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

007294

JUN 30 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg. No. 3125-340; Triadimefon (Bayleton)®

EPA Accession No's 407521-01,-02 and 408651-01
Caswell No. 862AA

FROM: George Z. Ghali, Ph.D. G. Ghali 6.22.89
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Susan Lewis, Acting PM 21
Fungicide-Herbicide Branch
Registration Division (H7505C)

THRU: John Quest, Ph.D. John A. Quest 6/22/89
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

and

Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Registrant: Mobay Chemical Corporation
Kansas City, MO 64120

Action Requested

Review and evaluation of an oncogenicity study in the NMRI mice submitted by Mobay Corporation in compliance with Section 6(a)(2) of FIFRA.

Conclusion and Recommendations:

Bayleton is a triazole fungicide produced by Mobay Corporation. It was registered in early 1980's for use on a variety of agricultural food commodities.

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Data submitted by the registrant in support of Bayleton registration indicated that the chemical was not oncogenic in both Wistar rats and the CF1 mice. In both studies the MTD was reached. However, a recent submission by Mobay Corporation indicated that dietary administration of Bayleton was associated with a statistically significant increase in the incidence of hepatic adenomas in both males and females of the NMRI mice at the highest dose tested. In both mouse studies, the same dose regimen was used, and the highest dose tested was adequete for oncogenic potential testing.

The study was reviewed by Dynamac Corporation and the Health Effect Division (HED) of the Office of Pesticide Programs (OPP). The report of the study, along with other relevant materials, will be submitted to the HED Peer Review Committeee (PRC) for further evaluation and a weight of the evidence determination.

Until the Committee,s final report becomes available, the Agency may consider tolerance petitions, including the currently pending or future petitions, on a case-by-case basis in light of the resulting increase in incremental risk.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

307294

EPA No.: 68D80056
DYNAMAC No.: 157-A
TASK No.: 1-57A
June 2, 1989

DATA EVALUATION RECORD

BAYLETON

Oncogenicity Feeding Study in Mice

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 6/2/89

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EPA No.: 68D80056
DYNAMAC No.: 157-A
Task No.: 1-57A
June 2, 1989

DATA EVALUATION RECORD

BAYLETON

Oncogenicity Feeding Study in Mice

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation
Signature: William L. McLellan
Date: June 5, 1989

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: Margaret E. Brower
Date: June 2, 1989

APPROVED BY:

Roman Pienta, Ph.D.
Department Manager
Dynamac Corporation
Signature: Roman Pienta
Date: June 2 1989

George Ghali, Ph.D.
EPA Reviewer, Science
Support Section
Toxicology Branch (TS-769C)
Signature: G.G.
Date: 6.21.89

John Quest, Ph.D.
EPA Section Head, Science
Support Section
Toxicology Branch (TS-769C)
Signature:
Date: 6/22/89

DATA EVALUATION RECORD

GUIDELINE §83-2

STUDY TYPE: Oncogenicity feeding study in mice.

ACCESSION/MRID NUMBER: 407521-01.

TEST MATERIAL: MEB 6447.

SYNONYM(S): Triadimefon; Bayleton; 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone.

STUDY NUMBER(S): 87287.

SPONSOR: Mobay Corp.

TESTING FACILITY: Bayer AG, Wuppertal, Federal Republic of Germany.

TITLE OF REPORT: MEB 6447 (Common Name: Triadimefon, the Active Ingredient of Bayleton) Carcinogenicity Study in NMRI Mice (21-Month Administration in the Feed).

AUTHOR(S): Bomhard, E., and Hahnemann, S.

REPORT ISSUED: April, 1986.

