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Thiophanate-Methyl, tech. Oral Carcinogenicity (83-2b)

EPA Reviewer: Linnea J. Hansen, Ph.D. Linnea J. Hansen Date 3/22/99
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Susan Makris, M.S. Susan Makris, Date 3/23/99
Toxicology Branch I(7509C)

Note: This executive summary is an addendum to a DER dated 9/21/94 (HED Doc. No. 010931)

DATA EVALUATION RECORD

STUDY TYPE: Oral Carcinogenicity (18-Month Dietary) - Mouse [§83-2(b)]

MRID NO.: 42607701

DP BARCODE: D241367
P.C. CODE: 102001

SUBMISSION CODE: S511876
TOX. CHEM. NO.: 375A

TEST MATERIAL (PURITY): Thiophanate-methyl (95.93% and 96.55%)

SYNONYMS: Topsin® M; Dimethyl 4,4'-o-phenylenebis (3-thioallophanate); 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene

CITATION: 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. Unpublished. (MRID 42607701).

SPONSOR: Elf Atochem (Formerly sponsored by Pennwalt Corporation)

EXECUTIVE SUMMARY: In a dietary carcinogenicity study (MRID 42607701), thiophanate-methyl (tech., 95.93 to 96.55% a.i.) was administered daily to 50 CD-1 mice/sex/dose at concentrations of 0, 150, 640, 3000 or 7000 ppm for 18 months (equivalent to average daily intakes of 0, 23.7, 98.6, 467.6 or 1078.8 mg/kg/day, males and 0, 28.7, 123.3, 557.9 or 1329.4 mg/kg/day, females). An additional 10 mice/sex/dose were administered these dose levels and sacrificed at 39 weeks.

At 640 ppm, increased incidence of hepatocellular hypertrophy was observed in females (8% vs. 0% affected, controls). At 3000 ppm, slightly decreased mean body weights in males, primarily during the middle of the study (<8% below controls; gain -12% below controls at week 53), transiently increased TSH (week 39, +100% above controls), increased abs/rel thyroid weights in males (+52%/+64% above controls, week 39 only), increased abs/rel liver weights (+20 to +26% above controls, males and females), increased

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DER for the Mouse 18-Month Feeding Carcinogenicity Study

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incidence of hepatocellular hypertrophy in males and females (25% affected vs. 10%, controls and 10% vs. 0%, controls, respectively) and increased incidence of atrial thrombosis in females (35% vs. 0%, controls) were observed. At 7000 ppm, decreased survival (males 52% vs. 82%, controls; females 54% vs. 76%, controls), decreased mean body weight and weight gain, primarily during the middle of the study (body weight -3% to -8% less than controls and gain at 53 weeks -12%, females and -16%, males); decreased mean body weight at termination in females (-8%) but not males; slightly decreased RBC count in males (-15%); decreased T4 in females (-28%, week 39); increased abs/rel liver weights (at wks 39 and 79, males +34%/+40% and +82%/+86%; females +57%/57% and +31%/31%), abs/rel thyroid weights (at wk 39, males >2-fold increase; females +30%) and abs/rel heart weights in females (+23%/+40%, wks 39 and 79); and increased incidence of hepatocellular hypertrophy in males and females (42% and 20% affected) and atrial thrombosis in males (16% vs. 2%, controls) and females (28%) were observed. **The systemic toxicity LOAEL is 640 ppm (123.3 mg/kg/day), based on hepatocellular hypertrophy in females. The NOAEL is 150 ppm (28.7 mg/kg/day). (The systemic toxicity LOAEL in males is 3000 ppm or 467.6 mg/kg/day, based on decreased body weight/weight gain, increased thyroid and liver weights and hepatocellular hypertrophy. The NOAEL is 640 ppm or 98.6 mg/kg/day).**

Hepatocellular adenoma showed statistically significant, dose-related increases in both sexes at 3000 and 7000 ppm (from control to high dose, 7%, 13%, 12%, 32% and 40%, males and 0%, 0%, 5%, 13% and 30%, females; all animals on study).

Dosing was considered adequate in both sexes based on body weight/weight gain decreases and increased thyroid weights in males, liver effects in both sexes and atrial thrombosis in females at 3000 ppm (and additional effects at 7000 ppm).

This study is classified **Acceptable (§83-2b)** and satisfies the guideline requirement for a carcinogenicity study in the mouse.

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

APR 21 1994

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: 102001. Thiophanate-methyl. Review of 18-Month Dietary Mouse Study as 6(A)(2) Data

PC Code 102001
Tox. Chem. No. 375A
Project No. D186825
Submission No. S433611
MRID No. 42607701

TO: Margarita Collantes, CRM Team # 53
Special Review and
Reregistration Division (7508C)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 4/13/94
Section I, Toxicology Branch I
Health Effects Division (7509C)

THRU: Roger L. Gardner, Section Head Roger Gardner
Section I, Toxicology Branch I 4/15/94 RLG 4/18/94
Health Effects Division (7509C)

Background and Request:

An 18-month dietary mouse oncogenicity study was submitted by Nippon Soda Co. Ltd. as part of the reregistration process. It was submitted as 6(A)(2) data. The Toxicology Branch (TB-I) has been asked to review the study.

Toxicology Branch Response:

TB-I has reviewed the mouse study and has determined that it satisfies the regulatory requirement for an oncogenicity study in the mouse (83-2(a)) for thiophanate-methyl. The study is classified as Core Guideline. Thiophanate-methyl was found to induce a statistically significant increase in hepatocellular adenomas under the conditions of the study. Therefore, the study satisfies the requirements for 6(A)(2) data. The following paragraphs summarize the results of the study.

Topsin M (technical thiophanate methyl) was fed to male and female CD-1 mice (60/sex/dose) at dietary levels of 0, 150, 640, 3000 or 7000 ppm for 18 months. The average daily intake values were 0, 23.7, 98.6, 467.6 or 1078.8 mg/kg/day for males and 0,

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28.7, 123.3, 557.9 or 1329.4 mg/kg/day for females. An interim sacrifice of 10/sex/dose was conducted at 39 weeks.

