150	300	50	50
High-Dose	300	600	50	50

2. Observations:

Mortality/moribundity checks and cageside observations for clinical signs of toxicity were performed twice daily. Daily cageside observations were made to determine signs of toxic effects and/or general condition of test mice. Detailed physical examinations for signs of local or systemic toxicity, pharmacologic effects and palpation for tissue masses were conducted twice a week.

3. Body Weight and Food Consumption:

Individual body weights were recorded at randomization, Week 0 and weekly through Week 14, once a week every fourth week through Week 94, and during Week 97.

Individual food consumption measurements were made daily through Week 14, then daily for a 1 week period every fourth week through Week 94.

4. Clinical Pathology:

After Week 53 (interim) and 97 (termination), blood samples were taken from the first 10 surviving mice (orbital sinus bleeding) and the following checked (x) clinical pathology parameters were determined. Samples from the vena cava were used to determine white blood cell differentials and cell morphology.

x Hematocrit (HCT)	x Leukocyte count (WBC)
x Hemoglobin (HGB)	x Platelet count
x Erythrocyte count (RBC)	x Leukocyte differential
x Mean corpuscular HGB (MCH)	x Mean corpuscular HGB Concentration (MCHC)
x Mean corpuscular volume (MCV)	x Cell morphology
Corrected leukocyte count (COR WBC)	x Reticulocyte count (if anemia was seen)

5. Sacrifice and Pathology

A complete gross postmortem examination was performed on these animals as well as on animals dying spontaneously, accidentally, and sacrificed in a moribund condition. Postmortem procedures included: examination of the external surface; all orifices; the cranial cavity; carcass; the external and sectioned surfaces of the brain and spinal cord; nasal cavity and paranasal sinuses; the thoracic, abdominal and pelvic cavities and their viscera and the cervical tissue. **Organs weighed from animals (from the first 10 animals/sex/group sacrificed at termination) were: brain, kidneys, liver (with gallbladder), testes (with epididymides) and ovaries.** Organ-to terminal-body weight ratios and organ-to-brain-weight ratios were also determined.

6. Histopathology

The checked (X) tissues from **all animals** were trimmed and processed for histopathological evaluation.

Digestive system	Respiratory System
x Salivary glands[a]	x Trachea[a]
x Esophagus[a]	x Lung[a]
x Stomach	Pharynx[e]
x Duodenum[a]	Larynx[e]
x Jejunum[a]	Nose[e]
x Cecum[a]	x Head [3 coronal sections]
x Colon[a]	
x Ileum[a]	
x Rectum[a]	Cardiovascular/Hemo.System
x Liver[ac]	
x Pancreas[a]	Aorta (thoracic)[a]
x Gall bladder[ab]	x Heart[a]
	x Bone marrow[a]
Neurological System	x Lymph nodes[a]
	x Spleen[a]
x Brain[ac]	x Thymus[a]
x Pituitary[a]	
x Sciatic nerve[a]	Urinogenital System
x Spinal cord (3 levels)[ab]	
x Eyes (optical nerve)[ab]	x Kidneys[ac]
	x Urinary bladder[a]
Glandular System	x Testes[ac]
	x Epididymides
x Adrenals[a]	x Prostate
Lacrimal glands	x Seminal vesicles
x Parathyroids[a]	x Uterus[a]
x Thyroids[a]	x Ovaries[ac]
	Others
	x Skin
	x Mammary glands
	x All gross lesions and masses
	x Skeletal muscle[a]
	x Bone (sternum and femur)

a. Required for subchronic and chronic studies.
b. In subchronic studies examined only if indicated by toxicity or target organ involvement.
c. Organ weights required in subchronic and chronic studies.
d. Organ weights required for nonrodent studies.
e. Required for chronic inhalation study.

9. Statistical Analyses

Body weight, body weight gain, food consumption, feed efficiency, hematology, clinical chemistry, and organ weight, were analyzed using appropriate statistical methods. Analysis were performed in the following order: 1) Bartlett's test for homogeneity of variance was performed if the variance proved to be homogenous, the data were analyzed by one-way classification analysis of variance [ANOVA], 2) If the variances proved to be heterogenous, $\log_{10}$ transformation of the data was performed which was followed by Bartlett's test and ANOVA, 3) If the $\log_{10}$ transformation was ineffective in removing variance heterogenicity, ANOVA of the untransformed data was completed, 4) In the case of significant overall variation as indicated by ANOVA of homogenous data, the Scheffe multiple comparison procedure was used for control vs. compound-treated groups mean comparisons, 5) In the case of significant overall variation as indicated by ANOVA of heterogenous data, the Games and Howel modification of the Tukey-Kramer honestly significant difference test was used.

