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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Dose Selection for an Oncogenicity Study in Mice with Thiophanate Methyl. Tox. Proj. No. 8-1053. Tox. Chem. No. 375A.

TO: Lois Rossi, Product Manager
Registration Division (TS-767C)

Thru: Ed Budd, Head
Review Section 1
Toxicology Branch 1, Insecticides/Rodenticides
Health Effects Division (TS-769)

FROM: Roger Gardner, Toxicologist
Review Section 1
Toxicology Branch 1, Insecticides/Rodenticides
Health Effects Division (TS-769)

Action Requested

Comments on proposed dose levels for an oncogenicity study with thiophanate methyl in mice.

Recommendations and Conclusions

Based on the results from 6-month and 2-year feeding studies conducted previously and the minimal toxicity indicated in mice given dietary levels up to 10,240 ppm for 13 weeks, the highest dose chosen for the chronic study should be greater than the 1280 ppm level proposed.

I. Background

In May of 1986, a draft Registration Standard which identified the need for an additional mouse oncogenicity study on thiophanate methyl was prepared by the Agency. That document discussed two previously conducted mouse feeding studies.

The first study (MRID No. 00055935) was a 6-month feeding study that showed significant body weight decreases in females given an 8000 ppm thiophanate methyl diet. There were also increased absolute liver weights and liver-to-body weight ratios in that group. The NOEL established in the study was 1600 ppm (24 mg/kg/day).

In the second study (MRID No. 00081611), five groups containing 50 male and 50 female ICR SLC strain mice were given diets containing 0, 10, 40, 160, or 640 ppm thiophanate methyl for two years. The results did not

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indicate the occurrence of toxic or oncogenic effects that could be attributed to administration of the test substance.

II. Submitted Information

On June 29, 1988, the Registrant (Penwalt Corp.) submitted a 13-week range-finding study that was used as the basis for proposing doses of 0, 40, 160, 640, and 1280 ppm in an 18 month feeding study. The Registrant's letter mentioned the following results from the range-finding study:

1. Compound-related clinical observations were limited to the high dose males and consisted of salivation.

2. Compound-related increases in thyroid and liver weights were observed. The thyroid and liver were affected in the 2,560 and 10,240 ppm male and female groups when compared to controls...

3. These increases in absolute organ weights were accompanied by increases in organ weights relative to body and brain weight ratios.

4. ...Hepatocellular hypertrophy was noted in one male in the 2,560 ppm group and in all mice in the 10,240 ppm group that was considered to be compound related. Two female mice in the 10,240 ppm dose group had epithelial hypertrophy of the thyroid gland, which was also considered to be compound related.

Based on these points, the June 29 letter concluded that a NOEL was established in this study at 640 ppm.

III. Discussion and Conclusions

The liver weight results (see Appendix below) indicated that a NOEL of 640 ppm is appropriate. However, without significant effects on body weight gain (>10% less in a treated group than the weight gain in controls), the histological changes reported in the thyroid and liver are of questionable toxicological significance. The increased organ weights and hypertrophy in the thyroid and liver are likely to be reversible changes or effects test animals in a chronic study could adapt to. These conclusions are plausable in view of results from the two previously submitted mouse feeding studies discussed above. Therefore, the proposed highest dose of 1280 ppm for the 18-month study in mice may not be adequate for assessing the oncogenic potential of thiophanate methyl in mice.

IV. References

00055935 Hashimoto Y., Noguchi, T., T. Makita, et al. 1970. Toxicological evaluation of thiophanate methyl: II. Studies on the subchronic oral toxicity of thiophanate methyl on mice. Unpublished report prepared by Nippon Soda Co., Ltd. Submitted by Pennwalt Corporation, Philadelphia, Pa.

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00081611 Nishibe, T., T. Mori, T. Nukui, et al. 1973. The report on the carcinogenesis studies of thiophanate methyl, dimethyl 4,4'-o-phenylene-bis(3-thioallophanate), in mice of ICR SLC strain for 24 months. Unpublished report prepared by Nippon Soda Co., Ltd. Submitted by Pennwalt Corporation, Philadelphia, Pa.

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APPENDIX

Data Evaluation Record for

Tompkins, E. C. June 6, 1988. 13-Week Dietary Range-Finding Study in Mice with Topsin M. Unpublished Report No. WIL-75023 prepared by WIL Research Laboratories, Inc. Submitted by Penwalt Corp., Agchem Division, Philadelphia, PA. MRID No. 407453-01

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Reviewed by: Roger Gardner R.G. 9-12-88
Section 1, Toxicology Branch 1 (Insecticides/Rodenticides) (TS 769C)
Secondary Reviewer: Judith W. Hammerstrom 11/7/88
Section 1, Toxicology Branch 1 (Insecticides/Rodenticides) (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (Range Finding) (Guideline §82-1)

MRID NUMBER: 407453-01 Caswell Number: 375A

TEST MATERIAL: Technical grade Topsin M with a stated purity of 95.2% was used.

