CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

THIOPHANATE-METHYL

FINAL REPORT

24-AUGUST-1999

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: _____
Linnea J. Hansen, Biologist

DOCUMENT PREPARATION: _____
Sanjivani B. Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke _____

William Burnam _____

Marion Copley _____

Vicki Dellarco _____

Virginia Dobozy _____

Linda Taylor _____

Esther Rinde _____

Joycelyn Stewart _____

Clark Swentzel _____

Yin Tak Woo _____

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Lucas Brennecke, Pathology Consultant _____

Lori Brunsman, Statistical Analysis _____

OTHER ATTENDEES:

Alberto Protzel HED/Toxicology Branch

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EXECUTIVE SUMMARY

On April 28, 1999 the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of thiophanate-methyl. The studies evaluated included a 2-year toxicity/carcinogenicity study in Fischer 344 rats and an 18-month carcinogenicity study in CD-1 albino mice as well as mechanistic studies submitted by the registrant to support the non-linear mode of action for thyroid tumor induction.

In the chronic toxicity/carcinogenicity study, 50 F344 rats/sex/dose received thiophanate-methyl in the diet at 0, 75, 200, 1200 or 6000 ppm equivalent to (0, 3.3, 8.8, 54.4 or 280.6 mg/kg/day for males and 0, 3.8, 10.2, 63.5 or 334.7 mg/kg/day, for females, respectively) for 24 months.
In a carcinogenicity study in mice, thiophanate-methyl was administered in the diet to male and female CD-1 albino mice (50/sex/group) at 0, 150, 640, 3000 or 7000 ppm (equivalent to 0, 4.0, 93.4, 393.6 or 943.5 mg/kg/day for males and 0, 6.5, 153, 634.9 or 1388.2 mg/kg/day for females, respectively) for 18 months.

The CARC concluded that

- Thiophanate-methyl was carcinogenic to rats because 1) male rats had statistically significant increases in the pair-wise comparison of 6000 ppm dose group with the controls, for thyroid follicular cell adenomas and carcinomas as well as combined adenomas and/or carcinomas. A statistically significant increasing trend was also observed. The increase in the incidence of adenomas at 1200 ppm was considered to be treatment-related because of the progressive nature of the tumor and evidence of a dose-response; 2) in female rats, although there were no significant differences in the pair-wise comparisons of the dosed groups with the controls, there was a significant increasing trend for thyroid follicular cell adenomas. Appropriate historical control data were not available for comparison; 3) the same spectrum of effects was seen in both sexes including same target organ effects, calcium deposition in various organs and progressive toxicity seen at 12 month onwards.

  The dosing was considered to be adequate in both sexes at 1200 ppm based on decrease in body weight gain (in males) and increase in liver, thyroid and kidney weights. Dosing was considered excessive in males at 6000 ppm due to high mortality and severity of toxicity in various organs. Some members of the CARC also considered this as an excessive dose in females due to large decreases in body weight gain and evidence of systemic mineralization.

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The CARC considered the thyroid tumors in males (at 1200 and 6000 ppm) and females (at 6000 ppm) to be treatment-related because a dose-response was seen in both sexes. The increase in TSH and decrease in T3 and T4 levels seen at 6000 ppm were suggestive of interference with thyroid-pituitary homeostasis. Registrant's submitted mechanistic studies did not demonstrate the reversibility of the effect on thyroid hormones and were conducted only at 6000 ppm, a dose that was excessively toxic to male rats in the 2-year study and also did not establish a dose-response.

- Thiophanate-methyl was carcinogenic to male and female CD-1 mice because 1) male mice had statistically significant increases in the pair-wise comparisons of the 3000 and 7000 ppm dose groups with the controls, for liver adenomas, and combined adenomas, carcinomas and/or hepatoblastomas (at 7000 ppm only). A significant increasing trend was also observed for these tumors; 2) female mice had statistically significant increases in the pair-wise comparisons of the 640, 3000 and 7000 ppm dose groups with the controls, for liver adenomas, and 3) the incidences of adenomas in males at 3000 and 7000 ppm and in females at 640, 3000 and 7000 ppm as well as carcinomas in males at 7000 ppm were outside the historical control ranges. The dosing was considered to be adequate based on decrease in body weight in both sexes, increase in liver and thyroid weights and histopathological changes in the liver, thyroid and heart at 3000 and 7000 ppm. Although the 7000 ppm dose may have been excessively toxic this dose level was not excluded from quantitative risk assessment because it was part of a continuing dose-response in both sexes and exceeded historical control values.

Thiophanate-methyl has been tested in *in vitro* and *in vivo* genotoxicity assays. It shows potential as an aneugen (i.e., causes changes in chromosome number) but does not appear to act as a clastogen (i.e., chromosome breaking). However, since the available studies do not satisfy the guideline requirements and do not exclude the possibility of direct DNA reactivity or mutagenicity, the CARC requested additional studies on thiophanate-methyl as well as on 2-aminobenzimidazole metabolite.

Structurally-related compounds including Benomyl and Methyl-2-benzimidazole carbamate are aneugenic and cause hepatocellular tumors; however, neither cause thyroid effects. Thiophanate-methyl shares a thiourea moiety with propylthiouracil, a known inducer of thyroid tumors in rats and mice.

The CARC classified thiophanate-methyl as **"likely to be carcinogenic to humans "** and recommended a linear low-dose approach for human risk characterization based on the most potent of the two tumor types i.e., liver tumors in mice.

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I. INTRODUCTION

On April 28, 1999, the Cancer Assessment Review Committee (CARC) met to evaluate the carcinogenic potential of thiophanate-methyl.

Dr. Linnea Hansen of the Toxicology Branch described the chronic toxicity/carcinogenicity study in Fisher 344 rats and an 18-month carcinogenicity study in CD-1 mice by detailing the experimental designs; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of dose levels tested; and presented the weight-of-the-evidence for the carcinogenicity of thiophanate-methyl. Dr. Hansen also discussed the findings of the mechanistic studies submitted by the registrant in support of the hormonal mechanism of thyroid tumor induction.

II. BACKGROUND INFORMATION

Thiophanate-methyl is a broad-spectrum systemic protectant fungicide. The PC Code No. is 102001 and the CAS No. is 23564-05-8. Its chemical name is dimethyl-4-4'-(o-phenylene)-bis(3-thioallophanate) or dimethyl[1,2-phenylene-bis(iminocarbonothioyl)]bis (carbamate). The chemical structure is shown below, Figure 1. The molecular weight is 342.4 g.

Current uses include a wide range of applications to food and feed crops, turf, ornamental plants and seed treatments. The current end-use products contain technical active ingredient (a.i.) at concentrations ranging from 1.04% to 90% and are supplied in wettable powder, granule, dust, concentrate and ready to use liquid formulations. Rates for most applications range from 0.034 to 4.2 lb/acre (0.025 to 0.05 lb/cwt for seeds). Applications may be done 1-5 times per year, depending on the use. Solubility in water is low (22 ppm), but it is moderately mobile in soil. Tolerances have been established for residues of thiophanate-methyl and metabolites on numerous commodities, including almonds, apples, apricots, bananas, beans, meat and meat by-products (cattle, goats, hogs, horses, poultry, sheep), eggs, milk, pecans, potatoes, squash, soybeans, sugar beets, sugarcane and wheat (grain, hay, straw) and several others.

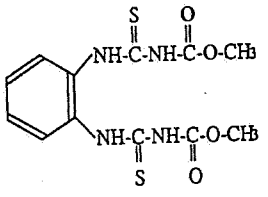

Figure 1: Thiophanate-methyl

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III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined 2-Year Dietary Chronic Toxicity/Carcinogenicity Study in F-344 Rats

Reference: Takaori, H. (1993) Thiophanate-methyl - Combined Chronic Toxicity/Oncogenicity Study in Rats. Toxicology Institute. Environmental Toxicology Laboratory, Nippon Soda Co., Japan. Study No. 0566. August 17, 1993. MRID 42896601. Unpublished study.

A. Experimental Design

Thiophanate-methyl (96.55% a.i.) was continuously administered to 50 Fischer 344 rats/sex/dose in the diet at dose levels of 0, 75, 200, 1200 or 6000 ppm for 2 years. An additional 10 rats/sex/dose group were assigned to an interim (12-month) sacrifice; however, at 6000 ppm, only 5 males were sacrificed at 12 months because 8 died from non-treatment-related injury during weeks 11 and 12. Average daily intake of test material was 0, 3.3, 8.8, 54.4 or 280.6 mg/kg/day for males and 0, 3.8, 10.2, 63.5 or 334.7 mg/kg/day for females.

B. Discussion of Tumor Data

A dose-related increase in the incidence of thyroid follicular cell adenoma was observed at 1200 and 6000 ppm in males and females. The incidence of these tumors and statistical analyses are shown below in Table 1, males and Table 2, females (extracted from Tables 3 and 4, L. Brunsman,1999):

Table 1. Thiophanate-methyl Fischer 344 Rat Study
Male Thyroid Follicular Cell Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	75	200	1200	6000
Adenomas (%)	1/50 (2)	0/46 (0)	0/45 (0)	3/47 (6)	12[a]/44 (27)
p =	0.000**	-	-	0.309	0.014*
Carcinomas (%)	0/47 (0)	0/44 (0)	0/42 (0)	0/47 (0)	3[b]/27 (11)
p =	0.002**	-	-	-	0.011*
Combined (%)	1/50 (2)	0/46 (0)	0/45 (0)	3/47 (6)	14[c]/44 (32)
p =	0.000**	-	-	0.309	0.001**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First adenoma observed at week 53, dose 1200 ppm, in an interim sacrifice animal. Interim sacrifice animals have been excluded from this analysis. Second adenoma observed at week 78, dose 6000 ppm.
[b]First carcinoma observed at week 86, dose 6000 ppm.
[c]One animal in the 6000 ppm group had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

In males, the incidence of follicular cell adenoma (control to high dose) was 2%, 0%, 0%, 6% and 27%. Statistical significance ($p<0.05$) was achieved only at 6000 ppm by pair-wise comparison with controls; a significant increasing trend was also observed ($p<0.01$). The incidence of follicular cell adenocarcinoma (control to high dose) was 0%,

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0%, 0%, 0% and 11%. A significant pair-wise increase ($p<0.05$) was observed along with a significant increasing trend ($p<0.05$). The combined incidence of these tumors (2%, 0%, 0%, 6% and 32%) was significant at 6000 ppm by pair-wise comparison with controls and also for increasing trend (both $p<0.01$).

