DATA EVALUATION REPORT

THIOPHANATE METHYL

(135)

STUDY TYPE: SPECIAL THYROID AND LIVER MECHANISTIC STUDIES

Prepared for

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

Prepared by

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Task Order No. 94-34A

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Attachment 5

DER of the Special Mechanistic Studies in the Thyroid and Liver

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DATA EVALUATION RECORD

STUDY TYPE: Special Thyroid and Liver Mechanistic Studies, Supplement to Chronic Feeding/Oncogenicity Study in Rats (§83-5)

DP BARCODE: D226120, D245095
P.C. CODE: 102001

SUBMISSION CODE: S505312
TOX. CHEM. NO.: 375A

TEST MATERIAL (PURITY): Thiophanate methyl (96.55%)

SYNONYMS: Topsin-M, dimethyl-4,4'-(o-phenylene)-bis(3-) thioallophanate, dimethyl[1,2-phenylene- bis (iminocarbonothioyl)]-bis(carbamate)

CITATION: Nishibe, T. and H. Takaori (1996) Mechanistic investigation in rats and mice of thiophanate-methyl on the thyroid and liver. Toxicology Institute, Environmental Toxicology Laboratory, Nippon Soda Co., LTD., 345 Takada, Odawara, Kanagawa, Japan. Laboratory Project ID 0248 and 8082 (Supplement to Project ID 0566), March 12, 1996. MRID 42896601b. Unpublished.

SPONSOR: Nippon Soda Co., LTD., 2-1, 2-Chome, Ohtemachi, Chiyodaku, Tokyo, Japan

EXECUTIVE SUMMARY: The mechanism for thiophanate methyl-induced thyroid changes was investigated in a series of six studies supplementing a two-year oncogenicity study (MRID 42896601). In the oncogenicity study, absolute and relative liver weights were significantly increased at 12 and 24 months in rats fed diets containing 1200 ppm or 6000 ppm thiophanate-methyl. Microscopic examination of the liver from both sexes showed centrilobular hypertrophy and lipofuscin pigmentation. In addition, the total cholesterol of male and female rats in the 6000 ppm treatment groups was elevated throughout the 2-year study. Other relevant findings included a significant dose-dependent increase of thyroid follicular cell adenomas; a non-statistically significant increase of thyroid follicular cell adenocarcinoma in male rats fed diets containing 6000 ppm test material; and a non-significant increase of follicular cell adenoma in female rats fed 6000 ppm. The thyroid neoplasia was correlated with various clinical, macroscopic, and microscopic changes such as decreased T_3 and T_4 and increased TSH levels, as well as

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diffuse and focal thyroid follicular cell hyperplasia and hypertrophy in male and female rats receiving diets containing 1200 ppm thiophanate-methyl.

The design of the supplementary studies follows: In the first study, treatment-related hormonal and organ weight changes were studied in male rats and mice following 8 days of dietary administration of thiophanate methyl (96.55%, Lot TIF-1016), phenobarbitol (PB), and propylthiouricil (PTU). In the second study, the reversibility of treatment-related increased thyroid weights was examined in male and female rats, with 8-day dietary administration of thiophanate-methyl followed by an 8-day recovery period. The third study was designed to determine if T_4 supplementation altered the effect of dietary treatment (changes in organ weights, TSH, and serum cholestrol levels) with thiophanate-methyl in male rats. In the fourth study, the mechanism of treatment-related liver hypertrophy was examined; microsomal protein concentration and liver enzyme levels (cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase, and UDP-glucuronosyltransferase) were evaluated following 8 days of thiophanate-methyl or phenobarbitol treatment in rats. The fifth study examined the effect of thiophanate-methyl or propylthiouricil on microsomal thyroid peroxidase in porcine thyroid tissue *in vitro*. The sixth study was performed to examine the mechanism of treatment-related liver hypertrophy in male rats and mice following 8 days of dietary treatment and 8 days of recovery, via histopathological examination of hepatic tissue sections stained for proliferating cell nuclear antigen.

