MEMORANDUM:


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: Peter 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 1260 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
indicate the occurrence of toxic or oncogenic effects that could be attributed to administration of the test substance.

II. Submitted Information

On June 29, 1986, the Registrant (Pennwalt Corp.) submitted a 13-week range-finding study that was used as the basis for proposing doses of 3, 60, 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 plausible 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 thiophenate methyl in mice.

IV. References

APPENDIX

Data Evaluation Report for

Tompkins, E. C. June 6, 1988. 13-Week Dietary Image-Finding Study in
Mice with Topcin M. Unpublished Report No. WIL-75023 prepared by WIL
Research Laboratories, Inc. Submitted by Penwalt Corp., Agchem Division,
Philadelphia, PA. MRID No. L07453-01
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: Pemwalt 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): Torpkins, 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.
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:

<table>
<thead>
<tr>
<th>Test groups</th>
<th>Dose (ppm)</th>
<th>Animals per sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Designation</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Low (LDT)</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>Mid (1)</td>
<td>640</td>
</tr>
<tr>
<td>4</td>
<td>Mid (2)</td>
<td>2560</td>
</tr>
<tr>
<td>5</td>
<td>High (HDT)</td>
<td>10,240</td>
</tr>
</tbody>
</table>

2. **Observations schedule**

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

C. **METHODS**

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

- Hematocrit
- Hemoglobin
- Red cell count
- Platelet count
- Total white cell count

- Differential white cell counts
- Mean corpuscular hemoglobin concentration
- Mean cell volume
- Mean corpuscular hemoglobin

b. **Blood chemistry**

- Total protein
- Albumin
- Globulin (calculated)
- Albumin/globulin ratio
- Blood urea nitrogen
- Calcium
- Creatinine

- Uric acid
- Glucose
- Alkaline phosphatase
- Total cholesterol
- Total bilirubin
- Lactate dehydrogenase
- Aspartate aminotransferase (AST)
- Triglycerides
- Alanine aminotransferase (ALT)

2. **Necropsy** Gross lesions were noted.

a. **Weighed organs**

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

The thyroid glands were weighed after fixation.

b. **Tissues fixed for microscopic examination:**

<table>
<thead>
<tr>
<th>Circulatory System</th>
<th>Hematopoietic System</th>
<th>Reproductive System</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Aorta</td>
<td>X Bone marrow</td>
<td>X Epididymides</td>
</tr>
<tr>
<td>X Heart</td>
<td>X Lymph nodes</td>
<td>X Mammary glands</td>
</tr>
<tr>
<td></td>
<td>X Spleen</td>
<td>X Ovaries</td>
</tr>
<tr>
<td></td>
<td>X Thymus</td>
<td>X Prostate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Digestive System</th>
<th>Musculoskeletal System</th>
<th>Nervous System</th>
<th>Respiratory System</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Cecum</td>
<td>X Bone</td>
<td>X Brain</td>
<td>X Lungs</td>
</tr>
<tr>
<td>X Colon</td>
<td>X Skeleton muscle</td>
<td>X Peripheral nerve</td>
<td>X Nasal turbanates</td>
</tr>
<tr>
<td>X Duodenum</td>
<td></td>
<td>X Spinal cord</td>
<td>X Trachea</td>
</tr>
<tr>
<td>X Esophagus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Ileum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Jejunum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Pancreas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Rectum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Salivary gland</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Stomach</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X Gallbladder</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
b. Tissues fixed for microscopic examination (continued)

<table>
<thead>
<tr>
<th>Endocrine System</th>
<th>Other</th>
<th>Urinary System</th>
</tr>
</thead>
<tbody>
<tr>
<td>X Adrenals</td>
<td>X All macroscopic abnormalities</td>
<td>X Kidneys</td>
</tr>
<tr>
<td>X Pituitary</td>
<td>X Eye</td>
<td>X Urinary bladder</td>
</tr>
<tr>
<td>X Thyroid with parathyroid</td>
<td>X Parotid gland</td>
<td>X Skin and subcutis</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Observation</th>
<th>Statistical Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weights, weight changes, clinical chemistry values, absolute and relative organ weights</td>
<td>One-way analysis of variance (ANOVA) followed by Dunnett's test</td>
</tr>
</tbody>
</table>

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 dose 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:
C. Test substance intake (continued)

<table>
<thead>
<tr>
<th>Dose level (ppm)</th>
<th>Calculated test substance intake (mg/kg/day)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>160</td>
<td>26.2</td>
<td>39.3</td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>107.2</td>
<td>182.0</td>
<td></td>
</tr>
<tr>
<td>2,560</td>
<td>423.0</td>
<td>562.6</td>
<td></td>
</tr>
<tr>
<td>10,240</td>
<td>1871.2</td>
<td>2200.7</td>
<td></td>
</tr>
</tbody>
</table>

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 \((1152 \times 10^3\) per cubic millimeter) compared to that for controls \((788 \times 10^3\) per cubic millimeter) \(p<0.01\). 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

<table>
<thead>
<tr>
<th>Observation</th>
<th>0</th>
<th>160</th>
<th>640</th>
<th>2560</th>
<th>10,240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>15.4</td>
<td>16.9 *</td>
<td>16.7 *</td>
<td>17.1</td>
<td>16.8 *</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>52.5</td>
<td>43.5 **</td>
<td>45.9 *</td>
<td>46.2 *</td>
<td>45.3 *</td>
</tr>
</tbody>
</table>

* 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
F. Clinical chemistry (continued)

the highest dose 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. The absolute
organ weight results are summarized as follows:

<table>
<thead>
<tr>
<th>Observation</th>
<th>0</th>
<th>160</th>
<th>640</th>
<th>2560</th>
<th>10,240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.0031</td>
<td>0.0027</td>
<td>0.0030</td>
<td>0.0044</td>
<td>0.0061 **</td>
</tr>
<tr>
<td>Liver</td>
<td>1.2181</td>
<td>1.3217</td>
<td>1.3539</td>
<td>1.4996 *</td>
<td>1.8048 **</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.0025</td>
<td>0.0033</td>
<td>0.0029</td>
<td>0.0040</td>
<td>0.0067 **</td>
</tr>
<tr>
<td>Liver</td>
<td>1.0951</td>
<td>1.1290</td>
<td>1.1409</td>
<td>1.3847**</td>
<td>1.7015 **</td>
</tr>
</tbody>
</table>

* 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.
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 (FRID 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).
III. DISCUSSION (continued)

In the second study (WRID 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
