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FINAL

DATA EVALUATION REPORT

THIRAM

Study Title:
Thirteen-Week Toxicity Study with
Thiram in Rats

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
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DATA EVALUATION REPORT

STUDY TYPE: Guideline Series (82-1): Subchronic oral toxicity in rats.

TEST MATERIAL: Thiram

MRID NUMBER: 407736-01

SYNONYMS: Thirame; TMTD

STUDY NUMBER: HLA 6111-110

SPONSOR: Uniroyal Chemical Company, Inc., Bethany, Connecticut.

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, Wisconsin.

TITLE OF REPORT: Thirteen-Week Toxicity Study with Thiram in Rats.

AUTHOR: Daniel F. Kehoe

REPORT ISSUED: March 23, 1988.

CONCLUSIONS: Thiram was fed to male and female Crl:CD(SD)BR rats at dietary levels of 0, 50, 500, or 1000 ppm. Mean body weight gains in the mid- and high-dose males and females were lower than those of the controls throughout the 13-week treatment period. Reductions in food consumption in these animals were also noted. Treatment-related changes noted in the mid- and high-dose animals included decreased red blood cell count, hemoglobin, and hematocrit in females; increased mean corpuscular volume and mean corpuscular hemoglobin in males and females; increased white blood cell count, neutrophil count, lymphocyte count, and monocyte count in females; decreased total protein and glucose in males and females; decreased albumin in females; and increased urea nitrogen and chloride in females. Gross pathological changes in the stomachs of the mid- and high-dose rats consisted of erosion/ulceration. Histopathological changes in the stomach noted in the mid- and high-dose rats included erosion/ulceration, mucosal hyperplasia, and inflammation/edema.

The LOEL is 500 ppm based on decreases in body weight gains, changes in hematology and clinical chemistry parameters, and pathological alterations in the stomach. The tentative NOEL is 50 ppm; however, a classification has to be made as to whether the 50 ppm diet was stored frozen or simply

refrigerated. If the latter, then additional analytical storage stability data have to be reported.

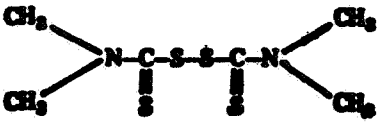
Core Classification: Core Supplementary. Data do not currently satisfy the the Guideline requirements for a subchronic feeding study (82-1) in rats. This classification is upgradeable provided an acceptable clarification and/or additional data are provided regarding stability of the test material in the diet at 50 ppm.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Thiram

Formula:



Lot number: HLA sample no. 70404831 (Lot no. 117)

Purity: 99.43% (Sponsor analysis)

Physical property: Crystalline

Stability: Stable

2. Test Article Analyses for Purity and Stability

Test diets were prepared fresh weekly according to the performing laboratory's standard operating procedures (not available for review) and refrigerated in polyethylene containers until fed. Dietary concentrations were based on ppm of test substance as supplied.

Although the study report states that the test diets were stored refrigerated, the results of dietary analyses were reported for diets stored frozen or at room temperature. There were no analytical data on diets stored refrigerated. Therefore, it is questionable as to whether or not analytical results were conducted on the diets as they were actually stored. The following information concerning the analyses of diets stored frozen or at room temperature was provided by the study report: the homogeneity of the test substance (samples derived from the top, bottom, and sides of the mixing vessel) was determined at week 1; the stability of the test substance in the diet was determined over periods of 7 days (diets stored at room temperature), 14 days (frozen diets), and 35 days (frozen diets); the concentrations of the test substance in the diet were determined weekly for a period of 14 weeks.

Results of stability analyses indicated that the test substance at concentrations of 50, 500, and 1000 ppm was stable in diets stored frozen. Mean stability assay results for diets stored frozen for 14 and 35 days ranged from 81% to 99%. The test substance in diets stored at room temperature for 7 days, in particular the 50 ppm diet, was less stable than diets stored frozen. Diets stored at room temperature for 7 days were 17%, 80% and 88%, respectively, of the nominal levels of 50, 500, and 1000 ppm. Mean concentrations of thiram in the diets at dose levels of 50, 500, and 1000 ppm were 75%, 95%, and 97% of target dose, respectively. Homogeneity analyses performed on the 50, 500, and 1000 ppm diets yielded mean nominal values of 80%, 97%, and 98%, respectively.