Table 2. Thiophanate-methyl Fischer 344 Rat Study
Female Thyroid Follicular Cell Tumor Rates[+] and
Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	75	200	1200	6000
Adenomas[#] (%)	0/50 (0)	0/49 (0)	0/50 (0)	1/50 (2)	2[a]/49 (4)
p =	0.031*	1.000	1.000	0.500	0.242

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.
[a]First adenoma observed at week 92, dose 6000 ppm.
[#]No carcinomas were observed.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

In females, the incidence of adenoma (control to high dose) was 0%, 0%, 0%, 2% and 4%. Pair-wise comparisons with controls did not show significant differences, but a significant increasing trend was observed ($p<0.05$).

Historical control data for thyroid follicular cell tumors in Fischer 344 rats from the testing laboratory were requested but were apparently not available. Instead, the study laboratory provided the following published studies on historical control data: (1) Fischer 344 rats compiled from studies conducted at NTP (Haseman *et al.*, 1990) using F-344/N rats from NIH genetic colonies. Data were collected from a total of 40, 2-year dietary studies conducted between 1977 and 1987; and (2) studies conducted at the National Institute of Hygienic Sciences, Tokyo, Japan (Maekawa, A. *et al.*, 1983) using F-344/DuCrj rats from Charles River Japan, the same source used in this study. Data were collected from 6, 2-year dietary studies conducted between 1975 and 1981. Findings are summarized below in Table 3, below:

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TABLE 3: SUMMARY OF AVAILABLE HISTORICAL CONTROL DATA FOR INCIDENCE OF THYROID FOLLICULAR CELL TUMORS IN F-344 RATS

Tumor type/parameter	NTP Data		Japanese NIHS Data	
	Males	Females	Males	Females
Thyroid follicular cell adenoma:				
No. examined	1904	1938	296	297
No. with tumors	13	12	2	2
Mean % incidence	0.7	0.6	0.7	0.7
Range % incidence	0-5	0-2	NG	NG
Thyroid follicular cell adenocarcinoma:				
No. examined	1904	1938	296	297
No. with tumors	10	7	3	0
Mean % incidence	0.5	0.4	1.0	0
Range % incidence	0-2	0-2	NG	NG

NG No data given

The incidence of thyroid follicular cell adenoma in male rats in the thiophanate-methyl study is slightly outside the reported incidence range from the NTP database at 1200 ppm and markedly so at 6000 ppm. The incidence of adenocarcinoma at 6000 ppm also exceeds historical control values. In females, the incidence of adenoma slightly exceeds the range of incidence from NTP database at 6000 ppm. The CARC determined that the historical control data were inappropriate for comparison with the rat study because both data sets were collected several years before the initiation of the study and in the case of the NTP data, were from a different supplier.

Statistically significant increases in adrenal pheochromocytoma were observed in males at 75, 200 and 1200 ppm (control to high dose, 0%, 18%, 12%, 10% and 1.8%). However, the incidence of these tumors did not show a dose-response and was within the range observed in the available historical control data for male Fischer 344 rats (mean 25.5% and range 6%-65%, NTP database; mean 18.9%, range not available, Japanese database).

C. Non-neoplastic Lesions

The incidence of selected non-neoplastic lesions is shown in the tables below, extracted from the DER: Table 4, microscopic lesions in interim sacrifice animals; Tables 5 and 6, in males and females of the main study, respectively:

TABLE 4. NONNEOPLASTIC LESIONS IN RATS ADMINISTERED THIOPHANATE METHYL AFTER 12 MONTHS (INTERIM SACRIFICE)[a]

Organs/Lesions	Doses (ppm)				
	0	75	200	1200	6000
Males					
Liver/Hypertrophy and Lipofuscin	0/10	0/10	0/10	10/10** (1.7)	5/5** (3.0)
Kidney/Nephropathy /Lipofuscin pigmentation	10/10 (2.0) 0/10	10/10 (1.9) 0/10	10/10 (2.0) 0/10	10/10 (2.5) 0/10	5/5** (3.0) 4/5* (1.0)
Thyroid//Hypertrophy and Hyperplasia	0/10	0/10	0/10	10/10** (1.0)	5/5** (2.2)
Adrenal cortex/ Lipidosis	0/10	0/10	2/10 (1.0)	0/10	4/5* (1.0)
Females					
Liver/ Hypertrophy and Lipofuscin	0/10	0/10	0/10	10/10** (1.1)	10/10** (2.0)
Kidney/ Nephropathy /Lipofuscin pigmentation	10/10 (1.0) 0/10	10/10 (1.0) 0/10	10/10 (1.1) 0/10	10/10 (1.1) 0/10	10/10** (1.9) 10/10** (1.0)
Thyroid/ Hyperplasia, focal /Hypertrophy and hyperplasia	0/10 0/10	0/10 0/10	0/10 0/10	0/10 5/10 (1.0)	2/10 (1.5) 10/10** (2.1)
Adrenal cortex/ Lipidosis	0/10	3/10 (1.0)	0/10	6/10* (1.0)	10/10* (1.0)

[a]Data was taken from Tables 31 and 32, MRID No. 42896601, and is presented as the number of animals showing a lesion/number of animals examined. The numbers in parentheses are the average severity rating or grade: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
Significantly different from control: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$,

TABLE 5. INCIDENCE OF SELECTED NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED THIOPHANATE METHYL (MAIN STUDY)a

Organs/Lesions	Doses (ppm)				
	0	75	200	1200	6000
Eye/ Inflammation, acute[b]	2/50	2/22	3/31	2/50	14/55**
/Calcification, corneal	29/50	5/12	15/31	40/50*	28/55
Heart/ Medial calcification[b]	2/50	2/17	1/24	2/50	24/55***
/Fibrosis[b]	6/50	10/17***	14/24***	17/50**	32/55***
Femur/ Resorption, osteoclastic[b]	2/50	2/17	2/24	7/50	35/55***
Sternum/ Resorption, osteoclastic[b]	2/50	2/17	2/24	7/49	34/54***
Liver/ Hypertrophy and lipofuscin pigmentation[b]	0/50	0/50	0/50	19/50***	46/55***
/ Focal fatty degeneration[b]	9/50	7/50	10/50	12/50	27/55***
/ Necrosis, focal[b]	0/50	2/50	2/50	2/50	6/55*
/ Multiple focal hyperplasia	2/50	6/50	9/50*	9/50*	5/55
Stomach/ Calcium deposition[b]	1/50	2/19	2/23	5/50	29/55***
Coagulating gland/ Calcium deposition[b]	0/48	0/17	0/24	0/50	7/53**
Thyroid/ Hypertrophy and Hyperplasia[b]	0/50	0/48	0/50	13/50***	53/55***
/Focal hyperplasia[b]	3/50	2/48	2/50	3/50	15/55**
Parathyroid/ Hypertrophy and Hyperplasia[b]	6/48	1/47	2/48	7/47	34/50***
Kidney/Lipofuscin pigmentation[b]	1/50	2/50	6/50*	8/50*	8/55*
Adrenal cortex/ Fat depletion[b]	3/50	0/50	4/49	14/50**	19/55***
/Focal necrosis[b]	0/50	0/50	0/49	0/50	5/55*

[a]Data taken from Tables 31 and 33, MRID No. 42896601, and is presented as the number of animals showing a lesion/number of animals examined. Statistical significance was calculated by the reviewer using the Fischer exact test. Significantly different from control: * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$

[b]Cochran-Armitage trend test indicated there was a dose-related response ($p < 0.05$) for the four tested doses.

TABLE 6. INCIDENCE OF SELECTED NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED THIOPHANATE METHYL (MAIN STUDY)a

Organs/ Lesions	Doses (ppm)				
	0	75	200	1200	6000
Liver/ Hypertrophy and lipofuscin[b]	0/50	0/50	0/50	28/50***	42/50***
Kidney/ Lipofuscin pigmentation[b]	4/50	5/50	6/50	18/50***	44/50***
Thyroid/ Calcium deposition	3/50	1/49	0/50	1/50	35/50***
/Hypertrophy and hyperplasia[b]	1/50	1/49	0/50	23/50***	49/50***
/Hyperplasia, focal[b]	0/50	1/49	0/50	4/50	6/50*
Pituitary/ Focal hyperplasia[b]	18/50	15/50	13/50	23/49	32/50**
Adrenal cortex/ Lipidosis[b]	5/50	8/50	13/50*	17/50**	14/50*

[a]Data taken from Tables 32 and 34, MRID No. 42896601, and is presented as the number of animals showing a lesion/number of animals examined. Statistical significance was calculated by the reviewer using the Fisher exact test.
Significantly different from control: * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$

[b]Cochran-Armitage trend test indicated there was a dose-related response ($p < 0.05$) for the four tested doses.

In males at the 12-month interim sacrifice (Table 4), statistically significant increases in the incidence and severity of liver hypertrophy and lipofuscin deposition and thyroid hypertrophy and hyperplasia were observed at 1200 and 6000 ppm (all animals affected). The severity of kidney nephropathy was increased and lipofuscin pigmentation was observed (80% vs. 0%, controls). The incidence of adrenal cortex lipidosis was also increased at 6000 ppm (80% vs. 0%, controls).

In the main study males (Table 5), thyroid hypertrophy/hyperplasia and liver hypertrophy and lipofuscin deposition were also observed, although lipofuscin deposition in the kidney, significantly increased at 640 ppm and above, did not show a dose-response. In the liver, focal fatty degeneration (49% vs. 18%, controls) and focal necrosis (11% vs. 0%, controls) were also significantly increased at 6000 ppm. Increased fat depletion at 1200 and 6000 ppm and focal necrosis of the adrenal cortex at 6000 ppm were also reported. In addition, parathyroid hypertrophy and hyperplasia was reported at 6000 ppm (78% vs. 12.5%, controls). Calcium deposition in the stomach, heart and coagulating gland and osteoclastic resorption of the sternum and femur were observed at high incidence at 6000 ppm and were considered to be secondary to hyperparathyroidism.

In females from the interim sacrifice group (Table 4), findings were similar to males. The incidence of thyroid hypertrophy/hyperplasia was lower at 1200 ppm than in males (50% vs. 100%) but comparable at 6000 ppm and the severity of kidney nephropathy was slightly lower than males. Adrenal cortical lipidosis was observed at 1200 ppm (60% vs. 0%, controls) as well as 6000 ppm (100%). Grossly visible swelling of the thyroid, pale granular kidneys and white areas of the heart were consistent with microscopic findings.

In the main study (Table 6), the incidences of liver hypertrophy and lipofuscin deposition, thyroid hypertrophy/hyperplasia and kidney lipofuscin pigmentation were significantly higher at 1200 and 6000 ppm compared to controls, affecting most animals at 6000 ppm. In addition, focal thyroid hyperplasia was increased (8% and 12% vs. 0%, controls) and thyroid calcium deposition was increased at 6000 ppm (70% vs. 6%, controls). At 6000 ppm, focal hyperplasia of the pituitary was significantly increased (control to high dose, 36%, 30%, 26%, 47% and 64%), suggesting a possible treatment-related effect. Increased adrenal cortical lipidosis was observed at 200 ppm and above, but the incidence did not show a dose-response and may be stress related and not strictly treatment-related. Gross findings of thyroid swelling and darkened kidneys at 1200 and 6000 ppm were consistent with microscopic findings in those organs.

