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

April 14, 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cancer Assessment Review Committee Meeting on Thiophanate-Methyl

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Thiophanate-Methyl prepared by Linnea Hansen.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday April 28,1999 at 10:00 am in Room 813, CM2.

Addressees

K. Baetcke
L. Brennecke
L. Brunsman
W. Burnam
M. Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
M. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor
Y. Woo

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TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Thiophanate-methyl.

Tox Chem No.: 375A
PC No.: 102001
CAS No.: 23564-05-8

FROM: Linnea J. Hansen, Ph.D.
Toxicology Branch I
Health Effects Division (7509C)

Alberto Protzel for 4/9/99

TO: Sanjivani Diwan, Ph.D.
Executive Secretary, Peer Review Committee for Carcinogenicity
Health Effects Division (7509C)

THROUGH: Alberto Protzel, Ph.D., Senior Scientist
Toxicology Branch I
Health Effects Division (7509C)

Alberto Protzel 4/9/9

Attached are Sections C, D, E and F for incorporation into the Peer Review Document on thiophanate-methyl.

The issues of concern are the occurrence of thyroid follicular cell adenomas and carcinomas in male rats and adenomas in female rats and hepatocellular adenomas in male and female mice.

The discussion of the use of the threshold model for evaluation of thyroid tumors in the Weight of the Evidence section of this document has been revised from the previous format to reflect wording contained in the current Agency science policy for assessment of thyroid tumors.

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A and B. Not included in this document. (To be prepared by the Peer Review Committee).

C. 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(iminocarbono-thioyl)]bis(carbamate). The chemical structure is shown below in 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.

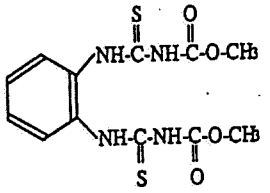

Figure 1: Thiophanate-methyl

D. Evaluation of Carcinogenicity Evidence

1. Rat 2-Year Dietary Chronic/Carcinogenicity Study

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. [See Attachment 1 for DER].

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. In males, the incidence of follicular cell adenocarcinoma was also increased. The incidence of these tumors is shown below in Table 1, males and Table 2, females (extracted from Tables 3 and 4, statistical analyses prepared by L. Brunsman in memorandum dated 3/31/99) [see Attachment 2]:

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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	0/46	0/45	3/47	12[a]/44
(%)	(2)	(0)	(0)	(6)	(27)
p =	0.000**	-	-	0.309	0.014*
Carcinomas	0/47	0/44	0/42	0/47	3[b]/27
(%)	(0)	(0)	(0)	(0)	(11)
p =	0.002**	-	-	-	0.011*
Combined	1/50	0/46	0/45	3/47	14[c]/44
(%)	(2)	(0)	(0)	(6)	(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 pairwise comparison with controls; a significant increasing trend was also observed ($p<0.01$). The incidence

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of follicular cell adenocarcinoma (control to high dose) was 0%, 0%, 0%, 0% and 11%. A significant pairwise increase was not observed but there was 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 pairwise 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 adenocarcinoma (control to high dose) was 0%, 0%, 0%, 2% and 4%. Pairwise 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[1] 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[2] 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:

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

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[1]Haseman, J.K. *et al.* (1990). Tumor Incidences in Fischer 344 Rats: NTP Historical Data. In: Pathology of the Fischer Rat (Boorman, G.A. *et al.* Ed), pp. 555-564, Academic Press, San Diego.

[2]Maekawa, A. *et al.* (1983). Spontaneous Tumors in F-344/DuCrj Rats. Gann, 74:365-372.

exceeds the range of incidence from NTP database at 6000 ppm.

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 (Attachment 1): 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: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$,

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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

Mortality in males is shown below in Table 7, extracted from Table 1 of the statistical analysis prepared by L. Brunsman.

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Table 7. Thiophanate-methyl Fischer 344 Rat Study
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[i]	53-78	79-106[f]	Total
0	0/60	0/60	10/60	1/50	12/49	13/50 (26)**
75	0/60	0/60	10/60	2/50	14/48	16/50 (32)
200	0/60	0/60	10/60	7/50	17/43	24/50 (48)*
1200	0/60	0/60	10/60	3/50	20/47	23/50 (46)
6000	0/52[a]	0/52	5[b]/52	4/47	41/43	45/47 (96)**

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 105.
[i]Interim sacrifice at week 53.
[a]Six accidental deaths at week 11, two at week 12, dose 6000 ppm.
[b]Five instead of ten animals were sacrificed at the week 53 interim sacrifice at the 6000 ppm dose due to the eight accidental deaths at weeks 11 and 12.
()Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Mortality was significantly increased at 6000 ppm by pairwise comparison with controls and an increasing trend was also observed (both $p<0.01$). Only 2 males remained alive at termination (96% mortality).