3. Animals

One hundred albino rats (Crl:CD(SD)BR strain) were received as weanlings from the Portage Michigan facility of the Charles River Laboratories, Inc., Wilmington, Massachusetts. Rats were acclimated to laboratory conditions for 14 days prior to treatment. Rats were housed individually in suspended, stainless steel cages in a room with temperature and humidity controls set at 72°F ± 3°F and 50% ± 20%, respectively, with a 12-hour light/dark cycle. Water and food were provided ad libitum. Prior to treatment, males weighed 127.0g-148.0 g and females weighed 111.9g-132.6g.

Rats were randomly assigned by body weights to the following test groups:

Dietary Levels (ppm)	13 weeks	
	Males	Females
0 (control)	10	10
50 (low)	10	10
500 (mid)	10	10
1000 (high)	10	10

4. Statistics

Initial body weights, cumulative body weight gain, food consumption, clinical pathology, and organ weights were analyzed by Levene's test for equality of variance. If variances were homogeneous, the standard one-way analysis of variance (ANOVA) was used to analyze the data. Rank transformations were used when the variances were not homogeneous. When the ANOVA was significant (for homogeneous or

ranked data) Dunnett's t-test or the Games and Howell Modified Tukey-Kramer test was used for pairwise comparisons between groups. Nonparametric tests were used when no transformation establishes variance homogeneity at $p \leq 0.001$. These tests included the Krushal-Wallis H-test, ANOVA, the Nemenyi-Kruskal-Wallis test for multiple comparisons or the Wilcoxon-Mann-Whitney Two-Sample Rank test.

Standard one-way analysis of covariance (ANCOVA) was used to analyze body weight with initial body weight as a covariate. Although Levene's test for variance homogeneity was performed, no transformations were used because covariance adjustment removed extraneous heterogeneity. If the ANCOVA was significant, Dunnett's t-test or the Games and Howell Modified Tukey-Kramer test was used for pairwise comparisons between groups.

5. Quality Assurance

A quality assurance statement was signed and dated March 23, 1988.

6. General Observations

(a) Mortality/moribundity/survival

All animals were observed at least twice daily for moribundity and mortality.

No deaths occurred during the study.

(b) Clinical observations

Rats were inspected once daily for signs of toxicity.

No clinical signs attributable to treatment were observed during the study period. Incidental findings included malocclusion, alopecia, mouth sores, bloody crust, lacrimation, and red or swollen paws.

(c) Body weights and food consumption

Body weights. Individual body weights were recorded prior to the initiation of the study, at initiation of treatment, weekly thereafter, and at study termination.

Tables 1 and 2 summarize data on mean body weights and mean body weight gains, respectively, at selected intervals. Mean body weights of the mid- and high-dose males and mid-dose females were significantly lower than controls during weeks 1 through 13. Mean body weights of the high-dose females were significantly lower than controls during weeks 0 through 13. Mean body weights of males receiving 50, 500, or 1000 ppm were 2%, 19%, and 28% lower, respectively, than those of controls at week 13. Mean body weights of females receiving 500 or 1000 ppm were 17% and 23% lower than those of controls at week 13.

TABLE 1. Mean Body Weights (g ± S.D.) at Selected Intervals for Rats fed Thiram for 13 Weeks[a]

Weeks	Dietary Level (ppm)			
	0	50	500	1000
		Males		
0	138.0±6.3	137.0±4.7	137.1±7.6	138.2±5.0
1	193.4±9.0	190.1±7.7	157.1±13.1*	137.8±13.6*
6	399.8±13.6	388.4±21.6	313.8±25.5	280.8±18.5*
13	506.9±31.0	497.9±32.9	409.0±37.6	367.5±26.2*
		Females		
0	125.1±4.1	123.0±5.5	123.6±4.5	118.4±4.7*
1	156.0±5.6	153.3±10.1	132.5±3.6*	117.9±10.7*
6	235.5±18.7	237.0±16.6	195.8±13.5*	179.0±11.0*
13	280.3±22.8	287.8±24.0	232.8±16.3*	216.1±12.8*

[a]Data extracted from Tables 11 and 12 of the study report.

*Significantly different from control values, $p<0.05$.