The systemic NOEL was 150 ppm (23.7 mg/kg/day in males and 28.7 mg/kg/day in females).

The systemic LOEL was 640 ppm (123.3 mg/kg/day in females) based on an increased incidence of hepatocellular hypertrophy in females.

At 3000 ppm (467.6 mg/kg/day in males and 557.9 mg/kg/day in females), males showed an increased incidence of hepatocellular hypertrophy and a small, but statistically significant decrease in body weight (<8%). Transient (observed only at week 39) increases in serum thyroid stimulating hormone (TSH) and in absolute and relative thyroid weights were also observed in males. Both males and females showed increased absolute and relative liver weights at week 39.

At the highest dose tested (7000 ppm; 1078.8 mg/kg/day in males and 1329.4 mg/kg/day in females), both males and females showed increased mortality and increased liver weight at both weeks 39 and 78. In addition, males showed enlarged thyroids at week 39 and decreased red blood cell count at week 79. Females at this dose also showed a small, but statistically significant decrease in body weight (<8%), decreased serum thyroxine (T_4) at week 39, and increased heart weight at weeks 39 and 78.

Under the conditions of the assay, a dose-related increase in the incidence of hepatocellular adenomas was observed in both sexes. The incidences were statistically significantly increased at both 3000 and 7000 ppm in both males and females.

The following table provides additional data on selected non-neoplastic and neoplastic lesions from the study. The additional tissues were selected on the basis of target organs for this chemical from other species and related organs of interest and other organs which are often affected by other chemicals. Amyloidosis tended to be present in the earlier sacrifices and deaths. At terminal sacrifice there was little present in the selected tissues. Therefore, the presence of amyloidosis at terminal sacrifice was not always included for the selected organs. It is possible that the animals which had it died earlier, as suggested in the Data Evaluation Record (DER).

Selected Neoplastic and Non-neoplastic Lesions

Dose (ppm) Observation	0	150	640	3000	7000
			Males		
Thyroid					
Amyloid deposition, interstitium					
Unscheduled deaths	1/9	1/11	6/13	5/16	15/24
Follicular hyperplasia					
Unscheduled deaths	0/9	1/11	0/13	0/16	0/24
Heart					
Amyloid deposition					
Unscheduled deaths	1/10	0/11	4/14	4/16	10/24
Kidneys					
Infiltrate cell, lymphocyte-interstitial					
Unscheduled deaths	0/10	4/11	4/14	3/16	8/24
Terminal sacrifice	14/40	16/39	16/36	16/34	18/26
Amyloid deposition, interstitial					
Unscheduled deaths	1/10	1/11	0/14	2/16	5/24
Terminal sacrifice	1/40	3/39	0/36	1/34	0/24
Amyloid deposition, glomerular					
Unscheduled deaths	1/10	6/11	5/14	6/16	13/24
Terminal sacrifice	8/40	8/39	6/36	2/34	2/24
Fibrosis, periglomerular					
Unscheduled deaths	0/10	0/11	0/14	0/16	3/24
Nephrosis					
Unscheduled deaths	0/10	2/11	0/14	2/16	5/24
Terminal sacrifice	4/40	3/39	10/36	8/34	1/24
Liver					
Amyloid deposition					
Unscheduled deaths	1/10	3/11	5/14	4/16	10/24
Hepatocellular Hyperplasia					
Unscheduled deaths	0/10	0/11	0/14	4/16	1/24
Hepatocellular Adenocarcinoma					
Terminal sacrifice	0/40	0/39	1/36	0/34	1/26
Pituitary					
Examined, unremarkable					
Unscheduled deaths	10/10	10/11	13/14	16/16	24/24
Terminal sacrifice	39/39	-	-	-	25/26

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Selected Neoplastic and Non-neoplastic Lesions

Dose (ppm) Observation	0	150	640	3000	7000
			Females		
Heart					
Amyloid deposition					
Unscheduled deaths	2/12	0/13	2/15	9/17	9/23
Kidneys					
Infiltrate cell, lymphocyte-interstitial					
Unscheduled deaths	2/12	2/13	0/15	2/17	9/23
Terminal sacrifice	12/38	10/37	11/35	18/33	11/27
Amyloid deposition, interstitial					
Unscheduled deaths	4/12	0/13	2/15	11/17	10/23
Terminal sacrifice	10/38	3/37	5/35	0/33	1/27
Amyloid deposition, glomerular					
Unscheduled deaths	4/12	3/13	6/15	12/17	12/23
Terminal sacrifice	12/38	7/37	7/35	1/33	1/27
Fibrosis, periglomerular					
Unscheduled deaths	0/12	1/13	0/15	0/17	2/23
Nephrosis					
Unscheduled deaths	2/12	1/13	3/15	1/17	1/23
Terminal Sacrifice	2/38	4/37	1/35	1/33	1/23
Liver					
Amyloid deposition					
Unscheduled deaths	1/12	0/13	5/15	9/17	8/23
Hepatocellular Hyperplasia					
Unscheduled deaths	0/12	0/13	0/15	0/17	0/23
Terminal sacrifice	1/38	0/37	3/35	1/33	2/23
Mammary gland					
Adenocarcinoma					
Unscheduled deaths	0/11	0/13	0/15	0/17	1/20
Terminal sacrifice	0/38	-	-	1/2	0/27
Pituitary					
Examined, unremarkable					
Unscheduled deaths	12/12	11/12	14/15	16/16	22/22
Terminal sacrifice	38/38	-	-	-	27/27
Thymus					
Atrophy					
Unscheduled deaths	1/11	2/12	0/14	0/17	3/20
Thyroid					
Amyloid deposition, interstitium					
Unscheduled deaths	3/12	1/13	4/15	12/17	13/22
Terminal sacrifice	9/38	3/37	6/35	2/33	1/27
Follicular hyperplasia					
Unscheduled deaths	0/12	0/13	0/15	0/17	0/22

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FINAL

DATA EVALUATION REPORT

Topsin M

Study Title:
18-Month Dietary Oncogenicity Study in Mice with Topsin M

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Vera Brankovan, Ph.D. Date 10/7/93