In addition to the above data, cumulative survival data were analyzed using the National Cancer Institute Package. The Cochran-Armitage test for linear trend and Fisher's "exact" test were performed on selected gross pathology and cataracts and retinal degeneration. If a significant trend was observed, Fisher's "exact" test was evaluated at one-tailed level, two-tailed otherwise. All analyses were evaluated at the 5% and 1% significance level.

10. Regulatory Compliances:

A signed statement of No Data Confidentiality Claim was provided that was dated March 23, 1992.

A signed statement dated 3/23/92 indicated that this study was conducted according to the principles of EPA's Good Laboratory Practice [40 CFR.160].

A statement for Potential Adverse Effects, signed and dated 3/12/92, indicated that this study neither meets nor exceeds any of the applicable criteria stipulated in 40 CFR 158.34.

A Quality Assurance Statement was signed and dated 3/10/92.

III. RESULTS

1. Analyses of Test Diets

The overall mean thiram dietary concentration and percent target values tabulated below show that the values were within the acceptable (± 10%) range. Stability analyses showed that thiram was stable in the diet for up to 7 days.

Table 2. Dietary Concentration of Thiram

Parameter Sample size 25/dose	Group 2 15 ppm M / F	Group 3 150 ppm Males	Group 3 300 ppm Females	Group 4 300 ppm Males	Group 4 300 ppm Females
Mean Concentration[a]	13	148	302	302	606
Mean % Target	88	99	101	101	101

2. Survival

There were no compound-related adverse effects on survival. The duration of this study was extended from 78 to 97 weeks, based on a criterion of 50% survival in a group for termination. Survival rates at termination were 60, 50, 64 or 58% for males at 0, 15, 150 or 300 ppm, respectively. Survival rates were 60, 57, 58 or 54% for females at 0, 15, 300, or 600 ppm, respectively.

Table 3. Adjusted Survival in Mice Fed Thiram for 97 Weeks.

Week	Male (ppm)				Female (ppm)			
	0	15	150	300	0	15	300	600
1	50/50	50/50	50/50	50/50	50/50	50/50	50/50	50/50
22	49/50	50/50	50/50	48/50	50/50	49/49	49/49	46/48
50	48/50	46/50	49/50	48/50	48/50	48/49	46/49	44/48
78	42/50	34/50	40/50	48/50	37/50	37/49	39/48	36/48
97	30/50	25/50	32/50	29/50	30/50	28/49	28/48	26/48
% Survival at Week 97	60	50	64	58	60	57	58	54

3. Clinical Observations

Except for skin lesions observed in both the control and treated mice, there were no treatment-related clinical signs of toxicity. The skin lesions were described as sores or reddened areas of the ears, head, and various body areas, consistent with bacterial dermatitis. These lesions were observed in males at the 300 ppm throughout the study and in females at the 600 ppm primarily during the later part of the study. The incidences are presented in Table 4.

Table 4. Clinical Observations: Sores Red and Swollen

Weeks	Males				Females			
	0	15	150	300	0	15	300	600
0	0	1	0	0	0	0	0	0
20	8	13	11	10	1	2	3	2
38	5	9	10	11	1	0	2	2
57	5	9	5	10	1	1	5	4
72	10	11	13	18	7	6	7	16
87	6	7	8	11	4	5	8	13

4. Body Weights and Body Weight Change

The mean body weight and body weight change data of both sexes of mice at 15 ppm were similar to control values. Statistically significant and dose-related decreases relative to control were seen in both sexes at the higher doses. These findings were seen consistently, beginning at Week 4 for males and at Week 5 for females.

Mean body weight changes are tabulated in Table 6. No treatment-related effects were seen at 15 ppm. At the higher doses, decreases in mean body weight gains were observed throughout the study in males, while it was apparent beginning at Weeks 0-13 in females. Mean body weight gain was significantly ($p<0.05$) decreased in both sexes of mice of the high-dose at 1-year and at termination. In males, body weight was 90% of controls at 1-year and 85% at termination. In females the values were 88% and 81% at 1-year and termination, respectively.

Table 5. Mean Body Weights [G] in Mice Fed Thiram for 97 Weeks.