SYNONYMS: Thiophanate methyl

STUDY NUMBER(S): WIL-75023

SPONSOR: Penwalt Corp., Agchem Division, Philadelphia, PA

TESTING FACILITY: WIL Research Laboratories, Inc.

TITLE OF REPORT: 13-Week Dietary Range-Finding Study in Mice with Topsin M

AUTHOR(S): Tompkins, E. C.

REPORT ISSUED: June 6, 1988

CONCLUSIONS: A 13-week feeding study with male and female Charles River Crl:CD-1(ICR) BR strain mice was conducted with dietary levels of 0 160, 640, 2560, and 10,240 ppm thiophanate methyl to determine dosage levels for a long-term oncogenicity study. The study suggested a no-observed-effect level of 640 ppm on the basis of increased liver weight only.

Based on the results from 6-month and 2-year feeding studies conducted previously and the minimal toxicity indicated in mice given dietary levels up to 10,240 ppm for 13 weeks, the highest dose chosen for the chronic study should be greater than the 1280 ppm level proposed.

Core Classification: Supplementary. The report described a range-finding study.

I. PROTOCOL

A. MATERIALS

1. Test species: Male and female 6-week-old Charles River Crl:CD-1(ICR) BR strain mice were used. Their weights ranged from 20.6 to 27.2 g for males and 17.2 to 23.6 g for females at the beginning of the study. The animals were placed on test diets 17 days after their arrival at the laboratory.

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2. Diet preparation: Basal diet consisted of Purina Certified Rodent Chow #5002, and the test substance was added in appropriate concentrations. Test diets were prepared weekly and stored under refrigeration. Samples of test diets were analyzed for stability, homogeneity and accuracy of test substance concentration at the beginning of the study, and analyses for accuracy of test substance concentration were conducted at test weeks 2, 3, 4, and 8.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

Test groups No.	Designation	Dose (ppm)	Animals per sex
1	Control	0	5
2	Low (LDT)	160	5
3	Mid (1)	640	5
4	Mid (2)	2560	5
5	High (HDT)	10,240	5

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	All	Three times a day during the week and twice a day on weekends.
Physical examination	All	Once a week
Body weight	All	On day of arrival at lab, at weekly intervals throughout the study, and just prior to sacrifice.
Food consumption	All	For all weighing intervals during the study.**
Blood samples	All	Just prior to termination of the study.
Necropsy	Animals found dead or moribund	When found.
	10 Survivors	At 12 months At 24 months

C. METHODS

1. Observation of blood samples: Blood was collected from the orbital sinus of animals fasted overnight.

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Range Finding

a. Hematology

- X Hematocrit
- X Hemoglobin
- X Red cell count
- X Platelet count
- X Total white cell count
- X Differential white cell counts
- X Mean corpuscular hemoglobin concentration
- X Mean cell volume
- X Mean corpuscular hemoglobin

b. Blood chemistry

- ___ Total protein
- ___ Albumin
- ___ Globulin (calculated)
- ___ Albumin/globulin ratio
- X Blood urea nitrogen
- X Calcium
- ___ Creatinine
- ___ Uric acid
- X Glucose
- ___ Total cholesterol
- ___ Total bilirubin
- X Aspartate amino-transferase (AST)
- X Alanine amino-transferase (ALT)
- X Alkaline phosphatase
- ___ Lactate dehydrogenase
- ___ Triglycerides

2. Necropsy Gross lesions were noted.

a. Weighed organs

- X Brain
- X Heart
- X Kidneys
- X Liver
- X Ovaries
- X Spleen
- X Testes
- X Thyroid gland

The thyroid glands were weighed after fixation.

b. Tissues fixed for microscopic examination:

Circulatory System

- X Aorta
- X Heart

Digestive System

- X Cecum
- X Colon
- X Duodenum
- X Esophagus
- X Ileum
- X Jejunum
- X Liver
- X Pancreas
- X Rectum
- X Salivary gland
- X Stomach
- X Gallbladder