In addition, decreased body weight/weight gain at 1200 ppm (16%/21% less than controls), 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. <u>Adequacy of Dosing for Assessment of Carcinogenic Potential</u>

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. The high dose of 6000 ppm was not considered to exceed the MTD; in addition to findings observed at 1200 ppm, decreased body weight/weight gain, increased TSH, pathology of the adrenal cortex were reported.

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, 3,000 or 7,000 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 1,388.2 mg/kg/day for females. [DER for this study is in Attachment 3].

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 7 and 8 of the statistical analysis prepared by Lori Brunsman dated 3/31/99 (see Attachment 2).

Table 8. 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 $p < 0.01$.

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 pairwise 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 pairwise comparison and an increasing trend was observed (all $p<0.01$).

Table 9. 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 pairwise 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 from the testing laboratory for these tumors in CD-1® mice were requested but at this writing have not been provided.

Historical control data on the CD-1® mouse have been published by the supplier, Charles River.[3] 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 is summarized below in Table 10:

TABLE 10: HISTORICAL CONTROL DATA FOR INCIDENCE OF HEPATOCELLULAR TUMORS IN CD-1® MICE

Tumor/parameter	Males	Females
Hepatocellular adenoma:		
No. examined	770	769
No. tumors	83	5
Mean % incidence	10.78	0.65
Range % incidence	0-19.3	0-2.00
Hepatocellular carcinoma:		
No. examined	770	769
No. tumors	38	3
Mean % incidence	4.94	0.39
Range % incidence	1.25-11.54	0-2.00

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

c. Non-neoplastic Lesions

[3]Charles River Laboratories, prepared by Patricia L. Lang, Ph.D., Consulting Toxicologist (1992) Spontaneous Neoplastic Lesions in the Crl:CD-1 BR Mouse.

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

TABLE 11. NONNEOPLASTIC LESIONS IN MICE ADMINISTERED THIOPHANATE METHYL FOR 18 MONTHS[a]

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

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, abs/rel) and increased relative liver weight at week 39 (26% above controls). Abs/rel liver weights of females were 24%/26% above controls at week 39.

Mortality in males is shown below in Table 12, below, extracted from statistical analysis prepared by L. Brunsman:

Table 12. Thiophanate-methyl CD-1 Mouse Study
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (ppm)	1-20	21-40	40[i]	41-60	61-80[f]	Total
0	0/60	0/60	10/60	2/50	8/48	10/50 (20)**
150	0/60	0/60	10/60	2/50	8[a]/47	10/49 (20)
640	0/60	2/60	10/58	2/48	10/46	14/50 (28)
3000	0/60	0/60	10/60	6/50	10/44	16/50 (32)
7000	1/60	3/59	10/56	5/46	15/41	24/50 (48)**

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 78.
[i]Interim sacrifice at week 40.
[a]One accidental death at week 68, dose 150 ppm.
()Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Mortality in males was significantly increased at 7000 ppm by pairwise 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.

20

Table 13. Thiophanate-methyl CD-1 Mouse Study
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-20	21-40	40[i]	41-60	61-79[f]	Total
0	1/60	1/59	10/58	1/48	9/47	12/50 (24)**
150	0/60	1/60	10/59	5/49	7/44	13/50 (26)
640	0/60	2/60	10/58	2/48	11/46	15/50 (30)
3000	0/60	0/60	10/60	3/50	14/47	17/50 (34)
7000	0/60	1/60	10/59	7/49	15/42	23/50 (46)*

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 79.
[i]Interim sacrifice at week 40.
()Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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.