TABLE 2. Mean Body Weight Gains (g ± S.D.) at Selected Intervals for Rats Fed Thiram for 13 Weeks[a]

Months	Dietary Level (ppm)			
	0	50	500	1000
		Males		
0-1	55.4±4.1	53.1±3.7	20.0±10.0*	-0.4±12.2*
0-6	261.8±11.5	251.4±17.6	176.7±20.8*	142.6±17.5*
0-13	368.9±30.7	360.9±29.0	271.9±32.6*	229.2±24.5*
		Females		
0-1	30.9±5.6	30.3±6.5	8.8±6.3	-0.5±8.2*
0-6	110.3±17.1	114.0±12.8	72.2±13.6*	60.6±7.4*
0-13	155.1±21.9	164.7±20.4	109.2±15.8*	97.7±10.6*

[a]Data extracted from Tables 6 and 7 of the study report.

*Significantly different from control values, $p<0.05$.

Reductions in mean body weight gains were noted in the mid- and high-dose males and females during weeks 1 through 13. Mean body weight gains of males receiving 50, 500 or 1000 ppm were 2%, 26%, and 38% lower, respectively, than those of controls at week 13. Mean body weight gains of females receiving 500 or 1000 ppm were 30% and 37% lower, respectively, than those of controls at week 13.

Food consumption. Individual food consumption was determined weekly from study initiation to study termination.

Table 3 summarizes selected data on mean food consumption.

Food consumption in the mid-dose males was significantly lower than that of controls during weeks 1 through 12 and in the high-dose males during weeks 1 through 13. Food consumption in the mid-dose females was significantly lower than that of controls during weeks 1 through 13 and in the high-dose females during weeks 1 through 8 and weeks 10 through 13.

(d) Ophthalmoscopic examination

Ophthalmological examinations were performed prior to study initiation and at termination.

There were no compound-related ophthalmological effects.

7. Clinical Pathology

Blood was collected from the retro-orbital plexus of all animals for clinical laboratory evaluations at study termination. Animals were fasted overnight prior to blood collections. Those parameters indicated by an "X" were examined:

(a) Hematology

X Hematocrit (HCT)*	X Leukocyte differential count
X Hemoglobin (HGB)*	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	X Coagulation: thromboplastin time (PT)
Reticulocyte count (RETIC)	
Red cell morphology	

* = Recommended by Subdivision F (November 1984) Guidelines.

Table 4 summarizes data on selected hematology parameters. Significant differences in hematology parameters were noted in males and females receiving 500 ppm or 1000 ppm. Effects in the mid- and high-dose females included decreases in red blood cell counts, hemoglobin levels, and hematocrit levels, and increases in mean corpuscular volume and white blood cell counts. Increases in neutrophil counts and decreases in lymphocytes were noted in the high-dose females. None of the females were overtly

TABLE 3. Mean Food Consumption (g ± S.D.) in Rats Fed Thiram for 13 Weeks[a]

Weeks	Dietary Level (ppm)			
	0	50	500	1000
		Males		
1	150.6±9.9	148.5±8.4	96.9±18.8*	73.8±16.8*"
6	189.8±13.3	187.4±15.7	153.9±17.6*	124.1±31.4*
13	176.9±17.9	183.0±14.0	158±32.5*	133.2±6.8*
		Females		
1	121.0±9.8	124.0±12.5	82.9±19.1*	57.9±12.1*
6	127±13.4	133.0±16.3	104.9±6.6*	100.8±8.7*
13	127.3±14.6	128.6±14.6	101.5±8.0*	99.9±7.1*

[a]Data extracted from Tables 11 and 12 of the study report.

*Significantly different from control values, $p<0.05$.

TABLE 4. Representative Results of Mean Hematology Parameters in Rats Fed Thiram for 13 Weeks[a,b]

	Dietary Level (ppm)							
	Males				Females			
Parameter	0	50	500	1000	0	50	500	1000
RBC (10^6 UL)	8.54	8.86	7.89	8.09	8.55	8.40	7.74*	7.10*
HGB (G/DL)	14.2	15.0	14.3	14.4	14.4	14.7	13.7*	13.7*
HCT (%)	46.8	49.6	47.1	47.2	48.2	48.4	45.0*	44.2*
MCV (FL)	55.0	56.0	60.0*	59.0*	56.0	58.0	58.0	62.0*
MCH (PG)	16.7	17.0	18.1*	17.9*	16.8	17.6	17.7*	19.3*
WBC (10^3/UL)	10.0	7.2*	8.9	9.3	4.2	5.8	6.4*	8.2*
LYMPHOCYTES (%)	76.0	83.0	84.0	76.0	83.0	83.0	84.0	76.0*
Neutrophils (%)	20.0	13.0*	14.0	22.0	13.0	14.0	12.0	19.0*

[a]Data extracted from Tables 12 and 15 of the study report.