Independent Reviewer John Liccione, Ph.D. Date 10/7/93

QA/QC Manager Sharon Segal, Ph.D. Date 10/7/93

Contract Number: 68D10075
Work Assignment Number: 2-05
Clement Number: 205.2.223.1
Project Officer: Caroline Gordon

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GUIDELINE Series 83-2

EPA Reviewer: William Greear, MPH
Toxicology Branch I, Review Section IV
Health Effects Division (H7509C)

Signature: WPC for WG
Date: 2/23/94

EPA Section Head: Marion Copley, DVM
Toxicology Branch I, Review Section IV
Health Effects Division (H7509C)

Signature: M Copley for
Date: 2/23/94

DATA EVALUATION REPORT

STUDY TYPE: Oral oncogenicity study in mice (Guideline 83-2)

TEST MATERIAL: Thiophanate methyl technical

Tox Chem. Number: 375A

P.C. Code: 102001

MRID Number: 426077-01

SYNONYMS: Topsin M

STUDY NUMBER: WIL-75024

SPONSOR: Nippon Soda Co. Ltd., Chiyoda-ku, Tokyo 100, Japan

TESTING FACILITY: WIL Research Laboratories, Inc., Ashland, Ohio

TITLE OF REPORT: 18-Month Dietary Oncogenicity Study in Mice with Topsin M

AUTHORS: E. Crosby Tompkins, Ph.D.

REPORT ISSUED: November 13, 1992

CONCLUSIONS: Topsin M was fed to male and female CD-1 mice (60/sex/dose) at dietary levels of 0, 150, 640, 3,000 and 7,000 ppm for 18 months. The average daily intake values for mice receiving diets containing 0, 150, 640, 3,000, and 7,000 ppm were 0, 23.7, 98.6, 467.6, and 1,078.8 mg/kg/day, respectively, for males and 0, 28.7, 123.3, 557.9, and 1,329.4 mg/kg/day, respectively, for females. An interim sacrifice of 10/sex/dose was conducted at 39 weeks.

NOEL (systemic) = 150 ppm (23.7 mg/kg/day in males and 28.7 mg/kg/day in females)

LOEL (systemic) = 640 ppm (123.3 mg/kg/day in females) based on an increased incidence of hepatocellular hypertrophy in females.

In addition, at 3,000 ppm (467.6 mg/kg/day in males and 557.9 mg/kg/day in females), males showed an increased incidence of hepatocellular hypertrophy and a small, but statistically significant, decrease in body weight (<8%). Transient (observed only at week 39) increases in serum thyroid stimulating

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hormone (TSH) and in absolute and relative thyroid weights were also observed in males. Both males and females showed increased absolute and relative liver weight at week 39.

At the highest dose tested (7,000 ppm; 1,078.8 mg/kg/day in males and 1,329.4 mg/kg/day in females), both males and females showed increased mortality and increased liver weight at both weeks 39 and 78. In addition, males showed and enlarged thyroid at week 39 and decreased red blood cell count at week 79. Females at this dose also showed a small, but statistically significant decrease in body weight (<8%), decreased serum thyroxine (T4) at week 39, and increased heart weight at weeks 39 and 78.

Under the conditions of this assay, a dose-related increase in the incidence of hepatocellular adenomas was observed in both sexes. The incidence was statistically significant at both 3,000 and 7,000 ppm in both males and females.

CORE CLASSIFICATION: Core Guideline. This study satisfies the requirements of EPA Guideline Series 83-2 for an oral oncogenicity study.

A. MATERIALS AND METHODS

1. Test Article Description

Name: Thiophanate methyl technical (Topsin M)

Lot number: TIF-1016 and TIF-01016

Purity: 95.93% (lot No. TIF-1016)
96.55% (lot No. TIF-01016)

Physical property: Tan powder

Stability: Stable at room temperature for at least 14 days (based on the results of stability analyses of the test material when mixed in the diet)

2. Diet Preparation and Analysis

Fresh test diets were prepared weekly. The premix was prepared by mixing the required amount of test material with a small amount of basal diet (Purina Certified Rodent Chow #5002) for 5 minutes in a Hobart mixer. The premix was then added to the pre-determined quantity of basal diet and mixed for an additional 15 minutes in a V-twin shell mixer rendering a final test diet mixture. The concentration of the test material was adjusted for purity. Homogeneity and 10-day stability analyses were done on 6 samples/dose level (from top, middle, and bottom) prior to study initiation. Fourteen-day stability analyses was performed on diet samples collected during weeks 0 (frozen), week 1 (room temperature), week 52 (frozen and room temperature), and week 79 (frozen and room temperature). Homogeneity of the test material was determined on

three samples (from the top, middle and bottom) from each dietary level during weeks 26, 52, and 79. A second set of samples was collected prior to the study initiation and during weeks 1, 2, 3, 7, 11, 23, 26, 35, 47, 52, 59, 71, and 79 for analysis of test compound concentration; at weeks 26, 52, and 79 the test compound concentration was determined only in the middle layer.

Results: The analyses of the test diets prior to study initiation and throughout the study showed that the test material in all diet preparations was homogeneously mixed with the basal diet and that the mixture was stable for 14 days at room temperature. The largest difference in the amount of test compound within a batch was 98.2% of targeted concentration. The means from the top, middle and bottom samples for each dose group were <10% different from the overall concentration mean for that group, and the overall mean was <15% different from the dose concentration for that group. Results were similar (<10% difference) for the 10-day stability analysis performed on the samples from the middle of the diet mix. Although there were no differences in 14-day stability data between diet samples stored at room temperature or frozen, a decrease in test material concentration was found during weeks 26 and 59 in samples of the 150 ppm diet stored at room temperature. During weeks 26 and 59, the mean test material concentrations from the 150-ppm diet samples stored at room temperature were 115 ppm and 129 ppm, respectively. However, these two concentrations were still within acceptable limits of ±15% of the target concentration.