D. Other Treatment-Related Toxicity

Among males, mortality was significantly increased at 6000 ppm by pair-wise comparison with controls and an increasing trend was also observed (both $p<0.01$; L. Brunsman, 1999). Only 2 males remained alive at termination (96% mortality). Although mortality was increased at 200 and 1200 ppm (significant at 200 ppm), a dose-response was not seen at these two dose levels.

In addition, decreased body weight and body weight gain in at 1200 ppm (16% and 21% less than controls, respectively), decreased food efficiency (22% less than controls), increased serum cholesterol and creatinine (at termination, 60% and 58% above controls, respectively), decreased albumin and A/G ratio (at termination, 27% and 32% less), decreased T3 and T4 (24% and 45% less, termination) and increased TSH at 18 months (22%) were observed. Abs/rel weights of liver, thyroid and kidney (25%/49%, 24%/50% and 20%/46% above controls, respectively) were also increased. At 6000 ppm, abs/rel thyroid weights were markedly increased: at 12 months, 150%/200% above controls and at 24 months (only 2 animals), 770%/1090%; increases in abs/rel liver weights were also pronounced (49%/108% above controls). Thyroid hormone levels were lowered (T3, 14% to 25% less and T4, 20 to 55% less than controls) and TSH increased (47% to 116% above controls) throughout the study (not evaluated at 24 months due to small number of surviving males). Decreases in red blood cell parameters (for Hct, Hgb and RBC, 4-21%; for MCH, MCV and MCHC, 2-8%) were statistically significant throughout the study and tended to increase with time and may have been a mild treatment-related effect. Increased urinary protein levels (3 to 7-fold above controls) were reported throughout the study and probably were related to pathology in the kidney.

There were no treatment-related increases in mortality in females (see Table 2 of memo in Attachment 2). At 1200 ppm, increased thyroid, liver and kidney abs/rel weights were observed at 12 and 24 months (at 24 months, 27%/43%, 24%/38% and 8.2%/19%, respectively). At 6000 ppm, body weight/weight gain were decreased (22%/31% less than controls at termination) and food efficiency decreased by 29%. Thyroid hormones effects were less pronounced than in males, but decreased T4 at 18 months (27% less than controls) and increased TSH from 18 through 24 months (37% to 80% above controls) were reported.

E. Adequacy of Dosing for Assessment of Carcinogenic Potential

In males, dosing was considered adequate at 1200 ppm based on decreased body weight, decreased T3/T4 and other clinical chemistry alterations, increased liver, thyroid and kidney weights and increased incidence of pathologic alterations of the liver, thyroid

and kidney. However, the high dose of 6000 ppm was considered excessive based on the high mortality (only 2 surviving animals at termination).

In females, dosing was considered adequate at 1200 ppm based on increased cholesterol, increased liver, thyroid and kidney weights and increased incidence of pathologic alterations of the liver, thyroid and kidney. In addition to findings observed at 1200 ppm, at 6000 ppm decreased body weight/weight gain, increased TSH, pathology of the adrenal cortex were reported. The opinion of the CARC was divided as to whether the high dose of 6000 ppm in females exceeded the MTD, based on the large decrease in body weight/weight gain and microscopic evidence of systemic calcification. The Committee concluded that the increased incidence of thyroid tumors at 6000 ppm was biologically significant since a dose-response was observed in both sexes.

2. Mouse 18-Month Dietary Carcinogenicity Study

Reference: Tompkins, E.C. (1992) 18-Month Dietary Oncogenicity Study in Mice with Topsin M. WIL Research Laboratories, Inc., Ashland, OH. Study No. WIL-75024. November 13, 1992. MRID 42607701. Unpublished study.

A. Experimental Design

Thiophanate-methyl (95.93% to 96.55% a.i.) was administered continuously in the diet to 50 CD-1 albino mice/sex/dose at dose levels of 0, 150, 640, 3000 or 7000 ppm for 18 months. An additional 10 animals/sex/dose group were assigned to a 39-week interim sacrifice group. Average daily intake of test material was 0, 4.0, 93.4, 393.6 or 943.5 mg/kg/day for males and 0, 6.5, 153, 634.9 or 1388.2 mg/kg/day for females.

B. Discussion of Tumor Data

The incidence of hepatocellular adenoma showed a dose-related increase at the highest two dose levels, 3000 and 6000 ppm in both males and females. Tables 8, males and 9, females, below show the incidence of hepatocellular adenoma. The tables are extracted from Tables 6 and 7 of the statistical analysis prepared by Lori Brunsman (1999).

Table 7. Thiophanate-methyl CD-1 Mouse Study

Male Liver Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	150	640	3000	7000
Adenomas	4/47	8/46	7/47	19/45	24[a]/42
(%)	(9)	(17)	(15)	(42)	(57)
p =	0.000**	0.098	0.123	0.000**	0.000**
Carcinomas[b]	0/40	0/39	1/36	0/34	1/26
(%)	(0)	(0)	(3)	(0)	(4)
p =	0.118	-	0.146	-	0.107
Hepato-blastomas	0/40	0/39	0/36	0/34	1[c]/26
(%)	(0)	(0)	(0)	(0)	(4)
p =	0.016*	-	-	-	0.107
Combined	4/47	8/46	8/47	19/45	24[d]/42
(%)	(9)	(17)	(17)	(42)	(57)
p =	0.000**	0.098	0.074	0.000**	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First adenoma observed at week 59, dose 7000 ppm.
[b]First carcinomas observed at week 80, simultaneously at 640 and 7000 ppm, in final sacrifice animals.
[c]First hepatoblastoma observed at week 80, dose 7000 ppm.
[d]One animal in the 7000 ppm dose group had an adenoma, a carcinoma and a hepatoblastoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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In males, the incidence of hepatocellular adenoma (control to high dose) was 0%, 17%, 15%, 42% and 57%. Incidence was statistically significantly increased at 3000 and 7000 ppm by pair-wise comparison to controls and an increasing trend was observed (all $p<0.01$). A statistically significant increase in carcinoma was not observed (incidence 0%, 0%, 3%, 0% and 4%); combined adenoma/carcinoma rates were significant at 3000 and 7000 ppm by pair-wise comparison and an increasing trend was observed (all $p<0.01$).

Table 8. Thiophanate-methyl CD-1 Mouse Study

Female Liver Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	150	640	3000	7000
Adenomas[#] (%)	0/43 (0)	0/39 (0)	3/38 (8)	8/34 (24)	18[a]/32 (56)
p =	0.000**	-	0.034*	0.001**	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First adenoma observed at week 74, dose 7000 ppm.
[#]No carcinomas were observed.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

In females, the incidence of hepatocellular adenoma (control to high dose) was 0%, 0%, 8%, 24% and 56%. Incidence was statistically significantly increased by pair-wise comparison to controls at 640 ppm ($p<0.05$) and at 3000 and 6000 ppm ($p<0.01$). An increasing trend was also observed ($p<0.01$). No carcinomas were reported.

Historical control data for hepatocellular tumors from the testing laboratory for these tumors in Crl:CD-1®(ICR) BR mice were submitted. Data were taken from two 18-month studies conducted at WIL Research Laboratories between November, 1985 and March, 1994 (a study on CD2F1/Crl:BR mice conducted between 11/7/85 and 5/19/87 was also submitted). All mice came from the Charles River Portage, MI facility.

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Historical control data on the CD-1® mouse have also been published by the supplier, Charles River. Twelve groups of animals from 18-month studies conducted at independent contract toxicology laboratories were evaluated between December, 1984 and March, 1991. Animals were supplied by Charles River Facilities in the United Kingdom, Portage, MI, Kingston, NY or Wilmington, MA. The incidence of hepatocellular tumors in males and females for both sets of data is summarized below in Table 9:

TABLE 9: HISTORICAL CONTROL DATA FOR INCIDENCE OF HEPATOCELLULAR TUMORS IN CD-1® MICE

	Charles River Data		WIL Research Laboratory Data[1]	
Tumor/parameter	Males	Females	Males	Females
Hepatocellular adenoma:				
No. examined	770	769	135	135
No. tumors	83	5	19	2
Mean % incidence	10.78	0.65	14.1	1.5
Range % incidence	0-19.3	0-2.00	12.5-16.4	0-1.3-1.8
Hepatocellular carcinoma:				
No. examined	770	769	135	135
No. tumors	38	3	2	0
Mean % incidence	4.94	0.39	1.5	0
Range % incidence	1.25-11.54	0-2.00	1.3-1.8	0

1 A third 18-month study on CD2F1/Crl:BR mice (75 males, 78 females) had an incidence of 2 (2.7%) adenoma and 0% carcinoma, males and no tumors in female mice.

The incidence of hepatocellular adenomas in male mice treated with thiophanate-methyl at 3000 and 7000 ppm exceeded the incidence observed in the available historical control data. The incidence of carcinomas was within historical control range. The incidence of hepatocellular adenomas in females markedly exceeded that of the historical control data at 640, 3000 and 7000 ppm.

C. Non-neoplastic Lesions

Selected non-neoplastic lesions in male mice are shown below in Table 11:

TABLE 10. NONNEOPLASTIC LESIONS IN MICE ADMINISTERED THIOPHANATE METHYL FOR 18 MONTHS[a]

Organs/Lesions	Doses (ppm)				
	0	150	640	3000	7000
Males					
Liver, centrilobular hepatocellular hypertrophy	5/10 (50)[2]	3/10 (30)	6/10 (60)	10/10 (100)	10/10 (100)
Interim sac	0/10	1/11 (9)	2/14 (14)	0/16	3/24 (13)
Preterminal deaths	1/40 (3)	2/39 (5)	1/36 (3)	5/34 (15)	12/26** (46)
Terminal	6/60 (10)	6/60 (10)	9/60 (15)	15/60 (25)	25/60** (42)
Total					
Heart, atrial thrombosis	NE	NE	NE	NE	NE
Interim sac	1/10 (10)	1/11 (9)	0/14	1/16 (6)	8/24 (33)
Preterminal deaths	0/40	½ (50)	NE	0/1	0/26
Terminal	6/60 (10)	2/12 (17)	0/14	1/17 (6)	8/50* (16)
Total					
Females					
Liver, centrilobular hepatocellular hypertrophy	0/10	1/10 (10)	5/10 (50)	6/10** (60)	10/10** (100)
Interim sac	0/12	0/13	0/15	0/17	2/23 (9)
Preterminal deaths	0/38	1/37 (3)	0/35	0/33	0/27
Terminal	0/60	2/60 (3)	5/60* (8)	6/60* (10)	12/60** (20)
Total					
Heart, atrial thrombosis	NE	NE	NE	NE	NE
Interim sac	0/12	2/13 (15)	1/15 (7)	6/17 (35)	12/23* (52)
Preterminal deaths	0/38	NE	NE	NE	2/27 (7)
Terminal	0/50	2/13 (15)	1/15 (7)	6/17** (35)	14/50** (28)
Total					

a Data was extracted from Study No. WIL-75024F, Tables 22-24.
b Numbers in parentheses indicate percent incidence.
* Significantly different from control; p≤ 0.05 using Fisher's Exact Test performed by the reviewers.
** Significantly different from control; p ≤ 0.01 using Fisher's Exact Test performed by the reviewers.
NE Not examined

In male mice, the incidence of centrilobular hepatocellular hypertrophy was increased at 3000 and 6000 ppm (total incidence 25% and 42% vs. 10%, controls). Atrial thrombosis was observed in a higher percentage of animals dying on study, but was only marginally higher as total incidence than controls.

