009528

DOC 920087

FINAL

82-16

DATA EVALUATION REPORT

THIRAM

Study Title:
Four-Week Range-Finding Study With Thiram in Dogs

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

January 3, 1992

Principal Author _____ Date 2/14/92
John Liccione

Reviewer _____ Date 2-14-92
Wayne Reichardt

QA/QC Manager _____ Date 2/14/92
Sharon Segal

Contract Number: 68D10075
Clement Number: 91-140
Work Assignment Number: 1-36A.1
Project Officer: Mr. Jim Scott

EPA Reviewer: Byron Backus, Ph.D.
Review Section I, Toxicology Branch I,
Health Effects Division

Signature: Byron T. Backus
Date: 2/27/92

Secondary EPA Reviewer: Clark Swentzel, Ph.D.
Section Head, Review Section I,
Toxicology Branch I, Health Effects Division

Signature: K. Clark Swentzel
Date: 2/27/92

DATA EVALUATION REPORT

STUDY TYPE: Four-week range-finding study in dogs

TEST MATERIAL: Thiram

SYNONYMS: Thirame; TMTD

MRID Number: 407797-01

STUDY NUMBER: HLA 6111-109

SPONSOR: Uniroyal Chemical Company, Inc.
Bethany, Connecticut

TESTING FACILITY: Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

TITLE OF REPORT: Four-Week Range-Finding Study with Thiram in Dogs

AUTHORS: Daniel F. Kehoe

REPORT ISSUED: March 24, 1988

CONCLUSIONS: Thiram was fed to male and female beagle dogs at dietary levels of 0, 125, 500, or 2000/1500 ppm. One female receiving 2000 ppm died during week 2. Consequently, the dietary level of 2000 ppm was reduced to 1500 ppm during weeks 3 and 4. The high-dose animals received control diet for 2 or 3 days prior to receiving the 1500 ppm diet. One high-dose male was sacrificed in a moribund condition during week 4. The causes of death and moribundity were not determined. Hepatocellular degeneration with sinusoidal proliferation was observed in the single surviving high-dose male sacrificed at week 4. Increases in alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase, decreases in red blood cell counts, and hemoglobin, and hematocrit levels, and an increase in MCV were also noted in the surviving high-dose male. There were no significant hemotological, clinical chemistry, or pathological findings in the single surviving high-dose female. Mean body weights and food consumption were lower than the controls in the surviving high-dose animals and in the mid-dose animals. Slight decreases in red blood cell count, hemoglobin, and hematocrit levels were also seen in the mid-dose males.

The LOEL is 500 ppm based on body weight decrement and changes in hematological parameters. The NOEL is 125 ppm.

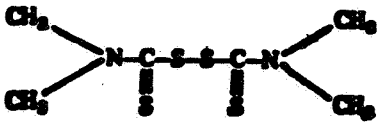
CORE CLASSIFICATION: Core Supplementary. This study provides information for setting dietary exposure levels to be used in a subchronic dog study.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Thiram

Formula:



Lot number: 117

Purity: 99.43% (determined by Sponsor)

Physical property: Off-white powder

Stability: Not reported

2. Test Article Analyses for Purity and Stability

Test diets were prepared by adding a specified amount of test material to a specified amount of Purina Certified Canine Diet #5007 and thoroughly mixed for 15 minutes. Test diets were prepared weekly. Diets were stored at room temperature. Samples from each mixed batch were to be retained below 0°C for 6 months and then returned to the sponsor or discarded.

The homogeneity of the test substance in freshly prepared diets (samples derived from the top, bottom, and sides of the mixing bowl) was determined at week 1. Stability analyses of the diets were not performed.

Mean values for method validation ranged from 93%-100% of the theoretical levels. Homogeneity assays ranged from 94%-96% of the theoretical levels. Mean results for verification of thiram levels in the diets at dose levels of 125-, 500-, 1,500-, and 2,000-ppm diets were 92%, 97%, 97%, and 97% of the target dose, respectively.

3. Animals

Dogs (8 males and 8 females, beagle strain) were received from Hazleton Research Products, Inc., Cumberland, Virginia. Dogs were identified with a numbered ear tag. Dogs were acclimated to

laboratory conditions for 22 days before being placed on test. Animals were housed individually in stainless steel cages within rooms with the temperature maintained at 73°F ± 6°, a relative humidity of 50% ± 20%, and a 12-hour light/dark cycle. Water and food (Purina Certified Canine Diet #5007) were provided ad libitum.

Before being placed on test, the dogs were stratified by body weight and two males and two females were randomly assigned to the following test and control groups:

Dietary Levels (ppm)	13 Weeks	
	Males	Females
0 (control)	2	2
125 (low)	2	2
500 (mid)	2	2
2,000/1,500[a] (high)	2	2

[a]Dose levels were decreased from 2,000 ppm to 1,500 ppm during Weeks 3 and 4.

4. General Observations

(a) Mortality/moribundity/survival

All animals were observed twice daily for moribundity and mortality.

One high-dose female died during week 2 while still receiving 2,000 ppm and one high-dose male became moribund and was sacrificed during week 4.