CONCLUSIONS: When MEB 6447 was fed to NMRI mice for 21 months at dietary levels of 50, 300, or 1800 ppm, there was a significant increase ($p < 0.05$) in hepatocellular adenomas in high-dose males and females; there was no increase in liver carcinomas. There was no effect of dosing on survival, toxic signs, food or water consumption, or hematology parameters. Mean weight gain was decreased in high-dose males and to a lesser extent in high-dose females. There were nonneoplastic and preneoplastic changes in the liver as well as increases in liver weights and correlating effects on serum enzymes. Hepatocellular hypertrophy was significantly ($p < 0.01$) increased in males receiving 300 or 1800 ppm and in females receiving 50, 300, or 1800 ppm. Hyperplastic nodules, altered cell foci, and microvesicular fatty changes were increased in both sexes ($p < 0.01$) at 1800 ppm. Kupffer cell proliferation, lipofuscin pigment in liver macrophages, and single cell necrosis were increased significantly ($p < 0.01$) in males receiving 1800 ppm and females receiving 300 and 1800 ppm. At study termination, liver weights were increased ($p<0.01$) in mid- and high-dose males and in high-dose females when compared to controls; serum alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase were increased in both sexes receiving 1800 ppm. The increases were significant compared to controls for all enzymes at 91 weeks and for some at 53 weeks. The LOEL in males and females is 300 ppm and 50 ppm in males and female mice, respectively. A NOEL was not achieved in females based on hepatocellular hypertrophy; the NOEL in males was 50 ppm.

Classification: CORE guideline.

A. MATERIALS:

1. Test Compound: MEB 6447, technical AI; description: colorless crystals; batch No.: F1 No. 125 and 138; purity: 90% [redacted]

2. Test Animals: Species: mouse; strain: Bor:NMRI (SPF Han); age: 5-7 weeks at initiation; weight: males--22 to 35 g, and females--20 to 27 g; source: Winkelmann, Borchen, FRG.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 8 days, identified by cage card, and assigned randomly by sex to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study (21 months) Males	Main Study (21 months) Females	Satellite Group/ Interim Sacrifice (12 months) Males	Satellite Group/ Interim Sacrifice (12 months) Females
1 Control	0	50	50	10	10
2 Low (LDT)	50	50	50	10	10
3 Mid (MDT)	300	50	50	10	10
4 High (HDT)	1800	50	50	10	10

Mice were individually housed in a single room controlled for temperature (22 ± 2°C), humidity (≈50%), and light (12 hours/day); air was exchanged ≈10 times per hour.

2. Diet Preparation: Dietary mixtures of the test substance at concentrations of 0, 50, 300, and 1800 ppm were prepared weekly in Altromin 1321 meal 1 week prior to feeding. Test compound in the diets was analyzed at 3-month intervals throughout the study. Homogeneity and stability were determined on low- and high-dose diets prior to study initiation.

   Results: As shown in Table 1, all diets were within 18% of target and ranged from a low of 84% (50 ppm, study month 16) to a high of 118% (300 ppm, study month 1). Low- and high-dose diets were homogeneous; the standard deviation ranged from 2 to 3% for three samples of each dietary concentration. After storage for 12 days at an unspecified temperature, 92 and 84% of the nominal concentration were recovered from low and high dietary preparations, respectively.

3. Food and Water Consumption: Animals received food (Altromin 1321 meal) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Body weight, clinical pathology, and organ weight were analyzed by the Mann and Whitney U-test and Wilcoxon test. Incidence data (mortality, clinical signs, etc.) were analyzed by Fisher's exact test. In addition, tumor incidence was evaluated according to the method of Peto et al. using the test for positive trend with respect to dose.

TABLE 1. Analysis of MEB 6447 in Test Diets at Representative Intervals

Study Month	Target Concentration (mg/kg/diet) 50		300		1800	
	Concentration in feed (mg/kg)	% of Target[a]	Concentration in feed (mg/kg)	% of Target[a]	Concentration in feed (mg/kg)	% of Target[a]
1	55	110	354	118	1908	106
4	50	100	267	89	1674	93
7	58	116	291	97	1710	95
16	42	84	279	93	1692	94
19	48	96	270	90	1530	85

[a]Calculated by our reviewers.

5. Quality Assurance: A quality assurance statement was signed and dated May 17, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for clinical signs and anomalies (once daily on weekends). Detailed physical examinations, which included external surface areas, orifices, posture, general behavior, respiration, and excretory products, were performed weekly.

Results: With the exception of piloerection, which occurred more frequently in treated than control groups and was not considered to be related to treatment, the report indicated that there were no effects of dosing on the incidence of clinical signs. Similarly, the authors stated that there were no effects of treatment derived from the frequency, site, and time of occurrence of palpable tissue masses.