In summary, treatment with thiophanate-methyl induced increased absolute and relative liver and thyroid weights, decreased circulating thyroid hormone levels (T_3 and T_4), and increased TSH. The results of these studies support the conclusion that the primary thyroid changes induced in rats following dietary exposure to 6000 ppm thiophanate methyl per day results from increased biliary clearance of T_3 and T_4 through the induction of UDP-glucuronosyltransferase. Because of the increased elimination of the thyroid hormones, TSH is increased and the prolonged stimulation of the thyroid results in the increased neoplasia. Although it was found that thiophanate methyl could inhibit thyroid peroxidase, a mechanism that would also induce primary thyroid changes, it was believed that this plays a minor role and is secondary to the increased metabolism and excretion of the thyroid hormones.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thiophanate methyl (TM)

Description: pale brown powder (MRID 42896601)
Lot/Batch #: TIF-1016
Purity: 96.55% a.i.
CAS #: 23564-05-8 (MRID 42896601)

2. Vehicle and/or positive control

The test material was ground and mixed into the diet. Phenobarbital (PB, drug metabolism inducer) and propyl-thiouracil (PTU, thyroid peroxidase inhibitor) were used as positive effect liver and thyroid controls, respectively.

3. Test animals

Source: Charles River Breeding Laboratories, Atugi, Kanagawa, Japan
Acclimation period: Not reported
Diet: basal (M Diet, Oriental Yeast Inc., Tokyo) plus test or control material, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 21-24EC
Humidity: 40-80%
Air changes: 10 times/hour
Photoperiod: 12 hour light/dark cycle
Species, strain, age, and weight at study dosing:
Study 1
Species and Strain: F344 Rats
Age and weight at dosing: Six week-old males (105-145 g)
Study 2
Species and Strain: F344 Rats
Age and weight at dosing: Seven week-old females (105-135 g)
Study 3
Species and Strain: F344 Rats
Age and weight at dosing: Eight week-old males (173-187 g)
Study 6
Species and Strain: ICR Mice
Age and weight at dosing: Eleven week-old males (39-47 g)
Species and Strain: F344 Rats
Age and weight at dosing: Eleven week-old males (238-262 g)

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B. STUDY DESIGN AND METHODS

1. In life dates

Start: November 1990; end: June 1992

2. Study designs

Study 1: This study was designed to measure the treatment-related hormonal and organ weight changes over an 8-day period. Four groups of 10 six-week-old male rats/group were fed either the basal diet or diets containing 6000 ppm TM, 500 ppm PB, or 1000 ppm PTU up to 8 days. Half the rats in each treatment group were sacrificed on study day 2 and the remainder on study day 8. At each sacrifice, the body, liver, and thyroid weights were measured and blood samples collected for TSH, T_4, T_3, and total cholesterol analysis.

Study 2: This study was designed to determine the reversibility of treatment-related increased thyroid weights. Three groups of 10 seven-week-old female rats/group were fed either the basal diet or diets containing 6000 ppm TM or 500 ppm PB for 8 days. All rats received basal diet for the remainder of the 16-day study. Half the rats in each group were sacrificed on study day 8 and the remainder on study day 16. At each sacrifice, the body and thyroid weights were measured.

Study 3: This study was designed to determine if T_4 supplementation altered the effect of TM treatment. Two groups of 10 eight-week-old male rats/group were fed either the basal diet or diets containing 6000 ppm TM for 8 days. Half the animals in the control group and treatment groups received no additional treatment while the other half received daily injections of 30 μ/kg L-thyroxine. On study day 8, all rats were sacrificed; the body, thyroid, and liver weights were measured, and blood samples were collected for TSH and serum total cholesterol analysis.

Study 4: This study was designed to investigate the mechanism of treatment-related liver hypertrophy. Homogenates and microsomes of four livers/group from the control, TM, and PB treatment groups collected on day 8 of Study 1 were prepared. The microsomal protein concentration and the activities of cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase, and UDP-glucuronosyltransferase were measured.