(b) Clinical observations

Dogs were inspected once daily for signs of toxicity.

Ptyalism was noted for the one high-dose male that was sacrificed in a moribund condition. The one high-dose female that died during week 2 exhibited convulsions.

(c) Body weights and food consumption

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

Table 1 summarizes data on mean body weights at weekly intervals. Body weights for the high-dose males and females at week 4 were 33% and 39% lower, respectively, than those of controls. Body weights

for the mid-dose males and females at week 4 were 14% and 16% lower, respectively, than those of controls.

There were no significant differences in body weights for the low-dose males or females when compared with those of controls.

Food consumption-- Individual food consumptions were recorded one week prior to the initiation of the study, at initiation of treatment, and weekly thereafter.

Table 2 summarizes data on mean food consumption at weekly intervals. During week 4, food consumption for low-, mid-, and high-dose males were 12%, 29%, and 92% lower, respectively, than those of controls. Food consumption for low-, mid-, and high-dose females during week 4 were 13%, 49%, and 78% lower, respectively, than those of controls.

(d) Ophthalmoscopic examination

No ophthalmoscopic examinations were performed.

5. Clinical Pathology

Hematology and clinical chemistry analyses were performed on all dogs prior to study initiation and at study termination. Animals were fasted overnight before blood sampling; water was provided ad libitum. Blood was collected from the jugular vein. Those parameters indicated by an "X" were examined.

(a) Hematology

X Hematocrit (HCT)
X Hemoglobin (HGB)
X Leukocyte count (WBC)
X Erythrocyte count (RBC)
X Platelet count
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)

Table 3 summarizes data on selected hematology parameters. Red blood cell count, hemoglobin and hematocrit levels were lower in the mid- and high-dose males. Slightly lower absolute lymphocyte count and slightly higher platelet count were seen in some mid- or high-dose dogs.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium
X Chloride
X Phosphorus
X Potassium
X Sodium

Other

X Albumin
X Albumin/globulin ratio
X Blood creatinine
X Blood urea nitrogen
X Globulins
X Glucose
X Total bilirubin

TABLE 1. Mean Body Weights (kg ± S.D.) in Dogs Fed Thiram for 4 Weeks[a,b]

Weeks	Dietary Level (ppm)			
	0	125	500	1500
		Males		
0	7.2±0.92	7.8±0.21	7.0±1.13	6.8±0.92
1	7.6±0.99	8.0±0.64	7.3±1.13	6.2±1.06
2	7.8±1.06	8.3±0.71	7.1±0.99	6.5±0.85
3	8.3±1.13	8.4±0.92	7.4±1.13	5.8±0.64
4	8.4±1.20	8.4±0.92	7.2±1.20	5.6[c]
		Females		
0	6.8±1.20	6.8±0.64	6.6±1.41	7.2±1.77
1	7.0±1.20	7.0±0.71	6.5±1.41	6.8±1.77
2	7.2±1.27	7.2±0.64	6.4±1.48	5.5[c]
3	7.4±1.20	7.2±0.57	6.4±1.48	4.9[c]
4	7.5±1.27	7.4±0.49	6.3±1.56	4.6[c]

[a]Data extracted from Tables 5 and 6 of the Study Report.

[b]Data based on 2 dogs/sex/group unless otherwise indicated.

[c]Data based on 1 dog.

TABLE 2. Mean Food Consumption (g ± S.D.) in Dogs Fed Thiram for 4 Weeks[a,b]

Weeks	Dietary Level (ppm)			
	0	125	500	1500
		Males		
-1	1958±284	1872±3	1715±232	1733±334
1	2131±276	1961±250	1676±52	671±93
2	2127±433	2012±380	1430±199	508±16
4	2258±278	1998±325	1601±195	186[c]
		Females		
-1	1728±407	1698±56	1596±140	1820±310
1	2068±450	1629±219	1087±209	896±153
2	1796±327	1607±38	1143±67	324[c]
4	1957±516	1709±215	998±194	424[c]

[a]Data extracted from Tables 7 and 8 of the Study Report.

[b]Data based on 2 dogs/sex/group unless otherwise indicated.

[c]Data based on 1 dog.

TABLE 3. Representative Results of Mean Hematology Parameters in Rats Fed Thiram for 4 Weeks[a,b]

Parameter	Dietary Level (ppm)							
	Males				Females			
	0	125	500	1500	0	125	500	1500
RBC (10^6/UL)	5.96	6.47	5.26	3.12	6.46	6.48	5.68	6.32
HGB (G/DL)	13.4	15.6	12.7	8.4	14.6	15.7	13.4	13.9
HCT (%)	40.2	45.5	37.5	24.5	43.5	45.5	40.0	41.9

[a]Data extracted from Tables 9 through 12.

[b]Data represent hematology values measured at week 4.

X Total protein

Enzymes

X Alkaline phosphatase (ALP)
X Serum alanine aminotransferase (SGPT)
X Serum aspartate aminotransferase (SGOT)

Table 4 summarizes data on selected clinical chemistry parameters. The activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were elevated in one high-dose male. Slightly higher total bilirubin and urea nitrogen levels were noted in some mid- or high-dose dogs.