3. Animals

Species: Mouse

Strain: CD-1 [Crl:CD-1(ICR)BR]

Age: Approximately 28-days-old on arrival; approximately 6-weeks-old at study initiation

Weight at initiation (week 0): Males: 23.1-34.9 g; females: 18.3-27.3 g

Source: The Charles River Breeding Laboratory, Inc. Portage, Michigan

Group assignment: Mice were acclimated to laboratory conditions for 13 days (males) or 14 days (females) and their health status was assessed. Animals were assigned to the following treatment groups using a computerized randomization procedure:

Test Group	Dietary Level (ppm)	Number of Animals: Oncogenicity Phase M	Oncogenicity Phase F	Interim Sacrifice (39 weeks) M	Interim Sacrifice (39 weeks) F
1 Control	0	50	50	10	10
2 Group	150	50	50	10	10
3 Group	640	50	50	10	10
4 Group	3,000	50	50	10	10
5 Group	7,000	50	50	10	10

Animals were housed one per cage in suspended wire-mesh cages in an environmentally controlled room with a temperature range of 62-77°F and relative humidity 22-89%. The room had a 12-hour light/dark cycle and was provided with approximately 10-15 air changes per hour. The basal diet and water were provided ad libitum.

Rationale for dose selection: The dietary levels were based on a 13-week dietary range-finding study in mice with Topsin M (study number WIL-75023, WIL Research Laboratories, June 1, 1988). This study was not available for review and details were not discussed in the present study. The high dose of 7,000 ppm was used to provide an upper limit dose of 1000 mg/kg/day.

4. Statistics

The data were analyzed by a two-tailed analyses for 5% and 1% significance levels. Statistical parameters, means and standard deviations were calculated using appropriately computerized methods. Dunnett's test was used for variance comparisons and analysis of weekly body weights, body weight changes, food consumption, absolute and relative organ weights, and clinical pathological values. The difference in terminal mortalities between groups were evaluated by Fisher's Exact Test. Since there were significant differences in mortalities in different groups, tumor incidences in control and treated groups were analyzed by the methods of Peto and by Fisher's exact test. A statistical method by Peto was also used for trend analysis of the occurrence of hepatocellular adenomas.

5. Quality Assurance

A signed quality assurance statement, dated November 13, 1992 as well as a signed compliance statement, dated November 9, 1992, were provided. A GLP certification statement and a flagging statement were provided.

B. METHODS AND RESULTS

1. General Observations

All animals were observed twice daily for moribundity and mortality and once daily for clinical signs of toxicity. In addition, detailed physical examinations that included palpation for masses were performed on each animal weekly.

Results: Table 1 summarizes data on mortality and percent survival. Treatment-related increases in mortality were observed in males and females at 7,000 ppm. At week 77, survival at 0, 150, 640, 3,000, and 7,000 ppm was 82, 78, 72, 68, and 52%, respectively in males, and 76, 74, 70, 66, and 54%, respectively in females.

Amyloidosis was present in animals from all groups but it was especially noticeable in mice from high-dose groups. Of the animals that were found dead or sacrificed in extremis prior to termination of the study, amyloidosis was diagnosed as the cause of death in 10%, 10%, 28.6%, 31.2%, and 58.3% of males and in 25%, 23%, 26.7%, 64.7%, and 47.8% of females in the 0-, 150-, 640-, 3,000-, and 7,000-ppm groups, respectively.

Physical examination of treated mice revealed no treatment-related clinical signs. The frequency of common signs such as urogenital staining or alopecia was not different in treated and control mice.

2. Body Weights/Food and Water Consumption/Test Material Intake

Individual body weights were recorded 1 week before the treatment started, at weekly intervals for the first 14 weeks and every 3 and 4 weeks thereafter. Food consumption was determined at weekly intervals for the first 14 weeks and every 4 weeks thereafter.

Results: Table 2 presents mean body weights at selected study intervals. Small (3-8%), but statistically significant decreases in body weight were observed in males at 3,000 and 7,000 ppm and in females at 7,000 ppm. These decreases were more frequently observed in 7,000-ppm animals than in 3,000-ppm animals, and occurred primarily during the middle of the study. Mean body weight gain over the period of weeks 0 to 53 was decreased by 12% in males at 3,000 ppm, 16% in males at 7,000 ppm, and 12% in females at 7,000 ppm. However, these differences were not statistically significant.

No treatment-related effects on food consumption were observed. Female mice at all doses showed statistically significant decreases in food consumption at several intervals, but no clear dose-response was observed. Therefore, these differences from control were considered to be incidental.

The mean test compound intakes were 23.7, 98.6, 467.6, and 1,078.8 mg/kg/day in males and 28.7, 123.3, 557.9, and

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TABLE 1. Mortality and Percent Survival in Mice Fed Topsin M for up to 18 Months[a,b]

Dietary Level (ppm)	Mortality and (Percent Survival) at Week:				
	59	64	69	74	77
			Males		
0	2 (96)	2 (96)	4 (92)	7 (86)	9 (82)
150	2 (96)	3 (94)	6 (88)	8 (84)	11 (78)
640	4 (92)	5 (90)	11 (78)	13 (74)	14 (72)
3,000	5 (90)	7 (86)	12 (76)	14 (72)	16 (68)
7,000	9 (82)	15 (70)	21 (58)	24 (52)	24 (52)
			Females		
0	3 (94)	6 (88)	6 (88)	8 (84)	12 (76)
150	5 (88)	9 (82)	9 (82)	12 (76)	13 (74)
640	4 (92)	7 (86)	10 (80)	15 (70)	15 (70)
3,000	3 (94)	7 (86)	14 (72)	17 (66)	17 (66)
7,000	8 (84)	13 (74)	14 (72)	21 (58)	23 (54)

[a]Data extracted from Study No. WIL-75024F, Table 1.
[b]Percent survival at all listed intervals is based on 50 mice/sex/group and is presented in parentheses. Ten mice per group of the original 60 per group were sacrificed by design at week 39.