In female mice, the incidence of centrilobular hepatocellular hypertrophy was increased at 640, 3000 and 6000 ppm (8%, 10% and 20%, vs. 0%, controls; all animals). The increase was primarily due to increased incidence in the preterminal sacrifice animals. Atrial thrombosis was also reported at 3000 and 6000 ppm (35% and 28% vs. 0%, controls) and was observed primarily in the preterminal death animals (interim sacrifice animals were not evaluated).

D. Other Treatment-Related Toxicity

At 3000 ppm, other effects in males included decreased body weight (<8% below controls), transient increased TSH (100% above controls), increased abs/rel thyroid weight at week 39 (50%/64% above controls, absolute/relative) and increased relative liver weight at week 39 (26% above controls). Absolute/relative liver weights of females were 24%/26% above controls at week 39.

Mortality in males was significantly increased at 7000 ppm by pair-wise comparison with controls and an increasing trend was also observed (both $p<0.01$). The increase in mortality did not occur until the last months of the study. Decreased RBC count (15% less than controls) was also observed at this dose level (L. Brunsman, 1999)

Mortality was increased in females at 7000 ppm ($p<0.05$) and an increasing trend was also observed ($p<0.01$). Most of this increase occurred during the latter part of the study. Other effects observed in females at 7000 ppm included decreased body weight (up to 8% less than controls), decreased circulating T4 (about 70% less than controls) and increased abs/rel weights of thyroid (30%, week 39), liver (57%/57% and 23%/23%, weeks 39 and 79) and heart (23% to 40%, week 39 and 79).

E. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dosing was considered adequate for assessing the carcinogenic potential of thiophanate-methyl in mice. In males, decreased body weight, increased TSH, increased thyroid weights, increased liver weights and increased incidence of hepatocellular hypertrophy were observed at 3000 and 7000 ppm. Decreased RBC count and increased mortality were observed at 7000 ppm.

In females, increased incidence of hepatocellular hypertrophy and atrial thrombosis were observed at 3000 ppm. Decreased body weight, decreased T4, increased thyroid, liver and heart weights, and increased mortality were observed at 7000 ppm.

The opinion of the CARC was divided as to whether the high dose exceeded the MTD in both sexes due to the doubling of mortality relative to controls. The Committee concluded that the increase in hepatocellular tumors at the high dose was biologically significant since a dose-responsive, statistically significant increase was observed in both sexes.

3. Mode of Action Studies

Six short-term experiments were conducted to provide information on the mechanism of thyroid and liver effects observed in the rat 2-year chronic toxicity/carcinogenicity study. These studies were summarized in Annex 5 of the rat study (MRID 42899601) and later submitted in a separate study report (MRID 42899601b).

Experiment 1: Evaluation of liver and thyroid weights, thyroid hormone levels and cholesterol levels - 10 male Fischer 344 rats/group were administered either basal diet or diets containing 6000 ppm thiophanate-methyl, 500 ppm phenobarbital (PB) or 1000 ppm propylthiouracil (PTU). Five animals/group were sacrificed after 2 days and the remaining 5 after 8 days. Body weight, thyroid and liver weights, serum thyroid hormones (T3, T4, TSH) and serum cholesterol were measured at sacrifice. Results are summarized below in Table 11:

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Table 11: Effects of Thiophanate-methyl, PTU and PB Treatment on Male Rats

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%)[2]	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	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 (%)	2	0.0127±0.002	0.0140±0.0009 (10.2%)	0.0186±0.0005*** (46.5%)	ND
	8	0.0135±0.0032	0.0314±0.0046*** (133%)	0.0462±0.0027*** (242%)	0.0146±0.0029 (8.1%)
T3 (ng/dl)	2	92.8±9.1	56.1±10.2*** (-39.2%)	38.0±5.1***[3] (-59.1%)	ND
	8	94.6±3.1	79.9±2.7*** (-15.9%)	24.5±2.1*** (-74%)	106.9±6.1** (13%)
T4 (μg/dl)	2	5.52±0.544	3.36±0.677*** (-39.2%)	4.81±0.918[3] (-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* (136%)	2.006±0.667*[3] (329%)	ND
	8	0.479±0.067	2.374±0.835** (396%)	5.429±1.288*** (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** (53.9%)	89.5±7.7*** (53.5%)	71.8±4.0*** (23.2%)

1 Table copied from review and from study report, MRID 42896601b).
2 Values in parentheses are percent change from control; calculated by reviewer.
3 4 animals/group - all others are 5 animals/group; ND Not determined
* $p \leq 0.05$; ** $p \leq 0.01$; ***$p \leq 0.001$. All by two-tailed Student's t-test except for TSH at 2 days, TM group and 2 and 8 days, PTU group; and cholesterol, day 8 TM group.

Rats treated with thiophanate-methyl had increased liver weights, increased thyroid weights, decreased circulating T3 and T4 and increased TSH and serum cholesterol (see Table 11, above, for values). Body weights were not affected by treatment. Similar thyroid and liver effects were observed with PTU treatment. The thyroid effects were more pronounced, but relative liver weights were not increased as much due to decreases in body weight. Phenobarbital caused increased absolute/relative liver weights and increased cholesterol but no biologically significant changes in thyroid parameters.

Experiment 2: Reversibility of thyroid weight increase - 10 female Fischer 344 rats/group were administered basal diet or diets containing 6000 ppm thiophanate-methyl or 500 ppm PB for 8 days. Five animals/group were sacrificed on day 8; the remaining 5 animals/group were placed on basal diet for an additional 8 days. Body and thyroid weights were measured at sacrifice; liver weight and circulating thyroid hormone levels were not measured.

Absolute/relative thyroid weights were statistically significantly increased on day 8 following treatment with thiophanate-methyl (140%/133% above controls), but returned to near control weights during the recovery period (19%/15% above controls). Thyroid weights were not increased in animals exposed to PB. Neither compound caused changes in mean body weights.

Experiment 3: Effect of T4 supplementation on liver and thyroid weights, thyroid hormone levels and cholesterol levels - 10 male rats/group were administered basal diet or diet containing 6000 ppm thiophanate methyl for 8 days. Five animals/group received daily injections of 30 μ/kg T4 (L-thyroxine); the remainder received no additional treatment. On day 8, animals were sacrificed and body weight, thyroid and liver weights, serum TSH and serum cholesterol were measured.

In rats treated with thiophanate-methyl, supplementation with T4 during treatment resulted in sharply reduced absolute/relative thyroid weight increases (10%/9% vs. 128%/136% above controls) and increases in TSH levels (55%, vs. 361% above controls). No changes in absolute/relative liver weights or cholesterol were observed.

Experiment 4: Effect of thiophanate-methyl on hepatic microsomal enzyme activity - Homogenates and microsomes from 4 livers/group from the control, thiophanate-methyl and PB treated groups collected on day 8 of Experiment 1 were prepared. Microsomal protein concentration and activities of cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase and UDP-glucuronosyltransferase were measured.

Thiophanate-methyl caused a pronounced increase in the activity of UDP-glucuronosyltransferase (236% above controls). Activities of cytochromes P-450 and b5 were also increased, each about 63%. Total protein was increased by about 18%, but there was no significant increase in NADPH-cytochrome c reductase. Rats treated with PB showed increases in all 4 enzymes (36% to 114% above controls) as well as increased protein (26%).

Experiment 5: Effect of thiophanate-methyl on thyroid peroxidase activity - The activity of microsomal thyroid peroxidase prepared from porcine thyroid after addition of 10^{-3} to 10^{-4} thiophanate-methyl compared to untreated preparations and preparations treated with 10^{-4} to 10^{-6} PTU, a known inhibitor of thyroid peroxidase and producer of thyroid adenomas/carcinomas in rats and carcinomas in mice (structure in Table 15), was evaluated.

Thiophanate-methyl caused a reduction in the activity of porcine thyroid peroxidase *in vitro*. The effective dose required to achieve 50% inhibition of activity (ED_{50}) was 6×10^{-4}, compared to an ED_0 of 8×10^{-5}. However, potency was about 30-fold lower than PTU, which has an ED_{50} of 2×10^{-5} and ED_0 of 4×10^{-7}.

Experiment 6: Effect of thiophanate-methyl on hepatocellular proliferation - 10 male Fischer 344 rats and 10 male ICR mice were administered basal diet or diet containing 6000 ppm thiophanate-methyl. After 2 days, 5 animals/group were sacrificed and the remaining animals were sacrificed on day 8. Livers were processed for immunohistochemical staining of proliferating cell nuclear antigen (PCNA).

In mice, the number of liver cells labeling positive for PCNA following treatment with thiophanate-methyl was increased by 2 days after treatment and remained increased after 8 days (≥9-fold higher than controls). Absolute/relative liver weights were also increased (between 20-28% above controls), but no effects on body weight were reported. In rats, the number of PCNA-labeled cells was increased at day 2 (by about 6-fold), but not day 8 and absolute/relative liver weights were increased at both days 2 and 8 (20%-35% above controls). Body weights were not affected. PB caused increased proliferation at day 2 and 8 in mice (less pronounced at day 8) but in rats was only increased on day 2 and the increase relative to controls was less pronounced than in mice.