[b]Data represent hematology values measured at week 14.

*Significantly later than controls, $p<0.05$.

anemic. Mean corpuscular volume and mean corpuscular hemoglobin were significantly higher in males receiving 500 or 1000 ppm.

(b) Blood (clinical) chemistry

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	X Albumin/globulin ratio
Magnesium*	X Blood creatinine*
X Phosphorus*	X Blood urea nitrogen*
X Potassium*	Cholesterol*
X Sodium*	X Globulins
	X Glucose*
Enzymes	X Total bilirubin*
	Direct bilirubin
Alkaline phosphatase (ALP)	X Total protein*
Cholinesterase	Triglycerides
Creatinine phosphokinase	
Lactic acid dehydrogenase	
X Serum alanine aminotransferase (SGPT)*	
X Serum aspartate aminotransferase (SGOT)*	
Gamma glutamyltransferase (GGT)	

* = Recommended by Subdivision F (November 1984) Guidelines.

Table 5 summarizes data on selected clinical chemistry parameters. Glucose levels were significantly lower than controls in the high-dose females and nonsignificantly lower than controls in the high-dose males. Total protein was significantly lower in the mid- and high-dose males and in the mid-dose females. Total protein was nonsignificantly lower than controls in the mid- and high-dose females. Albumin levels were significantly lower than controls in the mid- and high-dose females. The study author attributed the decreases in glucose, albumin, and total protein to decreases in food consumption or assimilation and lower body weights. Urea nitrogen levels were significantly higher than controls in the mid- and high-dose females. Chloride levels were significantly higher in the high-dose females.

(c) Urinalysis

Urinalysis was not performed.

8. Sacrifice and Pathology

After 13 weeks of treatment, all animals were fasted overnight, then anesthetized using methoxyflurane, weighed, exsanguinated, and necropsied. At necropsy, macroscopia were recorded, organ weights were obtained, and tissues placed in fixative. Microscopic examinations were performed on all tissues from control rats and high-dose rats. Those tissues indicated by an "X" were collected for histopathological examination; those organs indicated by "XX" were weighed:

TABLE 5. Representative Results of Mean Clinical Chemistry Parameters in Rats Fed Thiram for 13 Weeks[a,b]

Parameter	Dietary Level (ppm)							
	Males				Females			
	0	50	500	1000	0	50	500	1000
Glucose (mg/dL)	115.2	107.4	103.0	102.4	101.6	99.4	96.7	86.7*
Urea nitrogen (mg/dL)	16.6	13.2	16.1	16.7	15.1	15.3	18.1*	20.5*
Chloride (mmol/l)	102.0	104.0	103.0	104.0	104.0	105.0	106.0	108.0*
Total Protein (g/dL)	7.1	7.0	6.6*	6.6*	7.2	7.2	6.7*	6.8
Albumin (g/dL)	4.5	4.5	4.4	4.4	5.0	4.9	4.6*	4.6*

[a]Data extracted from Tables 14 and 15 of the study report.

[b]Data represent clinical chemistry values measured at week 14.

*Significantly different from control values, $p<0.05$.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	X Brain
X Salivary glands*	X Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	Spinal cord (three levels)
Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen	X Eyes (Optic nerve)*
X Jejunum*	X Thymus	
X Ileum*		
X Cecum*	Urogenital	Glandular
X Colon*		
X Rectum	XX Kidneys*	X Adrenals*
XX Liver*	X Urinary bladder*	Lacrimal gland
Gallbladder*	XX Testes*	X Mammary gland
X Pancreas*	X Epididymides	X Thyroids*
	Prostate	X Parathyroids*
Respiratory	Seminal vesicle	Harderian glands
	Ovaries	
X Trachea*	X Uterus	
X Lung*		

Other

X Bone (femur)*
Skeletal muscle*
Skin
X All gross lesions and masses

* = Recommended by Subdivision F (November 1984) Guidelines.