E. Additional Toxicology Data on Thiophanate-Methyl

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, 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 (section prepared by Nancy McCarroll)

The only 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 gene mutation or *in vivo* 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 aberrations in whole animals and caused cell transformation *in vitro*. The positive results from the *in vivo* micronucleus assay are consistent with the data from the common metabolite of benomyl and thiophanate-methyl (methyl-2-benzimidazolecarbamate, MBC) indicating that both compounds are confirmed inducers of aneuploidy (adverse effects on chromosome numbers). Since aneuploidy may be involved in carcinogenesis, 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). No correlation can be

made relative to the possible role of aneuploidy as a contributing factor to birth defects since the rat developmental studies were considered unacceptable (MRID Nos. 00106090, 00146643/92186011); however, there was no indication of a developmental effect in these studies.

The acceptable studies do not satisfy either pre-1991 or new mutagenicity guideline requirements. In consideration of the results from the open literature, however, it is concluded that the acceptable studies submitted to the Agency combined with the data from the open literature studies satisfy the pre-1991 mutagenicity test guidelines, and that no further testing is warranted. Summaries of the acceptable mutagenicity studies and studies from the open literature are presented below:

SUBMITTED STUDIES

CHROMOSOME ABERRATIONS

1) *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; ≥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 vivo* cytogenetic mutagenicity data (MRID No. 40980101).

OTHER MUTAGENIC MECHANISMS

2) *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).

OTHER INFORMATION

GENE MUTATIONS

In preincubation *Salmonella typhimurium* mammalian microsome gene mutation assays (Zeiger et al., 1992)[4], 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.

CHROMOSOME ABERRATIONS (SOMATIC CELLS)

Cytogenetic analysis performed by Barale et al. (1993)[5] 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

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[4]Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: IV Results of testing 311 chemicals. Environ and Mol Mutagen 19 [Suppl 21]:2-141.

[5]Barale, R., Scapoli, C., Meli, C., Casini, D., Minunni, M., Marrazzini, A., Loprieno, N. and Barrai, I. (1993). Cytogenetic effects of benzimidazoles in mouse bone marrow. Mutat Res. 300:15-28.

assays for detection of aneuploidy induction with benomyl (MRID No. 42911601) and MBC (MRID No. 42911602).

OTHER MUTAGENIC MECHANISMS--CELL TRANSFORMATION

In independently performed BALB/c3T3 *in vitro* cell transformation assays (Perocco et al., 1997)[6], 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.

CONCLUSIONS

Overall, the data from the submitted studies and the open literature indicate that thiophanate-methyl is neither clastogenic nor causes UDS in cultured mammalian cells. There is, however, reproducible positive results from *in vitro* cell transformation studies as well as micronuclei induction in the absence of structural chromosome aberrations *in vivo*. There is also convincing and supporting evidence that while both benomyl and the MBC metabolite induce micronuclei *in vivo*, neither compound is clastogenic. Hence, the available data for thiophanate-methyl are consistent with the genetic toxicology profiles for benomyl and MBC and indicate that thiophanate-methyl causes aneuploidy. Since it is generally acknowledged that somatic cell aneuploidy may be involved in carcinogenesis and the test article caused morphologically transformed cells *in vitro*, it is not surprising that the results from genetic toxicology testing with thiophanate-methyl correlate favorably with the data from the chronic feeding study demonstrating hepatocellular carcinomas in male and female mice (MRID No. 42607701). These data are also consistent with the results of the chronic mouse bioassay indicating that benomyl is a liver carcinogen (MRID No. 00096514). As an aneugen, the possible role of thiophanate-methyl in contributing to birth defects can not be determined at this time since both rat

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[6]Perocco, P., Del Ciello, C., Mazzullo, M., Rocchi, P., Ferreri, A.M., Paolini, M. Pozzetti, L. and Cantelli-Forti, G. (1997). Cytotoxicity and cell transforming activities of the fungicide methyl thiophanate on BALB/c 3T3 cells in vitro. Mutat. Res. 394:29-35.

developmental studies were considered unacceptable. There was, however, no indication of a developmental effect in these studies.

In light of the evidence indicating that thiophanate-methyl is an aneugen, it is concluded that no additional genetic toxicology testing is warranted. The acceptable studies combined with the open literature studies satisfy the Pre-1991 mutagenicity initial testing battery guidelines and provide adequate data to draw meaningful conclusions.