Weeks	Males				Females			
ppm	0	15	150	300	0	15	300	600
1	32	31	31	32	24	24	24	24
4	34	33	32*	32*	27	27	26	26
8	35	35	33*	32*	28	28	27*	27*
13	36	36	34*	34*	29	29	27*	27*
26	39	38	36*	35*	31	31	28*	28*
42	40	38	36*	25*	33	32	29*	29*
54	39	38	36*	35*	33	33	29*	29*
66	39	39	37*	35*	34	34	29*	29*
74	38	38	37*	35*	33	32	29*	28*
86	39	39	37*	34*	33	33	30*	28*
97	38	37	36*	33*	33	34	29*	27*

Significantly different from controls at p <0.05 [*].

Table 6. Mean Body Weight Change [G] in Mice Fed Thiram for 97 Weeks.

Weeks	Males				Females			
	0	15	150	300	0	15	300	600
0- 4	3.7	3.6	2.7*	2.4*	3.8	3.6	3.6	3.4
0-13	6.1	6.1	4.5*	3.9*	6.1	6.3	4.8*	4.7*
0-26	8.7	8.4	6.2*	5.1*	8.2	7.8	5.6*	5.5*
0-50	9.1	8.7	6.5*	5.4*	10.4	10.3	6.4*	6.8*
0-78	8.8	9.1	6.9*	4.8*	11.3	9.7	6.5*	5.8*
0-97	8.5	7.7	6.1*	3.2*	10.8	10.7	5.9*	4.4*

Significantly different from controls at p <0.05 [*].

5. **Food Consumption, Food Efficiency, and Compound Consumption**

Food consumption was similar between controls and Group 2. In Group 3 and 4, these values reflected the decreases seen in the body weight data. Statistical evaluation of total food consumption [Weeks 1-4, 1-13, 1-26, 1-50, 1-78, and 1-94) indicated a significant negative trend and decreased mean values in the mid-and high-dose groups for all intervals analyzed.

Food efficiency varied across all groups and there were no marked differences in mean body weight gains relative to food intake between control and test groups.

Mean compound consumption estimates through Week 94 show that the overall compound intake was 2.5 and 3.1 mg/kg/day for low dose males and females, respectively, 24 and 57 mg/kg/day for the mid-dose males and females, respectively, and 50 and 112 mg/kg/day for the high-dose males and females, respectively.

6. **Clinical Pathology**

At Week 97, mean values of MCV and MCH were significantly increased ($p<0.05$) in males at 150 ppm while those of MCHC were decreased at 150 or 300 ppm. In females, RBC was decreased at 300 or 600 ppm, HGB and HCT were decreased at 600 ppm and platelets counts were increased at 300 or 600 ppm. All of these changes were significantly different when compared to controls.

Table 7. Hematology Findings in Females Treated with Thiram

Parameter	0 ppm	15 ppm	300 ppm	600 ppm
RBC (mI/uL)	9.48	9.16	8.73*	8.46*
Hgb (g/dl)	14.6	14.3	13.9	13.4*
HCT (%)	42.9	42.2	40.8	39.6*

$p < 0.05$

7. **Gross pathology:**

No treatment-related gross pathology was seen. Frequently observed findings in both control and treated animals included an enlarged spleen, liver, or kidneys; pale kidneys; dark area(s) on the stomach; enlarged mesenteric lymph nodes; distended urinary bladder (mainly males); enlarged seminal vesicles; cystic ovaries and uterus; alopecia; and sores in

various body areas, mainly on the ears. Frequently seen at terminal sacrifice were lung masses; splenomegaly, liver masses (males mainly); thickened stomach mucosa; enlarged seminal vesicles; and cysts, masses, and/or distensions in the ovaries and uterus. Alopecia, sores and darkened ears were also noted.

8. Organ Weights

No treatment-related effects were observed in any of the organ weight parameters. The statistically significant decrease in absolute brain weight in females at 300 or 600 ppm was attributed to lower terminal weights. Similarly, the increases in both absolute and relative ovarian weight in females at 15 ppm were attributed to cystic ovaries in the mice.

9. Histopathology

(i) Non-neoplastic Lesions

Treatment-related non-neoplastic lesions, present in the eyes, non-glandular stomach and urinary bladder, spleen, and skin are presented in Table 8. The treatment-related findings were most remarkable, both in incidence and severity at the high-dose.

Retinal atrophy was characterized by a decrease to an absence of the outer nuclear cell layer of the retina. The lesion was not specifically associated with cataracts.

Hyperkeratosis of the non-glandular stomach was characterized by minimal to moderate increases in keratin in the nonglandular stomach, suggesting a mild irritant effect of Thiram in this region.