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TABLE 2. Mean Body Weight at Representative Intervals in Mice Fed Topsin M for 18 Months[a,b]

Dietary Level (ppm)	Mean Body Weight (g ± SD) at Weeks: 4	13	34	53	74	78
			Males			
0	33.2	37.8	39.8	40.4	40.8	40.1
150	33.0	37.5	38.5	39.2	38.9	38.6
(%)	(99.4)	(99.2)	(96.7)	(97.0)	(95.3)	(96.3)
640	33.4	37.6	39.4	40.1	39.7	39.1
(%)	(100.6)	(99.5)	(99.0)	(99.3)	(97.3)	(97.5)
3,000	32.7	36.6	37.9*	38.5	39.0	38.5
(%)	(98.5)	(96.8)	(95.2)	(95.3)	(95.6)	(96.0)
7,000	32.4	36.4	37.7*	37.9*	39.4	39.7
(%)	(97.6)	(96.3)	(94.7)	(93.8)	(96.6)	(99.0)
			Females			
0	26.7	29.1	32.1	33.5	34.2	34.7
150	26.1	30.0	32.9	33.8	34.5	34.7
(%)	(97.8)	(103.1)	(102.5)	(100.9)	(100.9)	(100)
640	26.1	29.5	31.6	33.1	33.9	33.3
(%)	(97.8)	(101.4)	(98.4)	(98.8)	(99.1)	(96.0)
3,000	26.1	28.6	31.8	33.0	32.8	32.9
(%)	(97.8)	(98.3)	(99.1)	(98.5)	(95.9)	(94.8)
7,000	25.9*	28.3	31.4	31.8	32.4	32.0**
(%)	(97.0)	(97.3)	(97.8)	(94.9)	(94.7)	(92.2)

[a]Data extracted from Study No. WIL-75024F, Table 5.
[b]Numbers in parentheses indicate percent control.

*Significantly different from control values, $p<0.05$.
**Significantly different from control values, $p<0.01$.

1,329.4 mg/kg/day in females receiving 150, 640, 3,000, or 7,000 ppm, respectively.

3. Ophthalmoscopic Examination

Ocular examinations were done on all animals prior to the study initiation and at the end of treatment with an indirect ophthalmoscope.

Results: No treatment-related ocular changes were found in any of the treatment groups.

4. Clinical Pathology

At 9 and 18 months, blood samples were collected from 10 mice/sex/group. The parameters checked (X) were examined:

(a) Hematology

X Packed cell volume (PCV)*	X Leukocyte differential count
X Hemoglobin (HGB)*	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	Coagulation: thromboplastin time (PT)
Reticulocyte count (RETIC)	
Red cell morphology	

*Recommended by Subdivision F (November 1984) Guidelines

Results: Results on selected hematological parameters are presented in Table 3. At 7,000 ppm (week 79) the mean red blood cell count was significantly decreased in males (15.2% lower than controls). Similar effects were not observed in females. The 7,000-ppm males also had significantly lower mean WBC count (46.2% lower than controls) at 79 weeks. However, this decrease was not statistically significant if the single male in the control group with an exceptionally high white blood cell count ($32 \times 10^3/\mu L$) was eliminated from the analysis.

(b) Thyroid Function

Blood from vena cava was collected from 10 mice/sex/group that were sacrificed at weeks 39 (interim) and 79 (terminal). Blood samples from each group were pooled and the sera separated for determination of thyroid hormones thyroxine (T4) and thyroid stimulating hormone (TSH). Serum assays for determination of triiodothyronine (T3) were performed on sera collected from individual animals. The method for thyroid hormone determination was not specified.

Results: Table 4 presents mean data on T3, T4 and TSH at weeks 39 and 79. These hormones were affected to a greater extent at week 39 than at week 79. At week 39, TSH values in males at

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TABLE 3. Summary of Selected Hematological Parameters at Terminal Necropsy (Week 79) in Mice Fed Topsin M for 18 Months[a,b]

Week Interval	Number of Cells (mean ± SD) by Dietary Level (ppm)				
	0	150	640	3,000	7,000
			Males		
WBC ($\times 10^3/\mu L$)	11.9 ± 7.6	9.0 ± 4.9 (76)	6.7 ± 3.2 (56)	7.1 ± 1.6 (60)	6.4 ± 1.9* (54)
RBC ($\times 10^6/\mu L$)	9.66 ± 1.58	8.82 ± 0.62 (91)	8.74 ± 1.41 (90)	8.39 ± 0.83 (87)	8.19 ± 0.87* (85)
			Females		
WBC ($\times 10^3/\mu L$)	5.3 ± 2.0	7.6 ± 4.2 (143)	4.3 ± 1.3 (81)	6.9 ± 2.8 (130)	5.5 ± 2.5 (104)
RBC ($\times 10^6/\mu L$)	8.75 ± 0.7	8.56 ± 1.6 (98)	8.64 ± 1.1 (99)	8.37 ± 0.96 (96)	8.61 ± 0.85 (98)

[a]Data extracted from Study No. WIL-75024F, Table 10.
[b]Numbers in parentheses indicate percent control.

*Significantly different from control values, $p<0.05$.
**Significantly different from control values, $p<0.01$.

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TABLE 4. Mean Serum Levels of Thyroid Hormones Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormones (TSH) in Mice Fed Topsin M for up to 18 Months[a,b]