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Study 5: The effect of 10^{-3} to 10^{-4} M TM (in DMSO) or 10^{-4} to 10^{-6} M PTU (dissolved in purified water) on porcine microsomal thyroid peroxidase using the Guaiacol method was investigated. The thyroids (20 g) were purchased from a meat packing facility and stored at -80°C until use.

Study 6: This study was performed to examine the mechanism of treatment-related liver hypertrophy caused by TM. Three groups of 10 eleven-week-old male ICR mice and three groups of 10 eleven-week-old F344 male rats were fed the basal diet or diets containing 6000 ppm TM or 500 ppm PB up to 8 days. Two days after the start of the study, half the animals in each group were sacrificed. The remainder of the animals were sacrificed on study day 8. At each sacrifice, the livers were removed, fixed in 4°C methanol, embedded in paraffin, sectioned, and stained for proliferating cell nuclear antigen (PCNA).

3. Statistics

Parametric and nonparametric analyses were done using the two-tailed Student's t-test or the Aspin-Welch test, respectively. Statistical analyses of PCNA were done using the Mann-Whitney U-test.

II. RESULTS

Study 1: Neither TM nor PB treatment significantly influenced the body weight of rats, although PTU treatment statistically ($p<0.05$) decreased the body weight 18%-19% from control at both observations (Table 1). The absolute liver weight of rats treated with PTU was statistically ($p<0.05$) decreased approximately 24% after 2 days of treatment. When expressed as the liver to body weight ratio, however, no PTU treatment-related effects on the liver were apparent. No statistical effect on liver weight was noted after 8 days of PTU treatment, although the liver to body weight ratio was statistically ($p<0.001$) increased. In contrast, both the liver weight and liver to body weight ratios of rats treated with TM and PB rats were statistically increased ($p<0.05$). The liver weight and liver to body weight ratios of rats treated with TM were increased 28-33% and 46-47% at the 2 and 8 day observations respectively, while the liver weight and liver to body weight of PB treated rats was increased 37% after 8 days of treatment. Likewise, the total cholesterol of rats treated with TM (day 2-33%, day 8-54%) and PB (day 8-23%) were statistically increased ($p<0.01$).

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Both TM and PTU had significant effects on the thyroid and indicators of thyroid function. After 8 days of treatment, the absolute and relative thyroid weights were increased >130% for both treatment groups. PB had no apparent effect on absolute or relative thyroid weight. Within 48 hours, TM treatment had statistically decreased T_3 and T_4 and increased TSH levels of the rats. These statistical differences persisted through study day 8 for T_3 and TSH, although no statistical difference of T_4 was found at this time. Likewise, PTU treatment statistically decreased T_3 and increased TSH levels at both the 2 and 8-day observations and T_4 at the study day 8 observation. Although increases in T_3 and T_4 were observed on day 8, no biologically significant indicators of thyroid dysfunction were found in rats treated with PB.

Table 1. Effect of TM, PTU, and PB treatment on male rats after 2 or 8 days of treatment[a]