Hematopoietic System

- X Bone marrow
- X Lymph nodes
- X Spleen
- X Thymus

Musculoskeletal System

- X Bone
- X Skeletal muscle

Nervous System

- X Brain
- X Peripheral nerve
- X Spinal cord

Reproductive System

- X Epididymides
- X Mammary glands
- X Ovaries
- X Prostate
- X Seminal vessicles
- X Testes
- X Vagina
- X Uterus with cervix

Respiratory System

- X Lungs
- X Nasal turbanates
- X Trachea

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b. Tissues fixed for microscopic examination (contnued)

Endocrine System	Other	Urinary System
X Adrenals	X All macroscopic abnormalities	X Kidneys
X Pituitary	X Eye	X Urinary bladder
X Thyroid with parathyroid	X Hardarian gland	
	X Skin and subcutis	

Only the liver and thyroid glands were prepared further for microscopic examination according to the report. These organs were selected based on organ weight results (See Section III. "Authors' conclusions" below for additional information).

D. STATISTICAL ANALYSIS

Observation	Statistical Test
Continuous Variables	
Body weights, weight changes, clinical chemistry values, absolute and relative organ weights	One-way analysis of variance (ANOVA) followed by Dunnett's test

II. REPORTED RESULTS

A. Mortality and clinical signs: There were no mortalities in the study, and most clinical signs were not related to treatment according to the report. One clinical observation the authors noted was apparently swollen testes which was seen in three of the five 10,240 ppm dose group males, and in one animal each from the 640 and 2560 ppm dose groups. In addition, the investigators noted occasional salivation in the highest dosed group males.

B. Body weight and food consumption: By the end of the 13-week feeding period, group mean body weights for treated male mice were slightly greater than that for the control group. The respective means for male mice given the 0, 160, 640, 2560, and 10,240 ppm diets were 28.8, 32.2, 31.4, 31.6, and 30.9 g at the end of the study. Similar results were observed in female mice. Group means for the 0, 160, 640, 2560, and 10,240 ppm dose groups at the end of the study were 25.7, 26.9, 27.0, 27.2, and 26.0 g, respectively.

C. Test substance intake: No effects were observed on body weight gain or food consumption, and the calculated test substance intake (based on body weight and food consumption results were reported as follows:

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C. Test substance intake (continued)

Dose level (ppm)	Calculated test substance intake (mg/kg/day) Males	Females
0	0.0	0.0
160	26.2	39.3
640	107.2	182.0
2,560	423.0	562.6
10,240	1671.2	2200.7

D. Hematology: The report stated that group means for hemoglobin and hematocrit in treated males were statistically significantly lower than those values for the control group ($p<0.05$). The investigators noted that there were two control group animals with unusually high values for the two parameters making that group's means much higher than expected. The investigators also pointed out the absence of a dose response for these results, and they concluded that there was no significant treatment-related effect on hemoglobin or hematocrit results (see Table 1 below).

The report stated that in female mice there was a statistically significantly increased platelet count in the highest dosed group (1152×10^3 per cubic milimeter) compared to that for controls (788×10^3 per cubic milimeter) ($p<0.01$). According to the report, the value from the highest dosed group was within the normal range for the strain of mouse, and no similar effect was seen in male mice in the study. No effects on differential white cell counts were noted.

Table 1

Selected Hematology Results from Male Mice Given Diets Containing Technical Grade Thiophanate Methyl for 13 Weeks

Observation	Dose level (ppm) 0	160	640	2560	10,240
Hemoglobin (g/dl)	18.8	16.9 *	16.7 *	17.1	16.8 *
Hematocrit (%)	52.6	43.5 **	45.9 *	46.2 *	45.3 *

* Statistically significantly different from controls ($p<0.05$).
** Statistically significantly different from controls ($p<0.01$).

E. Clinical chemistry: The report noted that serum glucose levels in female mice given the 10,240 ppm diet were statistically significantly increased above control values. The group mean reported for the highest dosed group was 93 mg/dl and that for the control group was 52 mg/dl. The investigators noted that the female control value was well below that observed in control group males (80 mg/dl), and they stated that

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E. Clinical chemistry (continued)

the highest dosed group's value was well within the normal range for the strain of mice tested. There was also no dose response noted (52, 77, 53, 80, and 93 mg/dl for the 0, 160, 640, 2560, and 10,240 ppm dose groups, respectively).

No other clinical chemistry results in treated groups of male and female mice were significantly different from control group values.

F. Necropsy: At necropsy only sporadic findings were reported in test animals, and no gross lesions were noted to occur in a dose-related manner.