IV. TOXICOLOGY

1. Metabolism

Thiophanate-methyl was rapidly absorbed, metabolized and excreted in rats at all dose levels and did not accumulate in tissues (MRIDs 42474802, 42602601). The highest concentrations were observed in thyroid (0.04-2.49 μg/g tissue) and liver (0.17-2.15 μg/g). Dose,

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but not sex-related differences in excretion were observed. Radioactivity was rapidly cleared from the blood: $T_{½}$ was 2.8, 2.2 and 7.8 hrs, males and 2.5, 1.6 and 4.0 hrs, females at single low repeated low and single high dose, respectively. T_{max} was achieved at 2-3, 1-2 and 4-7 hrs at single low, repeated low and single high dose, respectively. By 4 days post-exposure, 87-100% of the administered radioactivity was recovered; >90% was excreted within 24 hrs post-dosing. In the single low-dose group excretion was primarily urinary (70-72% of administered dose, vs. 28-29% in feces), but with repeated dosing, the percentage of fecal excretion increased (48-49% of recovered radioactivity, vs. 51-52% in urine). At high dose the primary route of excretion was fecal (67-70%, vs. 29-33% in the urine). Excretion in CO_2 as determined in the preliminary study was negligible. Metabolic profiles were qualitatively similar for all groups. The proposed metabolic pathway for thiophanate methyl in rats appears in Attachment 4. Twelve identified and 4 unknown urinary metabolites were found at levels between <0.1%-7.9% of recovered radioactivity, including MBC (methyl 2-benzimidazole carbamate; 0.2-2.2% of recovered radioactivity) and other sulfate-conjugated and hydroxylated derivatives of the parent compound. The major urinary metabolite was 5-OH-MBC-sulfate [5-(2-methoxycarbonylamino) benzimidazole sulfate; 13.9-42% of excreted radioactivity]. Parent compound constituted 0.2-0.7% of the recovered radioactivity. A total of 7 identified fecal metabolites (also in urine) and 2 unidentified metabolites were found, including MBC (0.5-2.7% of recovered radioactivity). In the feces, the major metabolite identified was 4-OH-TM or dimethyl[(1,2-(4-hydroxyphenylene)]bis (iminocarbonothioyl)bis(carbamate) (3.5-10.5% of recovered radioactivity). Parent compound constituted 1.1% of excreted radioactivity in the single low dose group, but in the repeated low and single high dose groups was the major excreted compound (21.4%-24%, repeated low and 52.2-55.7%, single high dose). Whether the unmetabolized parent compound in feces was due to poor intestinal absorption or biliary excretion was not determined since bile was not evaluated.

2. Mutagenicity

A. Overview: The acceptable genetic toxicology studies on thiophanate-methyl indicate that the compound is not clastogenic *in vitro* and did not cause unscheduled DNA synthesis in cultured rat hepatocytes. However, there are no acceptable *in vitro* gene mutation or *in vivo* genetic toxicology assays. In contrast to the negative findings from the acceptable *in vitro* studies, data from the open literature show that thiophanate-methyl is positive for the induction of micronuclei, but not structural chromosome damage, in whole animals and positive for cell transformation *in vitro*. There is also evidence of a weak equivocal response in the preincubation *Salmonella typhimurium* mammalian microsome gene mutation assay.

The positive results from the *in vivo* micronucleus assay are consistent with the data from MBC (methyl-2-benzimidazolecarbamate), the common metabolite of benomyl and thiophanate-methyl, and for benomyl itself, indicating that all three compounds are inducers of aneuploidy (adverse effects on chromosome numbers). However, conversion of methyl-thiophanate to MBC

proceeds at a slower rate than benomyl (Selling *et al.*, 1970). Thus, the slower breakdown of thiophanate-methyl to MBC, compared to benomyl, may explain the less efficient production of micronucleated polychromatic erythrocytes (PCEs) observed by Barale *et al.* (1993) with thiophanate-methyl. MBC and benomyl also caused liver tumors in male and female mice. Since it is generally acknowledged that somatic cell aneuploidy may be involved as an early event in carcinogenesis and the test article caused morphologically transformed cells *in vitro*, the weight-of-the-evidence from the genetic toxicology studies with thiophanate-methyl in conjunction with the findings for benomyl and MBC support the involvement of a genetic component in the data from 18-month chronic feeding study demonstrating hepatocellular carcinomas in male and female mice (MRID No. 42607701). Nevertheless, the possibility of a mutagenic component to the liver tumor induction has not been ruled out by the available data.

The acceptable studies do not satisfy either pre-1991 or new mutagenicity guideline requirements. Based on the mutagenicity data deficiencies and because, while thiophanate-methyl is probably aneugenic, the possible role of a direct DNA reactive mutagenic mode of action cannot be ruled out. The CARC concluded that additional genetic toxicology testing is warranted. Thiophanate-methyl should be tested for (1) gene mutation in the pre-incubation modification to the *S. typhimurium* mammalian microsome gene mutation assay to resolve the equivocal results from the literature. Thiophanate-methyl should also be investigated in the (2) mouse lymphoma L5178Y mammalian cell forward gene mutation assay; this assay should include colony sizing. In addition, (3) an *in vivo* mouse micronucleus assay should be performed, and the Agency prefers that this assay include immunofluorescent antikinetochore-specific antibody staining. Finally, (4) the 2-aminobenzimidazole metabolite should be tested at minimum in the *S. typhimurium* mammalian microsome gene mutation assay because of the structural alert for mutagenesis (i.e., the NH_2 group attached to the imidazole ring of the benzimidazole portion of the molecule).

B. Available studies: Summaries of the two acceptable mutagenicity studies and studies from the open literature are presented below:

I. Submitted Studies

a. Chromosome aberrations: *In vitro* mammalian cell cytogenetic assay in Chinese hamster ovary cells: The test is negative up to insoluble and cytotoxic doses (400 μg/mL -S9; $\geq$750 μg/mL +S9). Marked increases in mitotic delay were seen at >100 μg/mL -S9; >335 μg/mL +S9. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic mutagenicity data (MRID No. 40980101).

b. Other mutagenic mechanisms: *In vitro* unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes: The test is negative up to a cytotoxic and

insoluble level (1000 μg/mL). The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a UDS assay (MRID No. 40095503).

ii. Other Information--Open Literature

a. Gene mutations: In preincubation *Salmonella typhimurium* mammalian microsome gene mutation assays (Zeiger *et al.*, 1992), thiophanate-methyl ester (95.1%) produced weak equivocal responses (i.e., dose-related increases in revertant colonies of strains TA98 and TA100, which approximated ≥2-fold, at precipitating concentrations ≥3333.0 μg/plate in the presence of 30% hamster or rat S9 activation in one trial and negative results in a subsequent trial.

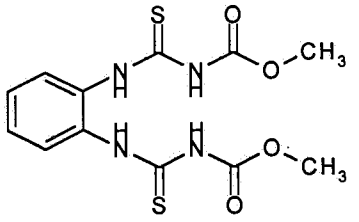
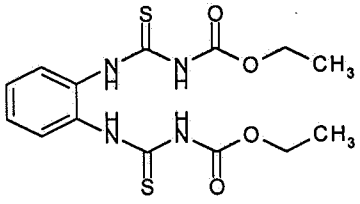
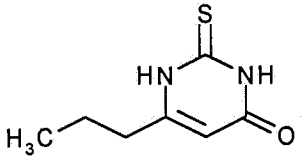
b. Chromosome aberrations (somatic cells): Cytogenetic analysis (Barale *et al.*, 1993) showed that thiophanate-methyl significantly ($p<0.0001$) increased the frequency of micronuclei in the bone marrow cells of male Swiss albino mice 24 hours after receiving a single oral gavage dose of 1 gm/kg. No increase in structural chromosome aberrations was seen but a borderline significant increase in polyploidy and hyperploidy cells was detected. The data further indicate that thiophanate-methyl was less effective than benomyl or the common metabolite of both benomyl and thiophanate-methyl, (methyl-2-benzimidazolecarbamate, MBC), in the induction of micronuclei. In this study, MBC induced micronuclei in PCE more efficiently that either parent with benomyl induction followed by thiophanate-methyl induction. The combined results of this series of tests (i.e., positive for micronuclei induction/negative for structural chromosome aberrations) suggest that thiophanate-methyl probably interferes with the mitotic spindle rather than causing structural chromosomal damage and is, therefore, aneugenic. This conclusion is supported by the positive findings of an *in vivo* bone marrow micronucleus assay (MRID No. 41051510) conducted with benomyl and MBC and the positive *in vivo* bone marrow erythrocyte immunofluorescent antikinetochore antibody assays for detection of aneuploidy induction with benomyl (MRID No. 42911601) and MBC (MRID No. 42911602).

c. Other mutagenic mechanisms--cell transformation: In independently performed BALB/c3T3 *in vitro* cell transformation assays (Perocco *et al.*, 1997), thiophanate-methyl induced a significant ($p<0.001$) and reproducible increase in morphologically transformed foci in the absence of S9 activation at 25 μg/mL and significant ($p\leq0.001$) and reproducible dose-related increases in transformed foci at 20, 100 or 200 μg/mL in the presence of S9 activation.

3. Structure-Activity Relationship

Thiophanate-methyl is a thioallophanate. Related chemicals available and their carcinogenicity effects are shown below in Tables 12 and 13:

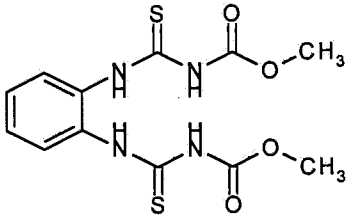
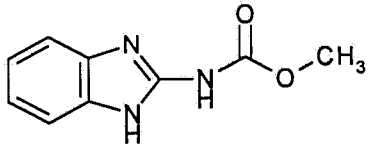
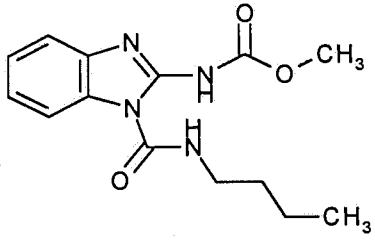
TABLE 12: STRUCTURAL COMPARISON OF THIOPHANATE-METHYL TO OTHER THIOUREA COMPOUNDS

Compound	Structure	Carcinogenic Effect
Thiophanate Methyl		Rat (F-344): thyroid adenoma, carcinoma (at excessive dose of 6000 ppm in males; marginal increase in thyroid adenoma in females and at lower doses in males) Mouse (CD-1): hepatocellular adenoma, males and females (≥3000 ppm)
Thiophanate Ethyl		Rat (S-D): No tumors reported (dosing up to 1000 ppm) Mouse (C57BL/6): No tumors reported (dosing up to 2000 ppm)
Propylthiouracil		Rat: thyroid adenoma, carcinoma Mouse: thyroid carcinoma, pituitary chromophobe adenoma (IARC monograph, 1974)

As shown in Table 12, thiophanate-methyl, a thioallophanate compound, shares a thiourea moiety with some other known thyroid carcinogenic agents. An example is propylthiouracil, which is a potent inducer of thyroid tumors in both rats and mice. The closely related compound thiophanate-ethyl did not cause tumors in rats (MRID 00032673) or mice (MRID 00032674) in the available submitted dietary studies, but may not have been tested at levels high enough to induce these tumors. However, it did induce thyroid hypertrophy and liver enlargement in the rat 2-year dietary study at 1000 ppm. The only effect reported in the mouse carcinogenicity study was decreased spermatogenesis at 2000 ppm.