Intracytoplasmic protein-like droplets in the epithelium of the urinary bladder were observed in males at 150 and 300 ppm and in females at 300 and 600 ppm of thiram.

Increases in hemosiderin-laden spleen cells (representing increased splenic red blood cell turnover) was seen only in females at 300 and 600 ppm. This finding corresponds with the decreased RBC mass [erythrocyte count, hemoglobin, and/or hematocrit] in females at 600 ppm of Thiram.

Skin lesions included necrosis and/or suppurative inflammation of both treated and non-treated animals which was most severe in the mid- and high-dose animals and was consistent with a staphylococcal dermatitis.

Table 8. Incidence and Severity of Mice with Non-neoplastic Lesions

	Males				Females			
No. Examined: 50/Sex/Dose	0 ppm	15 ppm	150 ppm	300 ppm	0 ppm	15 ppm	300 ppm	600 ppm
	Eyes: Retinal atrophy							
Incidence Severity	1 0.1	0 0.0	6 0.1	27 0.9	3 0.1	0 0.0	18 0.5	34 1.7
	Non-glandular Stomach: Hyperkeratosis							
Incidence Severity	2 0.1	2 0.1	5 0.1	8 0.3	8 0.3	7 0.2	15 0.4	28 0.9
	Urinary bladder: I-cytoplasmic protein-like droplets							
Incidence Severity	0 0.0	0 0.0	21 0.5	19 0.4	0 0.0	0 0.0	21 0.6	28 0.9
	Skin: Necrosis							
Incidence Severity	7 1.6	10 1.6	14 3.0	13 2.5	6 2.0	6 1.3	12 2.8	13 2.5
	Skin: Suppurative Inflammation							
IncidenceSe verity	8 1.9	12 1.9	15 3.1	14 2.6	5 1.7	6 1.3	13 3.0	15 2.7
	Spleen: Increased Pigment							
Incidence Severity	-	-	-	-	0 0.0	2 0.0	19 0.6	18 0.7

(ii). Neoplastic Lesions

No treatment-related neoplastic lesions were seen; histopathology revealed a variety of benign and malignant tumors at different sites in both control and treated animals, but there were no statistically significant increases in the incidence of individual tumor types in any treated group of either sex. The Report Table 14C which summarizes the neoplastic lesions are appended to this DER.

IV. DISCUSSION

Dietary administration of thiram at 0, 15, 150, or 300 ppm to male or 0, 15, 300 or 600 ppm to female CD-1 mice for 97 weeks caused no alteration in survival at any dose level. No treatment-related effects were observed in males or females at 15 ppm. At 150 and 300 ppm in males and at 300 and 600 ppm in females, thiram caused significant decreases in mean body weights, mean body weight gains and in food consumption. The mild but statistically significant decreases in RBC mass [decreases in RBC, HGB and HCT] in females at 600 ppm was corroborated with increased hemosiderin in the spleen of these mice at histopathology. Treatment-related non-neoplastic lesions observed at the higher doses [150 and 300 ppm in males and 300 and 600 ppm in females] included hyperkeratosis of the non-glandular stomach, retinal atrophy of the eyes, intracytoplasmic protein-like droplets in the superficial transitional epithelium of the urinary bladder, and hemosiderin deposits in the spleen. No treatment-related neoplastic lesions were seen; histopathology revealed a variety of benign and malignant tumors at different sites in both control and treated animals, but there were no statistically significant increases in the incidence of individual tumor types in any treated group of either sex.

V. CONCLUSION

In this study, **Thiram induced toxicological** [reduction in body weight/body weight gain and food consumption], **pharmacological** [anemia], **and histopathological** [non-neoplastic lesions in the eyes, urinary bladder and skin] **changes.** Therefore, it is concluded that the highest dose tested in this study [300 ppm in males and 600 ppm in females] was adequate to assess the carcinogenic potential of Thiram and establish that it does not induce carcinogenicity.

Under the conditions of this study, **for chronic toxicity a NOEL of 15 ppm [3 mg/kg/day for males and females]** and a **LOEL of 150 ppm [24 mg/kg/day in males] and 300 ppm [57 mg/kg/day in females]** was established based on decreases in body weight gain, hematological alterations, and non-neoplastic lesions in the eyes, nonglandular stomach, and urinary bladder].

VI. CORE CLASSIFICATION: Minimum; this study satisfies guideline requirement [83-2b] for a carcinogenicity study in mice and is acceptable for regulatory purposes.