All tissues (when present) from animals in the 0 and 1000 ppm groups were sectioned, stained, and examined microscopically. Microscopic examinations were done on all macroscopic lesions from all rats. Lung, livers, kidneys, and stomach were examined microscopically from rats receiving 50 or 500 ppm.

(a) Macroscopic

Erosion/ulceration of the nonglandular stomachs and diffusely red or mottled mesenteric lymph nodes were observed in rats receiving 500 or 1000 ppm. The incidences of these gross lesions observed at terminal sacrifice are as follows:

	Control		50 ppm		500 ppm		1000 ppm	
	M	F	M	F	M	F	M	F
Number examined	10	10	10	10	10	10	10	10
Stomach (nonglandular)								
Erosion/ulceration	0	0	0	0	2	2	4	2
Mesenteric lymph node								
Diffusely red	0	0	0	0	0	1	3	3
Mottled	0	0	0	0	7	5	6	6

(b) Organ weights and body weight ratios

There were no organ weight changes of toxicological importance in animals sacrificed at termination.

Statistically significant changes in relative organ weights (for example: liver and kidney) noted in the high-dose rats were attributable to lowered body weights of the animals when compared to the controls.

(c) Microscopic

An increased incidence of histologic changes in the mucosa of the nonglandular stomach was noted in the high-dose males and females. These changes consisted of focal areas of erosion/ulceration, mucosal (epithelial) hyperplasia, or both. Occasionally these were accompanied by submucosal inflammation and edema. The incidences of microscopic lesions in the stomach are summarized below:

	Control		50 ppm		500 ppm		1000 ppm	
	M	F	M	F	M	F	M	F
Number examined	10	10	10	10	10	10	10	10
Stomach (nonglandular)								
Erosion	1	0	1	0	1	2	1	0
Mucosal hyperplasia	0	0	0	0	2	3	3	4
Inflammation/edema	0	0	0	0	2	4	2	5
Ulceration	0	0	0	0	1	1	0	3

The mesenteric lymph nodes from the high-dose rats were frequently congested but otherwise normal. All other microscopic

observations were considered incidental findings with no major differences between control and treated animals.

B. REVIEWERS' DISCUSSION

The study protocol was acceptable for a subchronic oral study in rats. The conduct and reporting of the study were adequate except that there is a question as to whether or not the 50 ppm diet was stored frozen or simply refrigerated. If the latter, the additional storage stability data have to be provided, particularly as there was only a 17% recovery from the 50 ppm diet after one week storage at room temperature.

Administration of 500 or 1000 ppm thiram to rats affected body weight gains, food consumption, hematology and clinical chemistry parameters, and histopathology. Mean body weight gains in the mid- and high-dose males and females were lower than in controls during the 13-week study. Decreases in food consumption in these animals were also noted.

Assessment of hematology data indicated possible anemia in the mid- and high-dose females, as indicated by lower red blood cell counts, hemoglobin, and hematocrit. Increases in white blood cell counts were noted in mid- and high-dose females, while increases in neutrophil counts and decreases in lymphocytes were noted only in the high-dose females. Mean corpuscular volume and mean corpuscular hemoglobin were significantly higher in males receiving 500 or 1000 ppm. These changes in hematology parameters were treatment-related.

Treatment-related changes in clinical chemistry parameters consisted of decreases in glucose levels (high-dose males and females), decreases in total protein levels (mid- and high-dose males and females), and decreases in albumin levels (mid- and high-dose levels). Urea nitrogen levels were elevated in the mid- and high-dose females. Chloride levels were increased in the high-dose females.

There was no effect of dosing on organ weights, ophthalmology, urinalysis and mortality.

Possible treatment-related gross pathological findings in the mid- and high-dose males and females consisted of erosion/ulceration of the nonglandular stomach and diffusely red or mottled mesenteric lymph nodes. Histological changes in the stomach were noted only in the high-dose males and females. These changes included erosion/ulceration, mucosal hyperplasia, and submucosal edema and inflammation. The toxicological significance of these stomach lesions is unknown.

The reviewers agree with the study author's conclusion that the no-observed-effect level for subchronic effects was 50 ppm; however, this tentative NOEL may have to be changed on the basis of storage stability data. The LOEL is 500 ppm based on body weight gains, clinical pathology, and gross microscopic pathology.