Parameter	Serum Levels of Thyroid Hormones by Dietary Level (ppm)				
	0	150	640	3,000	7,000
			Males		
Total T3 (ng/dL)					
Week 39	110.1 ± 32.7	115.4 ± 26.6 (105)	131.1 ± 18.3 (119)	117.6 ± 39.2 (107)	113.7 ± 19.8 (103)
Week 79	99.0 ± 21.2	98.3 ± 51.8 (99)	101.5 ± 31.0 (103)	116.1 ± 27.3 (117)	124.4 ± 35.5 (126)
Total T4 (µg/dL)					
Week 39	3.1 ± 1.1	3.3 ± 0.8 (107)	3.2 ± 0.7 (103)	2.4 ± 1.0 (77)	2.4 ± 0.9 (77)
Week 79	2.3 ± 1.2	2.5 ± 1.0 (109)	3.2 ± 1.7 (139)	2.3 ± 0.9 (100)	2.3 ± 0.8 (100)
TSH (µg/mL)					
Week 39	0.39 ± 0.12	0.39 ± 0.08 (100)	0.53 ± 0.17 (136)	0.78 ± 0.13** (200)	1.00 ± 0.43** (256)
Week 79	0.14 ± 0.08	0.14 ± 0.11 (100)	0.13 ± 0.06 (93)	0.20 ± 0.07 (143)	0.21 ± 0.12 (150)
			Females		
Total T3 (ng/dL)					
Week 39	100.0 ± 13.0	108.2 ± 28.0 (108)	117.3 ± 27.8 (117)	113.4 ± 21.4 (113)	111.0 ± 32.6 (111)
Week 79	75.4 ± 27.7	76.2 ± 24.9 (101)	81.9 ± 21.2 (109)	80.5 ± 21.8 (107)	98.9 ± 28.9 (131)
Total T4 (µg/dL)					
Week 39	2.5 ± 0.7	1.9 ± 0.5 (76)	2.1 ± 0.4 (84)	2.4 ± 0.8 (96)	1.8 ± 0.5* (72)
Week 79	1.2 ± 0.6	1.1 ± 0.6 (92)	1.1 ± 0.5 (92)	0.8 ± 0.4 (67)	0.8 ± 0.5 (67)
TSH (µg/mL)					
Week 39	0.24 ± 0.11	0.16 ± 0.13 (67)	0.22 ± 0.07 (92)	0.30 ± 0.07 (125)	0.29 ± 0.10 (121)
Week 79	0.05 ± 0.01	0.04 ± 0.04 (80)	0.06 ± 0.03 (120)	0.06 ± 0.02 (120)	0.07 ± 0.04 (140)

[a]Data extracted from Study no. WIL-75024F, Table 12.
[b]Numbers in parentheses indicate percent control.

*Significantly different from control values, $p<0.05$.
**Significantly different from control values, $p<0.01$.

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3,000 and 7,000 ppm were significantly increased and females treated with 7,000 ppm had significantly decreased levels of T4. The T4 values in the 3,000-ppm and 7,000-ppm males at week 39 were 33.3% lower than controls, but were not significantly different than controls. At week 79, the TSH levels in the 3,000-ppm and 7,000-ppm males and females were elevated but were not significantly different from values in control animals. Other than enlargement of the thyroid gland in males, the effects on TSH and T4 were not supported by morphological changes in the thyroid gland. The changes in thyroid hormone levels may have been secondary to an increase in hepatic clearance of T4 which resulted from the induction of cytochrome P-450.

5. Sacrifice and Pathology

All animals that died, were sacrificed moribund, or were sacrificed by design received a complete gross examination. At the interim sacrifice (week 39) 10 mice/sex/dose were necropsied. The necropsy included examination of the external surface, all orifices, abdominal, pelvic, and thoracic cavities, and all the viscera. The tissues checked (X) below were preserved and stained for histologic examination and the double-checked (XX) organs were weighed:

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	XX Brain* (3 levels)
X Salivary glands*	XX Heart*	X Peripheral nerve* (sciatic nerve)
X Esophagus*	X Bone marrow*	X Spinal cord* (three levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	XX Spleen*	X Eyes* (Optic nerve)
X Jejunum*	X Thymus*	
X Ileum*		Glandular
X Cecum*	Urogenital	
X Colon*		XX Adrenals*
X Rectum*	XX Kidneys*	Lacrimal gland
XX Liver*	X Urinary bladder*	X Mammary gland
X Gallbladder*	XX Testes*	XX Thyroids*
X Pancreas*	X Epididymides	X Parathyroids*
	X Prostate*	Harderian glands
Respiratory	X Seminal vesicle	
	XX Ovaries*, oviducts	
X Trachea*	X Uterus* (including vagina)	
X Lungs* (including bronchi)		

Other

X Bone (sternum and femur)*
X Skeletal muscle*
X Skin*
X All gross lesions and masses*

*Recommended by Subdivision F (November 1984) Guidelines

(a) Organ Weights

The mean absolute and relative liver weights in male and female mice treated with 7,000 ppm of Topsin M were significantly ($p<0.01$) increased at 39 weeks (Table 5). Female mice in the 3,000-ppm group also had significantly increased absolute and relative liver weights, while the males from the same dose group had only significantly higher relative liver weights. At the end of the study (week 79) males from the 7,000-ppm group had significantly higher relative and absolute liver weights, while females at the same dose level had only significantly higher relative liver weights. These changes are considered to be treatment related because they occurred in both sexes, were dose dependent at week 39, occurred in high-dose animals at week 79, and are supported by histopathological findings such as increased incidences of centrilobular hypertrophy and hepatocellular adenomas.

Absolute and relative thyroid weights at week 39 were significantly higher in the 3,000- and 7,000-ppm males, while in the 7,000-ppm females only the relative thyroid weight was increased compared to controls. No thyroid weight changes were observed at week 79.

Females treated with 7,000 ppm had significantly ($p<0.01$) increased absolute and relative heart weights at the 39- and 79-week sacrifices. At the same dietary level, males had significantly increased relative heart weights only at 39 weeks and not at the end of the study. The increased heart weight may have been treatment related because atrial thrombosis was observed in 8/50 males and 14/50 females in the 7,000-ppm group versus 1/50 males and 0/50 females in the controls (see below).

(b) Macroscopic Pathology

A statistically significant treatment- and dose-related increase in liver masses as compared to controls was observed in 3,000-ppm and 7,000-ppm mice. In the 3,000-ppm group, liver masses were observed in 38.2% males and 21.2% females, while in the 7,000-ppm group, 65.4% males and 66.7% females had liver masses. In the control groups, 12.5% males and 2.6% females had liver masses. There were no significant differences in the incidence of liver masses among interim sacrifice animals or animals that were found dead or were euthanized in extremis. The liver masses observed during gross examination were identified as hepatocellular adenomas following histopathological analysis.