Parameter	Day	Control (0 ppm)	TM (6000 ppm)	PTU (1000 ppm)	PB (500 ppm)
Body Weight (g)	2	138.7 ± 17.6	133.2 ± 15.3	112.7 ± 7.4* (-18.7%)[b]	ND
	8	173.1 ± 23.0	171.4 ± 22.3	141.2 ± 11.9* (-18.4%)	173.6 ± 20.0
Liver Weight (g)	2	6.429 ± 1.031	8.217 ± 1.020* (27.8%)	4.908 ± 0.559* (-23.6%)	ND
	8	7.617 ± 1.184	11.097 ± 1.647** (45.7%)	7.300 ± 0.886 (-4.2%)	10.443 ± 1.414** (37.1%)
Liver to Body Weight Ratio[c]	2	4.62 ± 0.251	6.17 ± 0.197*** (33.5%)	4.36 ± 0.4407 (-5.6%)	ND
	8	4.39 ± 0.188	6.46 ± 0.128*** (47.1%)	5.16 ± 0.301*** (17.5%)	6.01 ± 0.264*** (36.9%)
Thyroid Weight (mg)	2	17.6 ± 3.5	18.7 ± 2.4 (6.3)	20.9 ± 1.2 (18.8%)	ND
	8	23 ± 4	53 ± 7*** (130%)	65 ± 2*** (183%)	25 ± 4 (8.7%)
Thyroid to Body Weight Ratio[c]	2	0.0127 ± 0.002	0.0140 ± 0.0009 (10.2%)	0.0186 ± 0.0005*** (46.5%)	ND
	8	0.0135 ±.0032	0.0314 ± 0.0046*** (133%)	0.0462 ± 0.0027*** (242%)	0.0146 ± 0.0029 (8.1%)
T_3 (ng/dl)	2	92.8 ± 9.1	56.1 ± 10.2*** (-39.2%)	38.0 ± 5.1***[d] (-59.1%)	ND
	8	94.6 ± 3.1	79.9 ± 2.7*** (-15.9%)	24.5 ± 2.1*** (-74%)	106.9 ± 6.1** (13.0%)
T_4 (µg/dl)	2	5.52 ± 0.533	3.36 ± 0.677*** (-39.2%)	4.81 ± 0.918[d] (-12.9%)	ND
	8	5.64 ± 0.630	4.97 ± 0.717 (-11.8%)	1.89 ± 0.273*** (-66.5%)	6.16 ± 0.353 (9.1%)
TSH (ng/100 µL)	2	0.467 ± 0.138	1.100 ± 0.531**[c] (136%)	2.006 ± 0.667**[d] (329%)	ND
	8	0.479 ± 0.067	2.374 ± 0.835**[c] (396%)	5.429 ± 1.288***[c] (1033%)	0.626 ± 0.059** (30.7%)
Cholesterol (mg/dl)	2	69.8 ± 6.3	92.7 ± 7.7 *** (32.8%)	69.6 ± 10.8	ND
	8	58.3 ± 3.3	89.7 ± 10.9**[c] (53.9%)	89.5 ± 7.7*** (53.5%)	71.8 ± 4.0*** (23.2%)

[a]From study report, page 6.
[b]Results in parenthesis are percent change from control calculated by reviewer
[c]Calculated by reviewer as (organ weight/body weight) × 100 followed by ANOVA and Dunnett's
[d]4 animals/group
N=5 animals/group; ND = Not determined
*p≤0.05, **p≤0.01, ***p≤0.001, two-tailed Student's t-test; [c]p≤0.05, [cc]p≤0.01, [ccc]p≤0.001, two-tailed Aspin-Welch test

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Study 2: Treatment of female rats with 6000 ppm TM in the diet for 8 days statistically ($p<0.001$) increased the absolute and relative thyroid weights (Table 2) while PB had no biologically significant effect during the same interval. On study day 16, 8 days after removal of the test material from the diet, the thyroid weights had regressed and no statistical difference in absolute or relative thyroid weight was found between TM and control rats. Neither TM nor PB treatment significantly affected body weight during the study.

Table 2. Reversible effect of TM on the thyroid weight of female rats[a]

Parameter	Day	Control(0 ppm)	TM (6000 ppm)	PB (500 ppm)
Thyroid weight (mg)	8	15 ± 5	36 ± 8*** (140%)[b]	17 ± 2 (13%)
	16	21 ± 5	25 ± 4 (19%)	20 ± 2 (-5%)
Thyroid to Body Weight Ratio[c]	8	0.012 ± 0.004	0.028 ± 0.005** (133%)	0.013 ± 0.002 (8%)
	16	0.013 ± 0.003	0.015 ± 0.003 (15%)	0.012 ± 0.001 (-8%)
Body weight (g)	8	127.9 ± 6.8	129.4 ± 5.8	135.3 ± 6.3
	16	158.4 ± 8.0	162.7 ± 12.1	165.4 ± 6.6

[a]From study report, page 7.
[b]Results in parenthesis are percent change from control calculated by reviewer
[c]Calculated by reviewer as (organ weight/body weight) × 100 followed by ANOVA and Dunnett's
N=5 for all groups
*p≤0.01, Dunnett's Test; ***p≤0.001, Student's t-test

Study 3: T_4 supplementation did not affect the body weight of TM-treated rats, however, liver weight and total cholesterol were significantly ($p<0.001$) increased 38-42% and 33-47%, respectively, relative to their corresponding controls (Table 3). The TSH of treated rats receiving daily T_4 injections was not statistically different from controls, although it was statistically increased ($p<0.05$) 361% in rats not receiving supplementation. Likewise, the absolute and relative thyroid weights of rats not receiving daily T_4 supplementation were statistically ($p<0.05$) increased approximately 130% over their respective control and treated rats.