In addition to structure-activity considerations related to the thiourea moiety, thiophanate-methyl is converted to methyl 2-benzimidazole carbamate (MBC), also a metabolite of benomyl, shown below in Table 13 (see previous section for discussion of mutagenicity structure-activity relationships):

TABLE 13. STRUCTURAL COMPARISON OF THIOPHANATE-METHYL TO MBC AND BENOMYL

Compound	Structure	Carcinogenic Effect
Thiophanate Methyl		Rat (F-344): thyroid adenoma, carcinoma (at excessive dose of 6000 ppm in males; marginal increase in thyroid adenoma in females and males at 1200 ppm) Mouse (CD-1): hepatocellular adenoma, males and females (≥3000 ppm)
MBC **Common meta-bolite with Benomyl**		Rat (Crl:CD-1): No tumors reported (dosing up to 5000 ppm) Mouse (CD-1): Hepatocellular adenoma (≥500 ppm) Mouse (Swiss): Hepatocellular blastoma (5000 ppm)
Benomyl		Rat: No study available Mouse (CD-1): Hepatocellular adenoma (≥500 ppm)

4. Subchronic and Chronic Toxicity

A. Subchronic Toxicity

Oral toxicity - In a 13 week toxicity study in the Fischer 344 rat (MRID 42001701), thiophanate-methyl was administered continuously in the diet at dose levels of 0, 200, 2200, 4200, 6200 or 8200 ppm (equivalent to average daily intakes of 0, 13.9, 155.0, 293.2, 426.9 or 564.7 mg/kg/day, males and 0, 15.7, 173.4, 323.0, 478.8 or 647.3 mg/kg/day, females). Effects were observed at 2200 ppm and higher in both sexes, including anemia, increased cholesterol (about 40% above controls, both sexes), increased weights of liver (27% males, 27%, females), thyroid (39%, males and 50%, females) and kidney (9%, males only) and incidence of microscopic lesions in these organs (liver swelling and lipofuscin deposition, thyroid hypertrophy and hyperplasia and in males only, glomerulonephrosis). At 4200 ppm, increased thymus weight and incidence of fatty degeneration of the adrenal cortex were observed in females only. At 6200 ppm, increased thymus weight and incidence of fatty degeneration of the adrenal cortex was also increased in males as well as females. At 8200 ppm, glomerulonephritis was observed in females as well as males. Food consumption was decreased only during the first 1-2 weeks at 4200 pm and higher (8-17%).

In a 90-day oral toxicity study in dogs (MRID 41982203), thiophanate-methyl was administered daily by gelatin capsule to 4 beagle dogs/sex/dose group at 0, 50, 200 or 800 mg/kg/day. The high dose was lowered to 400 mg/kg/day on day 50 due to excessive toxicity. At 50 mg/kg/day, minimal thyroid follicular cell hypertrophy was observed in 1 male and 1 female. At 200 mg/kg/day, decreased body weight/weight gain (15%/0.1 kg less than controls, males and 24%/1.0 kg less than controls, females), decreased food consumption during the early weeks of the study, anemia (10% to 14% decrease in RBC, Hct and Hgb), increased cholesterol (55%, males and 73%, females), decreased T3 and T4, females (26% and 47%), increased relative liver weight (26%, males and 32%, females), increased abs/rel thyroid weight, males (31%/53%), slight to minimal, 3 males and 2 females) slight thyroid follicular cell hyperplasia in 1 male, slight hypoplasia/atrophy of the testes in all males, spleen lymphoid cell depletion in 2 males and 2 females, and thymic involution/atrophy in males were also observed. Additional effects observed at 800/400 mg/kg/day included increased platelet count, increased activated thromboplastin time and minimal to marked thyroid follicular cell hyperplasia in almost all animals.

B. Chronic Toxicity

Long-term oral toxicity studies in the rat (chronic toxicity/carcinogenicity) and mouse (carcinogenicity) are summarized above in Section III.

A 12-month oral toxicity study was conducted in the beagle dog (MRID 42311801). Thiophanate-methyl was administered daily by gelatin capsule to 4 animals/sex/dose at 0, 8.0, 40 or 200 mg/kg/day. At 40 mg/kg/day, body weight/weight gain was decreased in both sexes (-7%/-19%, males and -6%/-19%, females. Thyroid effects included decreased serum T4 in males and markedly increased TSH in 1 male, increased absolute/relative thyroid weights (+33%/+42%, males and +28%/+10%, females) and thyroid follicular cell hypertrophy in females. Serum cholesterol was also increased in males at 6 and 12 months (+47% and +30%). At 200 mg/kg/day, tremors were observed post-dosing in most animals on day 1 and sporadically until day 17, but not later. Slight anemia (Hgb, Hct and RBC decreased by 13% to 14%), increased serum alkaline phosphatase in both sexes at 6 and 12 months (100% and 300%, males and 47% and 82%, females), increased absolute/relative liver weights (46%, males and 35%, females) and thyroid follicular cell hyperplasia in 1 male and 1 female were also observed. Decreased body weight and thyroid effects were more pronounced than at 40 mg/kg/day.

5. Reproductive Toxicity

Neither developmental nor reproductive toxicity was observed in the rat 2-generation reproduction study (MRIDs 42899101 through -05; 43624401), which tested at dietary exposures of 0, 200, 640 or 2000 ppm (average daily intakes of 0, 13.7, 43.3 or 138.9 mg/kg/day, males and 0, 15.5, 54.0 or 172.0 mg/kg/day, females). Parental systemic toxicity was observed at 200 ppm as hepatocellular hypertrophy and thyroid follicular cell hypertrophy/hyperplasia (threshold effect level). Offspring systemic toxicity was reported at 640 ppm (threshold effect level) as decreased F2b pup weights during lactation.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity: The CARC concluded that thiophanate-methyl was carcinogenic in male and female rats and mice.

- Thiophanate-methyl was carcinogenic to F344 rats because 1) male rats had statistically significant increases in the pair-wise comparisons of 6000 ppm dose groups with the controls, for thyroid follicular cell adenomas (12/44, 27%; $p<0.05$) and carcinomas (3/27, 11%, $p<0.05$), as well as combined adenomas

and/or carcinomas (14/44, 32%, $p<0.01$). A significant increasing trend ($p<0.01$) was also observed. The increase in the incidence of adenomas (3/47, 6%) at 1200 ppm was considered to be treatment-related because of the progressive nature of the tumor and evidence of dose-response, and 2) the increase in the combined incidence in males at 6000 ppm was partly driven by the incidence of carcinomas; 3) for female rats, although there were no significant increases in the pair-wise comparisons of the dosed groups with the controls for thyroid follicular cell adenomas, a significant increasing trend ($p<0.05$) was observed; and 4) the dosing was considered to be adequate in males and females at 1200 ppm based on decreased body weight gain (21% in males) and increase in liver, thyroid and kidney weights. Dosing in males at 6000 ppm was considered excessive due to high mortality (96%) and severity of toxicity in various organs. Some members of the CARC considered the toxicity in females at 6000 ppm to be excessive as well due to large decrease in body weight gain (31%) and evidence of systemic mineralization. The histopathological changes in liver, thyroid and kidney at 1200 ppm were consistent with those seen at 6000 ppm. The increase in TSH and decrease in T3 and T4 levels seen at 6000 ppm were supportive of the disruption of thyroid-pituitary homeostasis. Appropriate historical control incidences (same strain, from the study lab and/or within a few years of the study conduct) were not available for comparison. Females appear to be less sensitive at 6000 ppm because the incidence of tumors was lower compared to males. However, the same spectrum of effects was seen in both sexes including same target organ effects, calcium deposition in various organs and progressive toxicity seen at 12 month onwards. Based on the above weight-of-the-evidence, the CARC concluded that the thyroid tumors in male and female rats at 1200 and 6000 ppm were treatment-related. There was no evidence of other types of tumors in rats.

The registrant contended that the findings of the submitted mechanistic studies demonstrated that thiophanate-methyl causes thyroid tumors in rats by disruption of thyroid-pituitary homeostasis. The CARC, however, concluded that the registrant did not demonstrate the reversibility of the effect on thyroid hormones and the mechanistic studies were conducted only at 6000 ppm, a dose that was excessively toxic in male rats in the 2-year dietary study and also did not establish the mechanism at the next lower dose where the tumors were seen. No histopathology was conducted to examine the presence of precursor lesions. In addition, the CARC noted deficiencies in the current mutagenicity database. A discussion of the Agency policy on thyroid tumor assessment in relation to the available data on thiophanate-methyl is provided below (section V.5).

- Thiophanate-methyl was carcinogenic to male and female CD-1 mice because 1) male mice had statistically significant increases in the pair-wise comparisons of the 3000 and 7000 ppm dose groups with the controls, for liver adenomas (19/45,

42% and 24/42, 57%, respectively, both at $p<0.01$), and combined adenomas, carcinomas and/or hepatoblastomas (19/45, 42%; and 24/42, 57%, respectively, both at $p<0.01$). A significant increasing trend for these tumors ($p<0.01$) as well as for liver hepatoblastomas ($p<0.05$) was also evident; 2) female mice had statistically significant increases in the pair-wise comparisons of the 3000 and 7000 ppm dose groups with the controls, for liver adenomas (8/34, 24% and 18/32, 56%, both at $p<0.01$, respectively). A significant increasing trend was also observed. There was a significant difference in pair-wise comparison of the 640 ppm dose group with the controls for liver adenomas (3/38, 8%, $p<0.05$); 3) the incidences of adenomas in males at 3000 and 7000 ppm and in females at 640, 3000 and 7000 ppm as well as carcinomas in males at 7000 ppm were outside the WIL Laboratory historical controls ranges (adenomas: males 2.7-16.4; females: 0-1.8%; carcinomas: males 0-1.8%); 4) dosing was considered to be adequate in both sexes based on decreased body weight in both sexes (8% less than controls), increased liver and thyroid weights and histopathological changes in the liver, thyroid and heart at 3000 ppm. Some members of the CARC considered dosing in both sexes at 7000 ppm to be excessive based on significant increases in mortality but this dose level was not excluded from the quantitative risk assessment because it was part of continuing dose-response in both sexes. The same spectrum of effects was also seen in both sexes.