STRUCTURAL ACTIVITY RELATIONSHIP (SAR)

Thiophanate-methyl is metabolized in the rat to methyl-2-benzimidazolecarbamate (MBC, Attachment 4), which is also a metabolite of the fungicide pesticide benomyl, a known aneugen, hepatocellular carcinogen and teratogen. Both methyl-thiophanate and benomyl can convert to methyl-2-benzimidazolecarbamate (MBC), the active form. However, conversion of methyl-thiophanate to MBC proceeds at a slower rate than benomyl (Selling et al., 1970)[7]. 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) [8] with thiophanate-methyl.

3. Special Mechanistic Studies (Attachment 5)

A series of 6 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 2-year chronic toxicity/carcinogenicity study (MRID 42899601) and later submitted in a separate, more complete study report (MRID 42899601b).

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[7]Selling, H.A., Vonk, J.W., Kaars Sijpesteijn, A. (1970). Transformation of the systemic fungicide methyl thiophanate in to 2-benzimidazole carbamic acid methyl ester, in: Chemistry and Industry, Madley, London, pp.1625-1626.

[8]Barale, R. et al., . Mutat Res. 300 (1993):15-28.

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 14:

Table 14: 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≤0.05; ** p≤0.01; ***p≤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 14, 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 abs/rel 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.

Abs/rel 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 abs/rel thyroid weight increases (10%/9% vs. 128%/136% above controls) and increases in TSH levels (55%, vs. 361% above controls). No changes in abs/rel 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 cytochrome 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 (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). Abs/rel 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 abs./rel liver weights were increased at both days 2 and 8 (20%-35% above controls). Body weights were not affected. In both species, phenobarbital caused similar responses.

4. Developmental Toxicity

No developmental toxicity was observed in the rat oral developmental toxicity study (MRID 00106090); however, this study is presently classified as unacceptable but may be upgraded with submission of additional information. In this study, pregnant animals were administered test material at dose levels of 0, 100, 300 or 1000 mg/kg/day (limit dose) from GD 6-19. Maternal toxicity (transiently decreased body weight/weight gain) was observed at 1000 mg/kg/day.

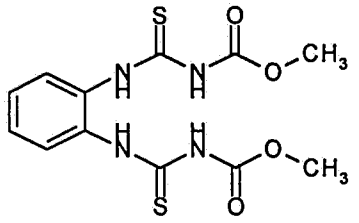
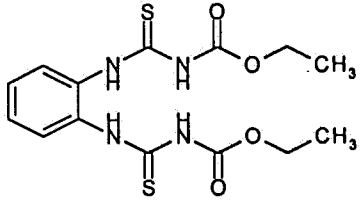
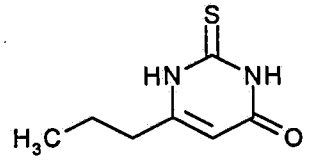
In the rabbit developmental toxicity study (MRID 40022901), pregnant females were dosed with 0, 2, 6 or 20 mg/kg/day, GD 6-19. A dose-related increased incidence of asymmetric pelvis, a skeletal variation considered an indication of developmental toxicity, was reported at 6 and 20 mg/kg/day. Maternal toxicity was observed as transient decreased body weight and food consumption at 20 mg/kg/day.

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, 630 or 2,000 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 the lowest dose, 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.

5. Structure Activity Relationship

Thiophanate-methyl is a thioallophanate. Related chemicals available and their carcinogenicity effects are shown below in Tables 15 and 16:

TABLE 15: STRUCTURAL COMPARISON OF THIOPHANATE-METHYL TO OTHER THIOUREA COMPOUNDS[a]

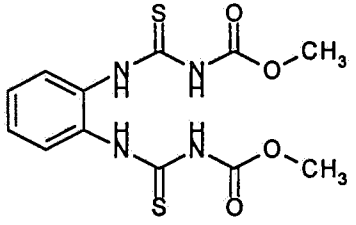
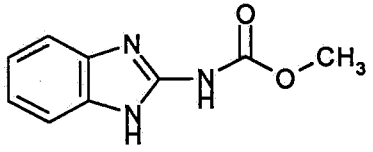
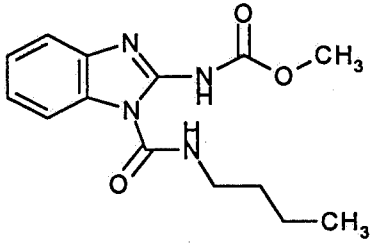
Compound	Structure	Carcinogenic Effect
Thiophanate Methyl		Rat (S-D): thyroid adenoma, carcinoma (at excessive dose of 6000 ppm in males; marginal increase in thyroid adenoma in females and at lower doses in males) Mouse (CD-1): hepatocellular adenoma, males and females (≥3000 ppm)
Thiophanate Ethyl		Rat (F-344): 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

[a] IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man: Some Anti-thyroid and Related Substances, Nitrofurans and Industrial Chemicals (1974) Vol 7:pp. 67-76.