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Table 3. Effect of daily T_4 supplementation on male rats fed TM[a]

Parameter	Control	Control + 30 μ g/kg T4	TM (6000 ppm)	TM (6000 ppm) + 30 μ g/kg T_4
Body Weight (g)	216.6 ± 7.6	208.5 ± 8.5	210.7 ± 8.1	207.3 ± 8.8
Thyroid Weight (mg)	19.2 ± 4.7	17.6 ± 2.5	43.8 ± 4.7*** (128%)[b]	19.4 ± 4.9 (10%)
Thyroid to Body Weight Ratio[c]	0.0088 ± 0.0020	0.0085 ± 0.0014	0.0208 ± 0.0024*** (136%)	0.0093 ± 0.0022 (9%)
TSH (ng/100 μ L)	0.503 ± 0.088	0.326 ± 0.022##	2.319 ± 0.93# (361%)	0.506 ± 0.158 (55%)
Liver Weight (g)	8.09 ± 0.389	7.563 ± 0.526	11.160 ± 0.697*** (38.0%)	10.734 ± 0.735*** (41.9%)
Liver to Body Weight Ratio[c]	3.73 ± 0.09	3.63 ± 0.14	5.29 ± 0.15*** (42%)	5.18 ± 0.24*** (43%)
Total Cholesterol (mg/dl)	50.8 ± 3.5	47.0 ± 3.8	67.8 ± 6.1*** (33%)	69.1 ± 5.2*** (47%)

[a]From study report, page 7.
[b]Results in parenthesis are percent difference from respective control, calculated by reviewer
[c]Calculated by reviewer as (organ weight/body weight) × 100
N=5
***$p \leq 0.001$, two-tailed Student's t-test; #$p \leq 0.05$, ##$p \leq 0.01$, two-tailed Aspin-Welch test

Study 4: The hepatic microsomal protein and enzyme activities of male rats receiving diets containing 6000 ppm TM for 8 days were induced similarly to the microsomal protein and enzyme activities of rats treated with PB (Table 4). In contrast with PB, however, the activity of NADPH-cytochrome c reductase was not increased with TM treatment.

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Table 4. Hepatic microsomal enzyme activities of rats fed TM or PB for 8 days[a]			
Parameter	Control (0 ppm)	TM (6000 ppm)	PB (500 ppm)
Cytochrome P-450[b]	0.62 ± 0.05	1.01 ± 0.05*** (62.9%)[c]	1.16 ± 0.09*** (87.1%)
Cytochrome b5[b]	0.44 ± 0.03	0.72 ± 0.05*** (63.6%)	0.6 ± 0.02*** (36.3%)
NADPH-cytochrome c reductase[c]	433 ± 45	453 ± 94 (4.6%)	590 ± 47** (36.3%)
UDP-glucuronosyltransferase[d]	20.58 ± 3.85	69.23 ± 19.53[#] (236%)	43.94 ± 6.69*** (114%)
Protein[e]	21.3 ± 0.9	25.1 ± 1.7** (17.8%)	26.9 ± 2.2** (26.3%)

[a]From study report, page 7.
[b]nmol/mg; [c]nmol/min/mg microsomal protein; [d]mg/g liver
[e]Results in parenthesis are percent difference from control, calculated by reviewer
**p≤0.01, ***p≤0.001 two-tailed Student's t-test; [#]p≤0.05 two-tailed Aspin-Welch test
N=4/group

Study 5: While TM decreased porcine thyroid peroxidase activity *in vitro*, the effective dose to achieve 50% inhibition (ED_{50}) was approximately 30-fold greater than that required by PTU (Table 5).