2. Mutagenicity: The CARC concluded that additional testing is required to characterize the mutagenic potential of thiophanate-methyl and that the current database does not satisfy guideline requirements for genotoxicity. The acceptable studies submitted by the registrant indicate that thiophanate-methyl is not clastogenic in cultured Chinese hamster ovary cells and did not cause unscheduled DNA synthesis in cultured rat hepatocytes. However, a published study demonstrated that thiophanate-methyl was positive for the induction of micronuclei, but not structural chromosomal aberrations, in mouse bone marrow cells *in vivo*. This finding suggests that it may interfere with the mitotic spindle rather than cause structural chromosomal damage and is therefore aneugenic. This is consistent with positive mouse micronuclei data for MBC (methyl-2-benzimidazole carbamate), a common metabolite with benomyl, and benomyl itself. Both of these compounds also cause mouse liver tumors and teratogenic effects. Since aneuploidy may be involved in carcinogenesis, these findings may support a genetic component for induction of tumors by thiophanate-methyl. Other published studies showed that thiophanate-methyl caused cell transformation in BALB/c 3T3 cells *in vitro* and a weak equivocal response in *S. typhimurium* strains T98 and T100. Because the available studies do not satisfy the guideline requirements and do not exclude the possibility of direct mutagenicity, the CARC requested that the following studies on thiophanate-methyl be submitted: (1) a preincubation *S. typhimurium* mammalian microsome gene mutation assay to resolve the equivocal results seen in the published study, (2) a mouse lymphoma

L5178Y mammalian cell forward gene mutation assay including colony sizing and (3) an *in vivo* mouse micronucleus assay including immunofluorescent antikinetochore-specific antibody staining. In addition, the 2-aminobenzimidazole metabolite should be tested at minimum in the (4) *S. typhimurium* mammalian microsome forward gene mutation assay because of the structural alert for mutagenesis (NH_2 group on the imidazole ring).

3. Structure Activity Relationship: A structurally related compound, thiophanate-ethyl, was not carcinogenic in rats and mice. However, it was not tested at adequately high dose levels and did cause thyroid and liver enlargement in rats at the doses tested. MBC, a metabolite of both thiophanate-methyl and benomyl, as well as benomyl, are both aneugenic and cause hepatocellular tumors in CD-1 mice but not thyroid tumors or antithyroid activity. Thiophanate-methyl, a thioallophanate compound, shares a thiourea moiety with other thyroid carcinogenic agents such as propylthiouracil which is a potent inducer of thyroid tumors in rats and mice. The available structure-activity data suggest distinct mechanisms of carcinogenesis for thyroid and liver carcinogenesis by thiophanate-methyl but additional information is required to establish this.

4. Mode of Action: A series of mechanistic studies were conducted to determine whether thyroid tumors in rats were due to antithyroid activity. In F344 rats, thiophanate-methyl at 6000 ppm for 2 or 8 days increased the liver and thyroid weights accompanied by increased circulating levels of TSH and decreased levels of T3 and T4. Thiophanate-methyl also induced hepatic microsomal UDP-GT, along with cytochromes p450 and b5, in male rats at 6000 ppm thiophanate-methyl in the diet for 2 or 8 days. Withdrawal of thiophanate-methyl treatment caused a reversal of the thyroid weight increases. However, the studies did not evaluate the effect of withdrawal on the levels of TSH and T3/T4 or changes in histopathology. The thyroid mechanistic studies also did not evaluate responses at other dose levels to establish a dose-response.

In another study, thiophanate-methyl reduced the activity of thyroid peroxidase in porcine thyroids *in vitro*. However, the thyroid peroxidase inhibiting activity was 30-fold less than that of propylthiouracil, a known antithyroid chemical.

The cellular proliferation data from the mechanistic study on thiophanate-methyl showed more sustained proliferating cell nuclear antigen (PCNA) labeling in rats than mice after short-term dosing, suggesting but not conclusively demonstrating that the effects on liver in two species may differ.

Although the available evidence is consistent with disruption of thyroid-pituitary homeostasis, the CARC concluded that additional information is needed to confirm this mechanism.

5. Consideration of the Use of the Non-linear Extrapolation Approach for Thiophanate-Methyl

A. Introduction: When evaluating thiophanate-methyl, the CARC considered whether use of a non-linear extrapolation approach for thyroid neoplasms was appropriate.

The quotations which follow are taken from the Agency's Policy Document entitled "Assessment of Thyroid Follicular Cell Tumors", March 1998 (EPA/630/R-97/002):

"Tumors of the thyroid gland follicular cells are fairly common in chronic studies of chemicals in rodents. Experimental evidence indicates that the *mode of action* for these rodent thyroid tumors involves (a) changes in the DNA of thyroid cells with the generation of mutations, (b) disruption of thyroid-pituitary functioning, or (c) a combination of the two. The only verified cause of human thyroid cancer is ionizing radiation, a *mutagenic* insult to which children are more sensitive than adults.

...Treatments of rodents that cause *thyroid-pituitary disruption* result in chronic reduction in circulating thyroid hormone levels, increase in TSH levels and the development of increased cell division, increased size and numbers of thyroid cells, increased thyroid gland weight and, finally, tumors of the thyroid. In some cases, there is also an increase in tumors of the pituitary cells that produce TSH. Cessation of treatment early in the process before tumor development results in reversal of processes back towards normal."

When assessing tumors of the thyroid, "For those cases where thyroid tumors arise from chemically induced disturbances in thyroid-pituitary functioning, tumors are considered to be secondary to the adverse effects on the thyroid gland function that precede them. As exposures to such agents decrease, the likelihood of cancer decreases; risks may be seen as minimal at doses where there is no effect on thyroid-pituitary homeostasis. Generally, homeostasis is considered to apply when serum T4, T3 and TSH levels and thyroid and pituitary morphology and growth are within their normal limits."

In the Science Policy Guidance section of this document, factors that should be considered in making this determination are discussed.

"Most of the focus in implementing this policy is devoted to answering the following questions: (1) Does an agent that shows thyroid carcinogenic effects have antithyroid activity? (2) Can modes of action other than thyroid-pituitary carcinogenic effects have antithyroid activity? (3) How can one express thyroid dose-response relationships?" The occurrence of tumors in tissues other than the thyroid is also considered in determining mechanism of carcinogenesis.

B. Determination of whether neoplasms are due to thyroid-pituitary imbalance

The Science Policy Guidance discusses the types of information necessary to characterize the mechanism of thyroid carcinogenesis. These are addressed as they apply to thiophanate-methyl, as follows:

i. Consideration of whether the thyroid tumors associated with administration of thiophanate-methyl can be attributed to disruption of the thyroid-pituitary hormonal balance (demonstration of antithyroid activity). In addressing this point, the Policy lists eight areas of inquiry for evidence demonstrating antithyroid activity (for additional details on the results described below, see individual study summaries presented earlier in this document or attached DERs for carcinogenicity and mechanistic studies):

a. Increases in cellular growth *in vivo* (evidence required):

In the 2-year chronic toxicity/carcinogenicity study in F344 rats, treatment with thiophanate methyl resulted in increased absolute and relative thyroid weights at dietary concentrations of 1200 and 6000 ppm in both males and females, with males showing a more pronounced effect than females. This increase was due to both follicular cell hypertrophy and hyperplasia, based on microscopic evaluation. Increased thyroid weight and follicular cell hypertrophy/ hyperplasia were also observed in the rat subchronic toxicity study and the rat 2-generation reproductive toxicity study, and increased thyroid weight in the special mechanistic study. Thyroid follicular cell hypertrophy and/or hyperplasia was also observed in other species. Increases in thyroid weight and thyroid follicular cell hypertrophy and hyperplasia were reported in both sexes in the dog subchronic toxicity study. Thyroid hypertrophy was also observed in the dog chronic toxicity study in males. Thyroid weights in the mouse carcinogenicity study were increased at week 39 but not termination in males and females.

b. Hormone changes (e.g., reduced thyroid hormones T3, T4 and increased TSH; evidence required):

In the rat 2-year chronic toxicity/carcinogenicity study, thiophanate-methyl induced significant decreases in circulating T3 and T4 in both sexes, as well as increases in circulating TSH. No significant alterations in thyroid hormones were reported in the rat subchronic study. In study 1 of the special mechanistic study, rats treated with 6000 ppm thiophanate-methyl had increased thyroid and liver weights, decreased circulating T3/T4 levels and increased TSH levels. Circulating T3/T4 levels were also measured in the dog subchronic study and were decreased; TSH was not evaluated.

c. Site of action (intra thyroidal, peripheral tissues, liver or other sites; evidence required):

The available data suggest that the primary site of action may be the liver, and to a lesser extent, the thyroid. Experiment 4 of the special mechanistic study demonstrated that in addition to increased liver weight, increased activities of rat liver microsomal enzymes UDP-glucuronosyltransferase, cytochrome P-450 and cytochrome b-5 were observed in liver homogenate and microsomal preparations taken from rats treated for 2 or 8 days with thiophanate-methyl at 6000 ppm in the diet. The activity of thyroid peroxidase in preparations from porcine thyroids was also reduced *in vitro* by thiophanate-methyl. Thiophanate-methyl was approximately 30-fold less potent at inhibiting thyroid peroxidase activity than propylthiourea (PTU), a known antithyroid chemical and inhibitor of this enzyme. These findings are consistent with alteration of thyroid-pituitary homeostasis due to increased metabolism of T4.

d. Dose correlations (evidence required):

The available data (2-year rat chronic toxicity and special mechanistic studies) indicate that the increase in thyroid follicular cell tumors is correlated with perturbance of thyroid hormone levels, hypertrophy and hyperplasia in both sexes. Increases in thyroid tumors were only observed at dose levels causing these effects.

e. Reversibility (evidence required):

Experiment 2 of the special mechanistic study demonstrated that 8 days after cessation of treatment with thiophanate-methyl for 8 days at a dietary concentration of 6000 ppm, the treatment-related increase in thyroid weight was reversed. However, the study did not evaluate reversibility of the increase in liver microsomal enzymes, thyroid peroxidase or circulating TSH, or the decreases in circulating T3/T4. Reversibility of the follicular cell hypertrophy/hyperplasia was also not evaluated.

f. Lesion progression (evidence desirable):

Some evidence exists for lesion progression (hypertrophy/ hyperplasia to adenoma to adenocarcinoma). In the short-term special mechanistic study, enlarged thyroid weights were observed within 8 days of treatment. However, histopathology was not evaluated. Thyroid hypertrophy/hyperplasia was observed at high incidence at termination in the rat 90-day toxicity study, along with increased thyroid weights. Thyroid follicular cell hypertrophy/hyperplasia was observed at high incidence in males and females the interim (12-month) sacrifice group of the rat 2-year chronic toxicity/carcinogenicity study at 1200 and 6000 ppm, the highest 2 dose levels tested. One follicular cell adenoma in a 1200

ppm male was observed at that time, whereas by termination, a dose-related increase in adenomas was observed at 1200 and 6000 ppm males (first adenoma in main study animals was observed at week 78). In addition, 3 follicular cell carcinomas were observed in males at 6000 ppm but not in any other group (first carcinoma observed at week 86). Females showed a slight, dose-dependent increase in thyroid follicular cell adenomas by termination at 1200 and 6000 ppm.

g. Structure-activity analysis (evidence desirable):

The ethyl analog, thiophanate-ethyl, did not cause tumors in rats (MRID 00032673) or mice (MRID 00081605) in the available submitted dietary studies, but may have not been tested at levels high enough to induce these tumors. However, it did induce thyroid hypertrophy in the rat 2-year dietary study at 1000 ppm. Thiophanate-methyl shares the thiourea moiety with propylthiouracil (PTU), a potent inducer of thyroid tumors in rats and mice. Both PTU and thiophanate-methyl inhibit the thyroid peroxidase enzyme. PTU is approximately 30-times more potent an inhibitor of this enzyme *in vitro* than thiophanate-methyl.

h. Other studies (evidence desirable):

Experiment 3 of the mechanistic study demonstrated that rats treated with 6000 ppm thiophanate-methyl for 8 days and supplemented with T4 during that time did not show increased thyroid weight or TSH levels. Effects on liver weight or serum cholesterol were unchanged (liver microsomal enzymes were not measured).