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TABLE 5. Summary of Absolute and Relative Organ Weights in Mice Fed Topsin M for up to 18 Months[a,b,c]

Organ	Organ Weight Means in Grams by Dietary Level (ppm)				
	0	150	640	3,000	7,000
Males					
Thyroid-wk, 39					
Absolute	0.0048	0.0060 (125)	0.0059 (123)	0.0072* (150)	0.0101** (210)
Relative	0.011	0.015 (136)	0.014 (127)	0.018** (164)	0.026** (236)
Thyroid-wk, 79					
Absolute	0.0058	0.0062 (107)	0.0061 (105)	0.0059 (102)	0.0065 (112)
Relative	0.015	0.016 (107)	0.015 (100)	0.015 (100)	0.017 (113)
Liver-wk, 39					
Absolute	2.0850	2.0131 (97)	2.1908 (105)	2.5043 (120)	2.7926** (134)
Relative	5.045	4.976 (99)	5.270 (104)	6.334** (126)	7.081** (140)
Liver-wk, 79					
Absolute	2.2693	2.2068 (97)	2.0638 (91)	2.8079 (124)	4.1279** (182)
Relative	5.676	5.789 (102)	5.213 (92)	6.893 (121)	10.555** (186)
Heart-wk, 39					
Absolute	0.2245	0.2195 (98)	0.2304 (103)	0.2230 (99)	0.2327 (104)
Relative	0.537	0.543 (101)	0.556 (104)	0.569 (106)	0.594* (111)
Heart-wk, 79					
Absolute	0.2467	0.2314 (94)	0.2426 (98)	0.2376 (96)	0.2404 (97)
Relative	0.622	0.607 (98)	0.613 (99)	0.590 (95)	0.618 (99)
Females					
Thyroid-wk, 39					
Absolute	0.0054	0.0050 (93)	0.0044 (81)	0.0062 (115)	0.0070 (130)
Relative	0.017	0.015 (88)	0.014 (82)	0.019 (112)	0.022* (129)
Thyroid-wk, 79					
Absolute	0.0053	0.0052 (98)	0.0045 (85)	0.0048 (91)	0.0064 (121)
Relative	0.017	0.015 (88)	0.014 (82)	0.015 (88)	0.020 (118)
Liver-wk, 39					
Absolute	1.6466	1.5908 (97)	1.8130 (110)	2.0358**(124)	2.5881** (157)
Relative	5.082	4.825 (95)	5.590 (110)	6.399** (126)	8.001** (157)
Liver-wk, 79					
Absolute	1.9587	2.0759 (106)	1.8615 (95)	2.0858 (106)	2.5753 (131)
Relative	6.086	6.025 (99)	5.698 (94)	6.422 (106)	7.965* (131)
Heart-wk, 39					
Absolute	0.1729	0.1648 (95)	0.1719 (99)	0.1785 (103)	0.2122** (123)
Relative	0.532	0.501 (94)	0.529 (99)	0.563 (106)	0.657** (123)
Heart-wk, 79					
Absolute	0.1756	0.2017 (115)	0.1897 (108)	0.1922 (109)	0.2444** (139)
Relative	0.547	0.585 (107)	0.581 (106)	0.595 (108)	0.766** (140)

[a]Data extracted from Study No. WIL-75024F, Tables 18-21.
[b]Relative organ weight refers to organ-to-body weight ratios in grams/100 grams.
[c]Numbers in parentheses indicate percent control.

*Significantly different from control values, $p<0.05$
**Significantly different from control values, $p<0.01$

TABLE 6. Summary of Selected Non-Neoplastic Observations in Mice Fed Topsin M for up to 18 Months[a,b]

Organ	Number of Animals by Dietary Level (ppm)				
	0	150	640	3,000	7,000
Males					
Centrilobular, hepatocellular hypertrophy					
interim	5/10 (50)	3/10 (30)	6/10 (60)	10/10 (100)	10/10 (100)
pre-terminal	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)
Atrial thrombosis					
interim	NE	NE	NE	NE	NE
preterminal	1/10 (10)	1/11 (9)	0/14	1/16 (6)	8/24 (33)
terminal	0/40	1/2 (50)	NE	0/1	0/26
total	1/50 (2)	2/12 (17)	0/14	1/17 (6)	8/50* (16)
Females					
Centrilobular, hepatocellular hypertrophy					
interim	0/10	1/10 (10)	5/10 (50)	6/10* (60)	10/10** (100)
pre-terminal	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)
Atrial thrombosis					
interim	NE	NE	NE	NE	NE
preterminal	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 extracted from Study No. WIL-75024F, Tables 22-24.
[b] Numbers in parentheses indicate percent incidence.

* Significantly different from control; $p \leq 0.05$ using Fisher's Exact Test performed by the reviewers.
** Significantly different from control; $p \leq 0.01$ using Fisher's Exact Test performed by the reviewers.

NE = Not examined

Number of Liver Masses and Percent (%) Incidence at 79 Weeks

	Number of Animals by Dietary Level (ppm)			
	0	640	3,000	7,000
Males	5/40	9/36	13/34*	17/26**
(%)	(12.5)	(25)	(38.2)	(65.4)
Females	1/38	3/35	7/33*	18/27**
(%)	(2.6)	(8.6)	(21.2)	(66.7)

*Significantly different from control values, $p<0.05$ by Fisher's Exact Test
**Significantly different from control values, $p<0.01$ by Fisher's Exact Test

In the 7,000-ppm males, 3/10 had enlarged thyroid glands at week 39. No treatment-related histopathological findings were present at interim sacrifice. At the week 79 necropsy, there was no difference in the incidences of enlarged thyroid glands in males or females in the 7,000-ppm group as compared to the control group.

(c) Microscopic Pathology

The results of histopathological analysis indicate that liver is the major target organ for Topsin M toxicity in mice following chronic oral exposure.

Nonneoplastic lesions

Table 6 presents the summary of nonneoplastic histopathological findings in the liver and heart of treated animals. Statistically significant increases in the incidence of hepatocellular centrilobular hypertrophy were found in 3,000-ppm and 7,000-ppm female mice at week 39, in 7,000 ppm males at terminal sacrifice, and in females at 640 ppm and above and in males at 7,000 ppm when all animals were considered together. Most of the lesions were graded as mild. A more severe degree of hypertrophy, graded as moderate, was present in some of the animals from the 3,000- and 7,000-ppm groups.