Table 5. Effect of TM and PTU on microsomal thyroid peroxidase activity[a]		
	TM	PTU
ED_{50}	6×10^{-4} M	2×10^{-5} M
ED_0	8×10^{-5} M	4×10^{-7} M

[a]From study report, page 8.

Study 6: Hepatic PCNA of rats and mice treated with TM was increased two days after treatment. This suggests the 20% increase of liver weight found following treatment was the result of hyperplasia (Table 6). In mice but not rats, the hyperplasia was sustained through study day 8.

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Table 6. Hepatocellular proliferation in mice and rats 2 and 8 days after treatment with TM and Pb[a]

Species	Parameter	Day	Control (0 ppm)	TM (6000 ppm)	PB (500 ppm)
Mice	PCNA[b]	2	3 ± 3	27 ± 11[ss]	175 ± 11[ss]
		8	0 ± 0	21 ± 13[ss]	22 ± 11[ss]
	Liver weight (g)	2	2.048 ± 0.183	2.495 ± 0.276* (21.8%)[d]	2.489 ± 0.169** (21.5%)
		8	2.095 ± 0.095	2.672 ± 0.200*** (27.5%)	2.847 ± 0.266[##] (35.9%)
	Liver to Body Weight Ratio	2	4.98 ± 0.41	5.95 ± 0.44** (19.5%)	5.89 ± 0.28** (18.3%)
		8	5.07 ± 0.28	6.33 ± 0.29** (24.9%)	6.82 ± 0.53*** (34.5%)
Rats	PCNA[c]	2	19 ± 6	113 ± 24[ss]	61 ± 21[ss]
		8	20 ± 6	12 ± 6	17 ± 6
	Liver weight (g)	2	9.740 ± 0.254	11.679 ± 0.520*** (19.9%)	11.273 ± 0.401*** (15.7%)
		8	10.109 ± 0.618	13.192 ± 0.601*** (30.4%)	12.665 ± 0.479*** (25.3%)
	Liver to Body Weight Ratio	2	3.75 ± 0.117	4.60 ± 0.174*** (22.7%)	4.25 ± 0.116*** (13.3%)
		8	3.62 ± 0.172	4.88 ± 0.178*** (34.8%)	4.53 ± 0.168*** (25.1%)

[a]From study report, page 8.
[b]0.28 mm² H 20 fields (total 5.6 mm²); N=5/group
[c]0.05 mm² H 20 fields (total 1 mm²); N=5/group
[d] Results in parenthesis are percent difference from control, calculated by reviewer
[ss]p≤0.01 Mann-Whitney U-test; *p≤0.05, **p≤0.01, ***p≤0.001 two-tailed Student's t-test; [##]p≤0.01 two-tailed Aspin Welch test

III. DISCUSSION

The above studies supplement a 2-year chronic rat feeding/oncogenicity study (MRID 42896601). In that study, the absolute and relative liver weights were statistically ($p<0.05$) increased at 12 and 24 months in rats fed diets containing 1200 ppm or 6000 ppm TM. Microscopic examination of the liver from both sexes showed centrilobular hypertrophy and lipofuscin pigmentation. In addition, the total cholesterol of male and female rats in the 6000 ppm treatment groups was elevated throughout the 2-year

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study. Other relevant findings included a statistical ($p<0.01$) and dose-dependent increase of thyroid follicular cell adenomas; a non-statistically significant increase of thyroid follicular cell adenocarcinoma in male rats fed diets containing 6000 ppm test material; and a non-significant increase of follicular cell adenoma in female rats fed 6000 ppm TM. The thyroid neoplasia was correlated with various clinical, macroscopic, and microscopic changes such as decreased T_3 and T_4 and increased TSH levels, as well as diffuse and focal thyroid follicular cell hyperplasia and hypertrophy in male and female rats receiving diets containing 1200 ppm TM.