1. Organ weight: The only organ weights significantly affected by the test substance were those of the thyroid and liver. Tho absolute organ weight results are summarized as follows:

Observation	Organ weight (g) 0	160	640	2560	10,240
		Males			
Thyroid	0.0031	0.0027	0.0030	0.0044	0.0061 **
Liver	1.2181	1.3217	1.3538	1.4996 *	1.8048 **
		Females			
Thyroid	0.0025	0.0033	0.0029	0.0040	0.0067 **
Liver	1.0951	1.1290	1.1409	1.3847**	1.7015 **

* Statistically significantly different from controls ($p<0.05$).
** Statistically significantly different from controls ($p<0.01$).

Similar statistically significant increases in relative organ to body and brain weights were also reported.

2. Histopathology: There was a dose related increase in the incidence of hepatocellular hypertrophy in mice given the 2560 and 10,240 ppm dose groups. One male in the 2560 ppm group exhibited mild hypertrophy, and all mice given the highest dose level had moderate to marked hypertrophy.

The authors noted that two females in the highest dosed group had mild epithelial hypertrophy in the thyroid gland, and they concluded the effect may have been related to treatment.

No other effects were observed microscopically according to the report.

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Range Finding

III. DISCUSSION

Authors' conclusions: The authors concluded that there was no compound-related effect on body weight, body weight gain, food consumption, hematology, blood chemistry, or gross necropsy findings. The report further concluded:

> Compound-related clinical observations were limited to the high dose males and consisted of occasional salivation. Compound-related increases in liver and thyroid weights were noted. The thyroid was affected in the 10,240 ppm males and females and the liver was affected in the 10,240 and 2560 ppm males and females. These increases in absolute organ weights were accompanied by corresponding increases in organ weights relative to body and brain weight ratios. A subsequent microscopic examination was conducted on the livers from all animals in the control, 640, 2560, and 10,240 ppm groups and on the thyroid glands from the control, 160, 640, 2560, and 10,240 ppm groups. Hepatocellular hypertrophy was noted in one male in the 2560 ppm group and in all mice in the 10,240 ppm group and was attributed to administration of the test article. Two female mice in the 10,240 ppm group had minimal epithelial hypertrophy in the thyroid gland, which indicated a possible test article effect. Although the thyroid weights (the absolute weight and the absolute weight relative to brain weight ratios) were numerically increased at the 2560 ppm level, the differences from control were not statistically significant and microscopically, no test article related changes were found at this level.
>
> Based on the results of this study, the no-observed-effect level for Topsin M when fed to mice for 13 weeks was 640 ppm.

It should be noted that the authors dismissed toxicological significance for swollen testes observed during the study since there was no increase in testes weight, testes to brain weight ratio, or testes to body weight ratio.

Reviewer's conclusions: The liver weight results indicated that a NOEL of 640 ppm is appropriate. However, without significant effects on body weight gain (>10% less in a treated group than the weight gain in controls), the histological changes reported in the thyroid and liver are of questionable toxicological significance. The increased organ weights and hypertrophy in the thyroid and liver are likely to be reversible changes or effects test animals in a chronic study could adapt to. These conclusions are likely in view of results from two previously submitted mouse feeding studies.

The first study (MRID No. 00055935) was a 6-month feeding study that showed significant body weight decreases in females given an 8000 ppm diet. There were also increased absolute liver weights and liver-to-body weight ratios in that group. The NOEL established in the study was 1600 ppm (24 mg/kg/day).

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III. DISCUSSION (continued)

In the second study (MRID No. 00081611), five groups containing 50 male and 50 female ICR SLC strain mice were given diets containing 0, 10, 40, 160, or 640 ppm thiophanate methyl for two years. The results did not indicate the occurrence of toxic or oncogenic effects that could be attributed to administration of the test substance.

Based on the results from the 6-month study conducted previously and the minimal toxicity indicated in mice given 10,240 ppm for 13 weeks, the highest dose chosen for the chronic study should be greater than the 1280 ppm proposed.

IV. REFERENCES

00055935 Hashimoto Y., Noguchi, T., T. Makita, et al. 1970. Toxicological evaluation of thiophanate methyl: II. Studies on the subchronic oral toxicity of thiophanate methyl on mice. Unpublished report prepared by Nippon Soda Co., Ltd. Submitted by Pennwalt Corporation, Philadelphia, Pa.

00081611 Nishibe, T., T. Mori, T. Nukui, et al. 1973. The report on the carcinogenesis studies of thiophanate methyl, dimethyl 4,4'-o-phenylene-bis(3-thioallophanate), in mice of ICR SLC strain for 24 months. Unpublished report prepared by Nippon Soda Co., Ltd. Submitted by Pennwalt Corporation, Philadelphia, Pa.

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