As shown in Table 2, percent survival in the treatment groups of both sexes were generally higher or comparable to the control groups.

2. Body Weight: Mice were weighed prior to the initiation of the study, weekly from weeks 1 to 12, and bimonthly from week 15 to study termination.

Results: Table 3 presents representative data on mean body weights in males and females. Body weights in high-dose males were significantly lower ($p<0.01$) than controls at week 2 and at the majority of weighings thereafter; the overall body weight gain (14 g) was lower than in controls (18 g). In mid-dose males, body weights in general were similar to controls. Mean body weights in low-dose males were significantly lower than control at the majority of intervals to week 65; however, the overall weight gain was comparable to controls. Body weights in the satellite groups of males (data not shown) were similarly increased in the high-dose groups. In high-dose females, sporadically lower body weights were recorded through week 28. Mean body weights were consistently lower (2-4 g, $p <0.01$) than in controls from weeks 29 to 83, however, the overall mean body weight gain (14 g) was only slightly lower than in controls (15 g). There were no effects in the low- and mid-dose groups of females.

TABLE 2. Cumulative Mortality and Percent Survival in Mice Fed MEB 6447 for 21 Months

Dietary Level (ppm)	No. of Mortalities and (percent survival) at Week			
	26[a]	52[a]	78[b]	89[b]
		Males		
0	0 (100)[c]	2 (97)	12 (76)	16 (68)
50	0 (100)	2 (97)	10 (80)	16 (68)
300	0 (100)	3 (94)	12 (76)	16 (68)
1000	0 (100)	1 (98)	9 (82)	14 (72)
		Females		
0	2 (97)	6 (90)	21 (58)	32 (32)
50	1 (98)	9 (85)	19 (62)	27 (46)
300	0 (100)	3 (94)	14 (72)	24 (52)
1000	0 (100)	5 (90)	16 (68)	24 (52)

[a]Percent survival was based on 60 animals/group/sex and includes satellite groups (10/sex/group) scheduled for interim sacrifice at 12 months.

[b]Percent survival was based on 50 animals/group/sex.

[c]Calculated by our reviewers.

TABLE 3. Representative Results of Mean Body Weights of Mice Fed MEB 6447 for 21 Months

Dietary Level (ppm)	Mean Body Weights (g ± S.D.) at Week						Overall Body Weight Gain (g)
	0	2	5	29	53	89	
				Males			
0	28 ± 1.4	33 ± 1.8	37 ± 2.2	47 ± 4.2	48 ± 4.7	46 ± 4.7	18
50	28 ± 1.7	32 ± 2.1	35 ± 2.6**	44 ± 4.7**	46 ± 4.8*	46 ± 3.7	18
300	28 ± 2.0	33 ± 2.5	37 ± 3.3	48 ± 6.4	50 ± 6.8	49 ± 6.1	21
1800	28 ± 1.9	31 ± 2.8**	34 ± 2.8**	40 ± 3.8**	42 ± 3.9**	42 ± 3.5**	14
				Females			
0	23 ± 1.6	25 ± 1.8	27 ± 2.0	35 ± 2.8	38 ± 3.6	38 ± 3.8	15
50	24 ± 1.5	25 ± 1.9	27 ± 2.1	35 ± 3.4	37 ± 3.9	40 ± 4.1	16
300	23 ± 1.5	25 ± 1.9	27 ± 1.9	34 ± 3.1	37 ± 3.9	39 ± 5.1	16
1800	24 ± 1.5	25 ± 1.4	26 ± 1.6**	32 ± 2.6**	35 ± 3.4**	38 ± 4.1	14

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

3. Food Consumption and Compound Intake: Food and water consumption were determined at the same intervals as the weighings. Mean daily diet consumption and compound intake were calculated.

   Results: Males receiving 1800 ppm consumed approximately 8% more food than the control mice at most intervals after study week 9 (Table 4). A similar trend of increased food consumption was noted in high-dose females from week 67 to study completion. Males receiving 50 and 300 ppm generally consumed more feed than the control males from week 61 to the end of the study; food consumption was not affected in females at the low and mid doses.