As shown in Table 15, thiophanate-methyl, a thioallophanate compound, shares a thionamide moiety with other known thyroid carcinogenic agents. An example is propylthiouracil, which is a potent inducer of thyroid tumors in both rats and mice[9]. The closely related compound thiophanate-ethyl did not cause tumors in rats (MRID 00032673) or mice (MRID 00081605) 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 16:

TABLE 16: 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)

6. Acute, Subchronic and Chronic Toxicity

a. Acute Toxicity Studies: Technical thiophanate-methyl is classified as Toxicity Category IV for acute oral toxicity (MRID 41644301) and primary eye and dermal irritation (MRIDs 40095501 and -02). It is classified as Toxicity Category III for acute dermal and inhalation toxicity (MRIDs 41644302 and 41482804). It is a dermal sensitizer (MRID 41482805).

b. Subchronic Toxicity Studies: 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 and included 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 showed decreases only during the first 1 or 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.

Dermal Toxicity - A 21-day dermal toxicity study (MRID 42110801) tested 5 New Zealand white rabbits/sex/dose group at dose levels of 0, 100, 300 or 1,000 mg/kg/day (moistened with distilled water and applied dermally 5 days/week, 6 hrs/day - total of 15 applications). Decreased food consumption was observed in females at 300 and 1000 mg/kg/day and in males at 1000 mg/kg/day. Although no other systemic toxicity was reported, this finding suggests that the test material is absorbed because it was dose-related in females and was observed in both sexes at the high dose.

c. Chronic Oral Toxicity Studies: Long-term oral toxicity studies in the rat (chronic toxicity/ carcinogenicity) and mouse (carcinogenicity) are summarized above in Section D.

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, 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. Decreases in body weight and thyroid effects were more pronounced than at 40 mg/kg/day.

d. Neurotoxicity Studies: Acute and subchronic neurotoxicity studies in the rat are not available.

F. Weight of Evidence Considerations:

Weight of the Evidence: The Committee is asked to consider the following facts regarding the toxicology data on thiophanate-methyl in a weight-of-the-evidence determination of carcinogenic potential:

1. Thiophanate-methyl, when administered in the diet to male Fischer 344 rats for 24 months at 0, 75, 200, 1200 or 6000 ppm (0, 3.3, 8.8, 54.4 or 280.6 mg/kg/day), was associated with a statistically significantly increased incidence of thyroid follicular cell adenomas, carcinomas and combined adenoma/adenocarcinoma by pairwise comparison of controls with the high dose group. The incidences (control to high dose) were 2%, 0%, 0%, 6% and 27%, adenomas; 0% all doses except 11% at high dose, carcinomas; and 2%, 0%, 0%, 6% and 32%, combined incidence. Significant increasing trends were also observed for these tumors. In females, tumors were not significantly increased by pairwise comparison with controls, but an increasing trend was observed (incidence from control to high dose was 0%, 0%, 0%, 2% and 4%). Dosing was equivalent to 0, 3.8, 10.2, 63.5, 334.7 mg/kg/day. No carcinomas were observed in females. The incidence of both adenomas and carcinomas exceeded the range of incidence observed in available historical control data for Fischer 344 rats (no data were available from the study laboratory itself); in females and in males at 1200 ppm, this increase was only slightly above the range but was more pronounced in males at 6000 ppm.

2. Dosing was considered adequate for male rats 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 exceeded the MTD: mortality was elevated during the last 6 months of the study and by termination only 2 males survived. This was the only dose level at which statistically significant increases in thyroid follicular cell tumors by pairwise comparison with controls were observed, although an increasing trend was also observed. Dosing was considered adequate for female rats up to 6000 ppm, based on increased cholesterol, increased liver, thyroid and kidney weights and increased incidence of pathologic alterations in the liver, thyroid and kidney at 1200 ppm and at 6000 ppm, decreased body weight/weight gain, increased TSH and pituitary pathology. The toxicity observed at 6000 ppm was not considered excessive in females.