Based on the overall judgement of the findings listed above, the CARC concluded that although the available data strongly suggest that the thyroid tumors in the rat associated with administration of thiophanate-methyl may be due to disruption in the thyroid-pituitary homeostatus, the evidence was not conclusive because reversibility of thyroid hormone effects was not demonstrated and the mechanistic studies were conducted only in animals exposed to 6000 ppm and not at lower dose levels to demonstrate a dose-response.

ii. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

Thiophanate-methyl has been shown to have genotoxic potential. In a published mouse *in vivo* micronucleus assay, thiophanate-methyl significantly increased the frequency of micronuclei in the bone marrow cells of male Swiss albino mice. In a second published study, BALB/c3T3 cells were transformed *in vitro* following exposure to thiophanate-methyl. Thiophanate-methyl did not induce chromosomal aberrations in CHO cells in

vitro or unscheduled DNA synthesis in primary rat hepatocytes *in vitro.* It produced weak equivocal responses in the Ames assay. Data to evaluate direct mutagenicity of thiophanate-methyl are inadequate and additional studies are required to address this deficiency.

iii. Consideration of the occurrence of tumors in other tissues in addition to the thyroid.

In the mouse 18-month carcinogenicity study, a statistically significant, dose-dependent increased in hepatocellular adenomas was observed in both sexes at the highest 2 dose levels.

iv. Consideration of the dose-response.

In the chronic toxicity/carcinogenicity study in rats, thyroid effects were observed at the same dose levels at which increases in thyroid tumors were observed in both sexes. However, the only statistically significant increase (pair-wise comparison to controls) was observed in males at an excessive high dose. Liver toxicity (hypertrophy) was also observed at these dose levels. The special mechanistic study provided evidence for perturbation of thyroid-pituitary homeostasis secondary to increased activity of hepatic microsomal enzymes and possibly thyroid microsomal peroxidase after short-term administration of thiophanate-methyl, although only a single dietary level (6000 ppm) was assessed. Increases in benign liver tumors were observed in the mouse carcinogenicity study in both sexes at high incidence at the highest two dose levels, and also in females at 640 ppm. Although tumors were observed at a lower dose in females than males, the incidence at the highest two dose levels was greater in males. These increases were statistically significant in both sexes at 2 or more dose levels, significantly exceeded the available historical control range and were not observed at doses causing excessive toxicity, although the high dose for each sex either approached or exceeded 1 g, the limit dose. Comparative PCNA labeling studies of rat vs. mouse liver suggest that proliferative effects of thiophanate-methyl in mice may be more sustained in mice than rats.

v. Conclusions: Based on the overall judgement of the 8 types of data evaluating evidence for antithyroid activity, the CARC concluded that there are insufficient data to determine whether or not the thyroid tumors in the rat associated with administration of thiophanate-methyl may be due to a disruption in the thyroid-pituitary homeostasis. The criteria for a threshold effect have largely been met; however, reversibility of thyroid hormone effects was not demonstrated, the mechanistic studies were conducted only at a single dose, 6000 ppm, data gaps for genotoxicity were identified and the presence of a second tumor type (liver, in mice) was observed. In addition to evidence supporting disruption of the thyroid-pituitary homeostasis, the following factors were considered in evaluating the

carcinogenic potential of thiophanate-methyl: (1) the incidence of thyroid tumors in rats was statistically significantly increased above controls and above the range observed in available historical control range only in males at 6000 ppm, a dose that exceeded the MTD; at 1200 ppm in males and 6000 ppm in females, incidence was only marginally above the historical control range; (2) thiophanate-methyl demonstrated mutagenic potential in a mouse bone marrow micronucleus assay and a BALBc/3T3 cell transformation assay, but additional genotoxicity testing is required to meet guideline requirements; (3) in addition to thyroid tumors in the rat, hepatocellular adenomas were increased in mice treated with thiophanate-methyl. These tumors were observed at high incidence at the highest 2 dose levels in both sexes as well as in females at 640 ppm; (4) Liver tumors were also observed in mice treated with the pesticide benomyl, which shares a common metabolite (MBC) with thiophanate methyl, and in mice treated with MBC itself. Both benomyl and MBC are also aneugenic. However, benomyl and MBC did not affect the thyroid or induce thyroid tumors in rats. Neither of these compounds have the thiourea moiety. The available, *albeit* limited, cellular proliferation data from the mechanistic study on thiophanate-methyl, showing more transient PCNA labeling in rats than mice after short-term dosing, suggests but do not conclusively demonstrate that the effects on liver in the two species may differ.

When considered together, although the CARC determined that the available information suggests that thiophanate-methyl disrupts thyroid-pituitary homeostasis, it was concluded that there was insufficient evidence to conclusively demonstrate this mechanism, based on incomplete reversibility data, lack of demonstration of a dose-response in the mechanistic studies and the need for additional mutagenicity studies. However, if mutagenicity plays a role in initiation of thyroid carcinogenesis by thiophanate-methyl, it would appear to be limited given the low rate of tumor formation at non-excessive doses.

C. Factors to be Considered in Determining Method to be Used in Estimating the Risks of Thiophanate-Methyl

Guidance given in the EPA policy for proceeding with the quantitation of risk when evaluating thyroid tumors is as follows:

"Some chemicals that have produced thyroid follicular cell tumors in laboratory rodents appear to work by producing a derangement in thyroid-pituitary homeostasis; others appear to act primarily through a mutagenic mode of action; and still others seem to show a combination of both modes of action. The question then becomes how to evaluate the risks of thyroid tumors for humans given exposure to any of these chemicals. If the animal tumors are due to chemical doses that produce imbalances in thyroid-pituitary functioning, it is anticipated that the chance of cancer is minimal under conditions of hormonal homeostasis. Tumors seeming to arise from relevant mutagenic

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influences...without perturbation in thyroid-pituitary status may pose some chance of cancer across a broader range of doses. Consequently, until such time that biologically based models and data become available, EPA adopts the following science policy for conducting dose-response assessments of chemical substances that have produced thyroid follicular cell (and related pituitary) tumors in experimental animals:

I. A linear dose-response procedure should be assumed when needed experimental data to understand the cause of thyroid tumors are absent and the mode of action is unknown...

ii. A linear dose-response procedure should be assumed when the mode of action underlying thyroid tumors is judged to involve mutagenicity alone.

iii. A margin of exposure dose-response procedure based on nonlinearity of effects should be used when thyroid-pituitary disruption is judged to be the sole mode of action of the observed thyroid and related pituitary tumors...Thyroid-pituitary perturbation is not likely to have carcinogenic potential in short-term or highly infrequent exposure conditions. The margin of exposure procedure generally should be based on thyroid-pituitary disruptive effects themselves, in *lieu* of tumor effects, when data permit...

iv. Consistent with EPA risk characterization principles, both linear and margin of exposure considerations should be assumed when both mutagenic and thyroid-pituitary disruption modes of action are judged to be potentially at work...The weight of evidence for emphasizing one over the other should also be presented...

v. Dose-response relationships for neoplasms other than the thyroid (or pituitary) should be evaluated using mode of action information bearing on their induction and principles laid out in current EPA cancer risk assessment guidelines. There is an association between thyroid and liver tumors in rodent cancer studies (McConnell, 1992; Haseman and Lockhart, 1993). The reason(s) for this relationship has not been generically established but should be carefully assessed for chemicals on a case-by-case basis. Some may be due to induction of hepatic microsomal enzymes."

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified thiophanate-methyl as **"likely to be carcinogenic to humans"** by the oral route based on the following weight-of-the-evidence considerations:

1. Tumors were seen in both sexes of two species. Liver tumors were seen in male and female mice and thyroid tumors were seen in male and female rats. A dose-

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HED DOC Number: 012444
DP Barcode: D240618
SignOff Date: 1/7/1998

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response in tumor incidence and progressive development of lesions were evident. Moreover, the same spectrum of effects was seen for both sexes in each species.

2. The relevance of the observed tumors to human exposure cannot be discounted

3. Thiophanate-methyl appears to be aneugenic. Methyl-2-benzimidazole carbamate (MBC), a metabolite of thiophanate-methyl, is a known aneugen, hepatocellular carcinogen and teratogen. MBC is also a metabolite of benomyl, an aneugen, which also produces liver tumors in mice. The potential for a direct DNA reactive mutagenic potential can not be dismissed by the available data.

4. There was some evidence of interference with thyroid pituitary homeostasis, an effect seen with other thiourea compounds. However, reversibility of the hormonal effect was not demonstrated, the studies were conducted only at an excessive dose and not at lower dose levels to demonstrate a dose-response. Additional studies are required to evaluate the direct mutagenic potential of thiophanate-methyl.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended the following approach for human risk characterization:.

- For human risk characterization, the extrapolation of risk using the linear low-dose (Q_1*) default approach for liver tumors was recommended. This extrapolation was supported by the lack of confirmation of the mode of action, concern for mutagenicity and dose-dependent increases in the incidence of liver tumors in male and female mice.

VIII. BIBLIOGRAPHY

Submitted studies

MRID No.	Citation
00032673	Noguchi, T. Hashimoto, Y., Makita, T. *et al.* Chronic oral toxicity studies of thiophanate, diethyl 4,4'-o-phenylenebis (3-thioallophanate), in Sprague-Dawley strain rats. Nippon Soda Co. and Nara Medical University, Second Dept. Of Pathology. No study number or date (report received June 27, 1980). Unpublished report.
00032674	Hashimoto, Y., Makita, T., Nishibe, T. *et al.* Toxicological evaluation of thiophanate (X): The final report on the carcinogenesis studies of thiophanate. Diethyl 4,4'-o-phenylenebis (3-thioallophanate), in mice of C57BL strain for full life span. Nippon Soda Co. and Nara Medical University, Second Dept. of Pathology. No study number or date (report received June 27, 1980). Unpublished report.
40095503	Myhr, B.C. (1981) Evaluation of pure thiophanate-methyl in the primary rat hepatocyte unscheduled DNA synthesis assay. Litton Bionetics, Inc., Kensington, MD. Study No 211191. October, 1981. Unpublished report.
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