A dose-related increase in the incidence of atrial thrombosis was observed in male and female mice that died or were sacrificed in extremis prior to study termination. The heart was not examined at the interim sacrifice and was not routinely examined in mice from the 150, 640, or 3,000-ppm groups at terminal sacrifice. However, mice at 7,000 ppm did not show an increase in atrial thrombosis at terminal sacrifice.

Amyloid deposits were found in many tissues. Although amyloidosis was found in animals of all groups, the incidence was especially high in the 3,000-ppm and 7,000-ppm mice. Of the 3,000-ppm and 7,000-ppm mice that were found dead or euthanized in extremis, 31.3% and 58.3% of males and 64.7% and 47.8% of females died of amyloidosis. Therefore, the amyloid deposits contributed in part to the increased mortality in the mid- and high-dose animals observed towards the end of the study.

Neoplastic Lesions

A treatment- and dose-related increase in the incidence of hepatocellular adenomas was observed in male and female mice treated with 3,000 and 7,000 ppm of Topsin M (Table 7). The incidences of hepatocellular adenomas at lower doses were not statistically significant and were within historical control ranges for this strain of mice. The incidence of hepatocellular adenomas correlates well with the macroscopic observations of significantly increased liver masses (3,000- and 7,000-ppm mice). The incidence of hepatocellular adenomas in mice that died, were sacrificed in extremis, or at the interim sacrifice was not significantly different from that in control animals. A significant trend for increased occurrence of hepatocellular adenomas in treated male and female mice was confirmed using the Peto analysis method.

There was no indication of an increased incidence to hepatocellular carcinomas; a total of two were observed in male mice, one in the 640-ppm group and the second in the 7,000-ppm group. A rare form of liver neoplasm, hepatoblastoma, was found in one 7,000-ppm male. Histopathological analysis of the thyroid revealed one malignant lymphoma in a 150-ppm female and one thyroid adenoma in a 7,000-ppm treated male. These changes were considered to be within historical range values and independent of Topsin M treatment.

C. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

The data reporting was acceptable, and the summary means that were validated were supported by individual animal data. The histopathologic analysis was audited for accuracy, consistency, and completeness by an independent pathologist (Quality Assurance Pathologist, QAP).

Oral Topsin M treatment in mice resulted in a statistically significant increase in the incidence of hepatocellular adenomas in 3,000- and 7,000-ppm male and female mice. The incidences of hepatocellular adenomas in these groups were higher than the historical control incidence compiled by the Charles River Laboratories. There was also a significant increase in hepatocellular centrilobular hypertrophy in male mice treated with 3,000 ppm and above, and in female mice treated with 640 ppm and above. Increased incidences of hepatocellular adenomas and centrilobular hypertrophy were reflected in increased liver weights in male and female mice from the 3,000- and 7,000-ppm groups.

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TABLE 7. Incidence of Neoplastic Lesions in the Livers of Mice Fed Topsin M for up to 18 Months[a,b]

Organ	Number of Animals by Dietary Level (ppm)				
	0	150	640	3,000	7,000
			Males		
Hepatocellular adenoma (benign)					
interim	0/10	0/10	0/10	0/10	0/10
pre-terminal	0/10	0/11	0/14	2/16 (13)	6/24 (25)
terminal	4/40 (10)	8/39 (21)	7/36 (19)	17/34** (50)	18/26** (69)
total	4/60 (7)	8/60 (13)	7/60 (12)	19/60** (32)	24/60** (40)
			Females		
Hepatocellular adenoma (benign)					
interim	0/10	0/10	0/10	0/10	0/10
pre-terminal	0/12	0/13	0/15	0/17	2/23 (9)
terminal	0/38	0/37	3/35 (9)	8/33** (24)	16/27** (59)
total	0/60	0/60	3/60 (5)	8/60** (13)	18/60** (30)

[a]Data 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 performed by the reviewers.
** Significantly different from control, $p<0.01$ using Fisher's Exact performed by the reviewers.
Test

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Topsin M treatment transiently affected the mean serum levels of thyroid hormones in 3,000-ppm mice at week 39. Significantly increased TSH levels were present in 3,000- and 7,000-ppm males, and mean serum T4 levels in the 7,000-ppm females were significantly decreased for that same time period. In addition, the thyroid weight increases were observed in 3,000- and 7,000-ppm males and 7,000-ppm females at the 39-week interim sacrifice. These changes were not significant at week 79. No significant histopathological changes were observed in the thyroid at either the interim or final sacrifices. Although the precise mechanism of thyroid hormone changes following Topsin M treatment is not known, it is possible that the mild hepatocellular hypertrophy that was observed in 3,000- and 7,000-ppm mice is an indication of cytochrome P-450 induction. Cytochrome P-450 in turn can lead to an increased hepatic clearance of T4, resulting in higher serum levels of TSH and thyroid hypertrophy. The information on the cytochrome P-450 levels was not provided in this study, but would be helpful in elucidating the mechanism of this effect.

The increase in the heart weight in the 7,000-ppm males and females was accompanied by an increase in the incidence of atrial thrombosis.

A significantly increased mortality was observed in 7,000-ppm males and females as compared to controls. Amyloidosis was present in all groups. However, it was especially high in the 3,000- and 7,000-ppm mice and may have been a contributing factor in the increased death rate.

The observed dose-related decrease in the number of RBC in male mice was not considered to be treatment related because the values were within the historical range for this strain of mice and were not accompanied by changes in either hemoglobin or hematocrit.

In summary, there was a dose-related increase in the incidence of hepatocellular adenomas in both sexes. Although there was no evidence of increased incidence of hepatocellular carcinomas following Topsin M treatment, the increased incidence of hepatocellular adenomas is an indication of a possible neoplastic response.

The study is classified as Core Guideline and satisfies the requirements for an oncogenicity study in mice.