(c) Urinalysis

Urinalysis was not performed.

6. Sacrifice and Pathology

A necropsy was done on the high-dose female that died during week 2 and on the high-dose male that was sacrificed in a moribund condition during week 4.

After 4 weeks of treatment, all surviving animals were fasted overnight, then weighed, anesthetized, exsanguinated, and necropsied. A complete necropsy was performed. All tissues from all animals including the animal that died and the animal sacrificed in moribund condition were preserved in 10% phosphate-buffered formalin. The checked (X) tissues were collected for histological examination. The double-checked (XX) organs were weighed:

Digestive System

X Salivary glands
X Esophagus
X Stomach
X Duodenum
X Jejunum
X Ileum
X Cecum
X Colon
X Rectum
XX Liver
X Gallbladder
X Pancreas

Cardiovascular/Hematologic

X Aorta
X Heart
X Bone marrow
X Lymph nodes
X Spleen

Urogenital

XX Kidneys
X Urinary bladder
XX Testes
X Epididymides
X Ovaries
X Uterus

Neurologic

X Brain
X Peripheralnerve (sciatic nerve)
X Pituitary

Glandular

X Adrenals
XX Thyroids

Respiratory

X Trachea
X Lung

Other

X Bone (sternum and femur)

TABLE 4. Representative Results of Mean Clinical Chemistry Parameters in Dogs Fed Thiram for 4 Weeks[a,b]

	Dietary Level (ppm)							
	Males				Females			
Parameter	0	125	500	2000/1500	0	125	500	2000/1500
AST/SGOT (MG/DL)	21	28	18	38	22	26	18	16
ALT/SGPT (IU/L)	20	21	18	229	25	24	16	20
ALK PHOS (IU/L)	86	95	74	221	121	98	115	96
T BILI (MG/DL)	0.2	0.2	0.4	0.5	0.2	0.1	0.2	0.2
UN (MG/DL)	11.8	11.1	15.0	15.9	11.6	13.2	15.1	20.3

[a]Data extracted from Tables 15 and 16 of the study report.
[b]Data represent clinical chemistry values measured at week 4.

X All gross lesions and masses

A complete histopathological examination was conducted on all control and high-dose dogs and on gross lesions from all dogs. The liver was also examined in the low- and mid-dose dogs since it was a possible target organ.

(a) Macroscopic

There were no compound-related gross observations.

(b) Organ weights and body weight ratios

The surviving high-dose male and female sacrificed at week 4 had notably lower terminal body weights and corresponding lower absolute organ weights.

(c) Microscopic

Hepatocellular degeneration with sinusoidal cell proliferation and pigmentation was noted in the one surviving high-dose male. There were no compound-related microscopic findings in the remaining dogs.

B. REVIEWER'S DISCUSSION

The study protocol was acceptable for a 4-week repeated oral dosing study in dogs. The conduct and the reporting of the study were adequate.

Mortality and moribundity were observed in the high-dose dogs. One female receiving 2000 ppm died on test, while one male receiving 2000/1500 ppm was sacrificed in a moribund condition. The causes of death and moribundity in these dogs were not ascertained. However, convulsions in the female that died and excessive salivation in the moribund male were noted. The male that became moribund also had slightly lower absolute lymphocyte count and potassium levels, slightly higher urea nitrogen levels, and moderately higher glucose levels. There were no treatment-related macroscopic or microscopic findings in this male or in the high-dose female that died during treatment.

Hepatocellular degeneration with mononuclear cell infiltrates and sinusoidal cell proliferation was observed in the surviving high-dose male sacrificed at week 4. Moderate amounts of pigment, identified as hemosiderin, were present in many of the sinusoidal cells within and adjacent to the areas of degeneration. Increases in alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were seen in the high-dose male. The significance of the hepatic lesions in this individual male dog is difficult to assess since these lesions did not occur in the single surviving high-dose female or in the low- and mid-dose animals. Decreases in the red blood cell counts, hemoglobin and hematocrit levels, and an increase in MCV were also noted in this particular male; these results suggest the presence of anemia. There were no remarkable hematological, clinical chemistry, or pathological findings in the one surviving high-dose female. Mean body weights and food consumption were lower in the surviving high-dose males and females.

Slight decreases in red blood cell count, hemoglobin, and hematocrit levels were also seen in the mid-dose males. Mean body weights and food consumption were lower than controls in the mid-dose males and females. There were no major macroscopic or microscopic findings in the mid-dose animals.

The reviewers agree with the study author's conclusion that the no-observed-effect level (NOEL) is 125 ppm. Mean body weights of the low-dose animals were similar to those of the controls at week 4. However, examination of the individual body weight data indicated that one low-dose female displayed a slightly lower body weight gain when compared to control animals at week 4. The slightly decreased body weight gain in this particular female corresponded to a slight decrease in food consumption noted during the 4-week treatment period. There were no significant effects on pathology, clinical chemistry, or hematology parameters noted in the low-dose animals.

In summary, the NOEL is 125 ppm based on changes in clinical pathology and body weight decrements. Dose levels to be used in the subchronic toxicity study can be determined from the results of this repeated dosing study.