3. Thiophanate-methyl, when administered in the diet to male and female CD-1 mice for 18 months at dose levels of 0, 150, 640, 3000 or 7000 ppm (0, 4.0, 93.4, 383.6 or 943.5 mg/kg/day, males and 0, 6.5, 153, 634.9 or 1388.2 mg/kg/day, females) was associated with a statistically significant increase in the incidence of hepatocellular adenomas at the highest two dose levels in males and the highest 3 dose levels in females by pairwise comparison with the controls. From control to high dose, the incidences were 9%, 17%, 15%, 42% and 57%, males and 0%, 0%, 8%, 24% and 56%, females. A significant increasing trend was also observed. The combined incidence of adenomas and carcinomas was also significantly increased in males only at the highest two dose levels (9%, 17%, 17%, 42% and 57%) along with an increasing trend; however, the incidence of hepatocellular carcinomas alone was not increased. The incidence of adenomas that were statistically significant were outside of the range of spontaneous incidence in the available historical control data for both male and female CD-1 mice from the supplier. These tumors were not observed at the interim (12-month) sacrifice.

4. Dosing in mice was considered adequate. 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. Increased mortality and decreased RBC count were observed at 7000 ppm. In females, increased incidence of hepatocellular hypertrophy and atrial thrombosis were observed at 3000 ppm. Increased mortality, decreased body weight, decreased T4 and increased thyroid, liver and heart weights were observed at 7000 ppm.

5. In the rat chronic toxicity/carcinogenicity feeding study, statistically significant increases in the incidences of thyroid follicular cell hypertrophy/hyperplasia were observed in both sexes at 1200 and 6000 ppm. The incidences of thyroid follicular cell focal hypertrophy in males and focal hyperplasia in females were also increased in both sexes at 6000 ppm. Absolute and relative thyroid weights were increased in both sexes. Statistically significant perturbations of circulating thyroid hormones T3 and/or T4 and TSH were observed in males at 1200 and 6000 ppm and to a lesser extent in females at 6000 ppm. Effects on the thyroid were therefore observed at the same dose levels at which tumors were observed. In the mouse carcinogenicity feeding study, thyroid hypertrophy and hyperplasia were not observed, although thyroid weights were increased in males at 1200 and 7000 ppm and in females at 7000 ppm in the interim sacrifice animals. Statistically significant perturbations in circulating T4 and TSH were observed but were less consistent than in rats.

6. In the rat chronic toxicity/carcinogenicity feeding study, statistically significant increases in the incidence of hepatocellular hypertrophy were observed in both sexes at 1200 and 6000 ppm. Absolute and relative liver weights were increased at these dose levels. Hepatocellular hypertrophy was also observed in the mouse carcinogenicity feeding study in males at 3000 and 7000 ppm and in females at 640 ppm and above, along with focal necrosis and focal fatty degeneration in males at 7000 ppm. Liver weights were increased at 7000 ppm in both sexes (and to a marginal extent at 3000 ppm).

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7. Additional studies to assess the mechanism of thyroid tumor induction demonstrated that after short-term dietary administration (2 to 8 days) of thiophanate-methyl at 6000 ppm: (a) both liver and thyroid weights are significantly increased; (b) this increase is accompanied by increased circulating levels of TSH and decreased T3 and T4 and increased serum cholesterol; © PCNA labeling of rat and mouse livers from animals treated for 2 vs. 8 days demonstrated that mouse liver activity was increased at both time points, whereas activity in rats was increased only at day 2 (thyroid was not evaluated for cell proliferation, although microscopic evaluation in the rat subchronic and chronic studies indicated hyperplasia as well as hypertrophy); (d) liver microsomes from thiophanate-methyl rats show increased activity *in vitro* of UDP-glucuronosyltransferase and to a lesser extent, cytochrome P450 and cytochrome b5. Porcine thyroid peroxidase activity *in vitro* was decreased, but thiophanate-methyl was approximately 30-fold less potent that propylthiourea, a known antithyroid agent; (e) supplementation of thiophanate-methyl treated rats with T4 prevented the increases in thyroid weight and TSH but not increases in liver weight or serum cholesterol and (f) withdrawal of thiophanate-methyl treatment caused a reversal of the thyroid weight increases (however, this study did not evaluate the effect of withdrawal on circulating levels of TSH and T3/T4 or on histopathology). The above studies also did not evaluate response at other dietary dose levels.