The chronic oncogenicity and supplemental studies clearly show TM to have significant effects on thyroid function that likely lead to the formation of thyroid follicular cell adenomas and adenocarcinomas. Based on the results of the supplemental studies, the study authors speculated the underlying mechanism of tumor formation was PTU-like inhibition of thyroid hormone synthesis. It is the opinion of the reviewer that although TM thyroid peroxidase inhibition may contribute, it is not the principal cause for the induced thyrotoxicity. The reviewer feels the primary cause of the induced thyroid changes arises from increased hepatic thyroid hormone excretion.

In studies 1, 3, and 6, TM had no significant effect on body weight but statistically increased the absolute and relative liver weight of male rats 20-46% in a time-dependent manner after 2 and 8 days of treatment. The results were similar to those obtained with PB. In contrast, PTU decreased the body weight of rats and induced a more modest 18% increase of relative liver weight after 8 days of treatment. All three compounds statistically increased serum cholesterol, indicative of hepatic congestion; however, it is questionable whether any of the increases were biologically relevant.

Both TM and PTU treatment had significant effects on thyroid function indicators. PTU and TM increased the absolute and relative thyroid weights of treated rats; however, the increase by TM was not as dramatic as PTU (Table 1). There were also differences between the two compounds in the manner they decreased thyroid hormone concentration. After two days of PTU treatment, the serum concentration of T_3 and T_4 were decreased 59% and 13% of control, respectively. Their concentrations further declined to 74% and 66% of control, respectively, by day 8. In contrast, TM initially decreased both T_3 and T_4 concentrations approximately 40% by day 2, but by day 8 the concentrations of these hormones, while still decreased, were a modest 12-16% reduction relative to control. Both TM and PTU increased TSH concentration; however, the increase induced by PTU was

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approximately 2.5 fold greater than that of TM. PB did not significantly alter indicators of thyroid function.

Study 4 demonstrated that the hepatic microsomal enzyme activities of TM-treated rats were all increased. The induction of the microsomal enzymes and the increase of hepatic protein were consistent with PB and phenobarbital-like inducers. Of particular note was the 236% increase of UDP-glucuronosyl-transferase activity of TM-treated rats relative to controls. The induction of this enzyme is of particular importance since approximately half of the T_4 is eliminated in the bile following glucuronidation of the phenolic hydroxyl group in rats. Sulphation also occurs, particularly with T_3, but unfortunately the activities of these enzymes were not investigated. Among other effects, phenobarbital-like inducers typically produce a significant increase in liver weight arising from the proliferation of hepatic endoplasmic reticulum and hyperplasia. They also affect thyroid function by altering the peripheral disposition of T_4 by increasing bile flow, hepatic binding of T_4, the bile to plasma T_4 ratio, and the biliary clearance of T_4 in rats. The increased T_4 turnover results in the enhanced and prolonged secretion of pituitary TSH. It has been generally accepted that the hormonal feedback mechanism leading to increased TSH plays a significant role in the etiology of thyroid carcinogenesis due to thyroid follicular cell hypertrophy and hyperplasia.

Other than showing that TM can inhibit thyroid peroxidase activity, study 5 did little to help determine the mechanism. The study did show that the ED_{50} of PTU was 30 fold greater than TM. But the study did not determine the effect of PB on the enzyme or explain whether the inhibition was reversible or nonreversible and competitive or noncompetitive. Without knowing the reversibility or type of inhibition, a useful interpretation of the results of study 5 can not be made.

In conclusion, the reviewer feels that the results of the supplemental studies indicate that the primary thyroid changes induced by TM is a result of increased biliary excretion of T_3 and T_4. This conclusion is supported by the increased liver weight and liver to body weight ratios found in studies 1, 3, and 6; documentation from study 6 that the increased liver weights were a function of both hypertrophy and hyperplasia; and the dramatically increased activity of microsomal UDP-glucuronosyltransferase. Additional support comes from the modest decrease of circulating T_3 and T_4 and increase of TSH relative to PTU two days after treatment followed by the partial recovery of circulating levels by day 8 in TM-treated animals (Study 1). These prolonged perturbations of thyroid homeostasis

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from TSH result in the increased incidence of thyroid neoplasia found in the oncogenicity study.

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