8. Thiophanate-methyl shows genotoxic potential as an aneugen, but not clastogen, as demonstrated by positive responses in an *in vivo* mouse bone marrow micronucleus assay and a BALBc/3T3 cell *in vitro* cell transformation assay (published studies).

9. Thiophanate-methyl is metabolized to methyl-2-benzimidazole carbamate (MBC), which is also a major metabolite for the fungicide benomyl. Both benomyl and MBC are aneugenic but not clastogenic and cause increased incidence of hepatocellular tumors in CD-1 mice in

lifetime dietary studies. Benomyl has been reviewed by the Cancer Peer Review Committee and classified as a C carcinogen with a Q_1^* of 0.0042 mg/kg/day^{-1}, based on increased incidence of hepatocellular tumors in male and female mice in studies on both benomyl and MBC. However, there is no evidence of thyroid effects in the rat in the chronic toxicity/carcinogenicity studies available on benomyl or MBC. The metabolic pathways are not identical in that (1) The rate of production of MBC appears to be more rapid with benomyl compared to thiophanate-methyl and (2) besides MBC and hydroxylated/sulfated derivatives, benomyl and thiophanate-methyl also produce a different spectrum of other metabolites.

Consideration of the Use of the Threshold Model for Thiophanate-Methyl

When evaluating thiophanate-methyl, the Committee is asked to consider whether use of the threshold model for thyroid neoplasms is 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 © 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.

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:

Consideration of whether the thyroid tumors associated with administration of thiophanate-methyl can be attributed to disruption of the thyroid-pituitary hormonal balance (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 of the thyroid. 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 (via peroxidase inhibition). 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 P450 and

cytochrome b5 were observed in liver homogenate and microsomal preparations taken from rats treated for 8 days with thiophanate-methyl at 6000 ppm in the diet. Experiment 5 demonstrated that 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 propylthiouracil (PTU), a known antithyroid chemical, inhibitor of this enzyme and thyroid carcinogen in rats and mice. 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 perturbation of thyroid hormone levels, hypertrophy and hyperplasia in both sexes. Increases in thyroid tumors were only observed at dose levels causing these effects. However, in males at 6000 ppm, toxicity was excessive and the MTD was exceeded.

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. 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.

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 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 not have 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 (Table 15) shares the thionamide 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, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of thiophanate-methyl may be due to disruption in the thyroid-pituitary status.

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

Thiophanate-methyl has been shown to have mutagenic 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.

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 incidence of hepatocellular adenomas was observed in both sexes at the highest 2 dose levels tested.

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 (pairwise 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 the proliferative effect of thiophanate-methyl in mice may be more sustained in mice than in rats.

Conclusions: As indicated above, based on the overall judgement of the 8 types of data evaluating evidence for antithyroid activity, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of thiophanate-methyl may be due to a disruption in the thyroid-pituitary homeostasis. Despite some experimental omissions in the special mechanistic study, the criteria for a threshold effect have largely been met except for genotoxicity and the presence of a second tumor type. In addition to evidence supporting disruption of the thyroid-pituitary homeostasis, the following factors should therefore be 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; (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, none of which exceeded the MTD; (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 mutagenic. However, benomyl did not affect the thyroid or induce thyroid tumors in rats. 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, the available information suggests that although thiophanate-methyl appears to disrupt thyroid-pituitary homeostasis, the carcinogenic effect to the thyroid is relatively weak. If genotoxicity 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. The Committee is asked to determine whether (1) despite evidence supporting perturbance of the thyroid-pituitary homeostasis, the low incidence of thyroid tumors at non-excessive doses indicates lack of significant carcinogenic potential and cancer risk should be based solely on incidence of hepatic tumors in mice or whether (2) the thyroid tumors should be considered together with the liver tumors in assessment of cancer risk of thiophanate-methyl.

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 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:

1. 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...
2. A linear dose-response procedure should be assumed when the mode of action underlying thyroid tumors is judged to involve mutagenicity alone.

3. 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...
4. 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...
5. 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."

G. <u>Not in this document</u> (to be prepared by the Peer Review Committee).

Attachment 1

DER of the Rat 2-Year Feeding Chronic Toxicity/Carcinogenicity Study