   Compound intake was 13.5, 76.0, and 550.1 mg/kg/day in males receiving 50, 300, or 1800 ppm, respectively, and was 19.6, 119.4, and 765.0 mg/kg/day in females in the same groups.

   Water consumption in dosed males tended to be increased; total water intake for males receiving 50, 300, and 1800 ppm was 180.6, 180.8, and 198.8 g/kg/day, respectively, as compared to 166.1 g/kg/day for the control males. In relation to body weight, high-dose males consumed an average of 20% more water than the control males. Water consumption was similar in all female groups.

4. Ophthalmological Examinations: The frequency and extent of ophthalmological examinations were not reported. The authors stated that the test material had no adverse effect on the eyes.

5. Hematology and Clinical Chemistry: Blood was collected from either the caudal vein or the retro-orbital venous plexus from 10 mice/group for hematology and clinical analyses at week 53, from 20 mice/group for hematology at week 90/91, and from 10 mice/group for clinical analysis at week 90/91. The CHECKED (X) parameters were examined:

a. Hematology:

X	Hematocrit (HCT)[+]	X	Leukocyte differential count[a]
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)		
X	Red cell morphology		

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 4. Representative Food Intake of Mice Fed MEB 6447 for 21 Months

Dietary Level (mg/kg/day)	Mean Food Intake (g/animal/day) at Week 1	29	53	67	89	Total Intake
			Males			
0	13.2	9.3	10.1	9.7	12.3	10.6
50	13.3	10.1	10.7	10.6	14.5	11.4
300	13.1	9.9	9.8	11.3	14.4	11.4
1800	12.7	10.9	10.9	11.6	14.8	11.8
			Females			
0	13.2	13.2	11.7	12.6	14.0	13.4
50	13.2	12.7	12.2	13.0	15.9	13.2
300	13.1	12.4	11.6	13.7	15.4	13.2
1800	12.8	12.8	12.7	14.2	16.8	13.7

Results: Mean values for hemoglobin, hematocrit, and platelets are summarized in Table 5. All erythrocyte parameters in the low- and mid-dose groups of both sexes were similar to controls, and there were no effects in high-dose males at either interval (53 or 91 weeks). The hemoglobin concentration was significantly decreased ($p < 0.05$) in high-dose females at week 51 but not at week 91. The hematocrit value was significantly decreased in dosed females at 91 weeks, but the decreases were slight, there was no dose-related trend, and values were within the normal laboratory range (see Appendix A). MCHC was decreased in high-dose females at 53 weeks but increased in all dosed groups of females at week 91. These changes were not considered of toxicologic importance since the values were within the normal laboratory range. Erythrocyte morphology was not affected by dosing.

Platelets were increased in high-dose females at 53 ($p < 0.05$) and 91 weeks (not significant); however, there were no effects on platelets in dosed males. There were no effects of biological importance on total leukocyte or differential leukocyte counts. Although leukocyte counts were reduced compared to controls in mid-dose males at week 53, they were increased at week 91 in the mid-dose and high-dose groups and there was no apparent dose-related trend.

b. Clinical Chemistry:

	Electrolytes		Other
	Calcium[+]		Albumin[+]
	Chloride[+]		Albumin/globulin ratio
	Magnesium[+]	X	Blood creatinine[+]
X	Phosphorus[+]	X	Blood urea nitrogen[+]
	Potassium[+]	X	Cholesterol[+]
	Sodium[+]		Globulins
		X	Glucose[+]
	Enzymes	X	Total bilirubin[+]
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein[+]
	Creatinine phosphokinase[+]		Triglycerides
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)[+]		
X	Serum aspartate aminotransferase (SGOT)[+]		
	Gamma glutamyltransferase (GGT)		

[+]Recommended by Subdivision F (October 1982) Guidelines.

TABLE 5. Selected Hematology Parameters (Mean ± S.D.) in Female Mice Fed MEB 6447 for 21 Months

Parameter/ Interval	Dietary Level (ppm) 0	50	300	1800
Hemoglobin (g/L)				
53 weeks[a]	151 ± 8.4	146 ± 10.4	150 ± 7.9	142 ± 7.3*
91 weeks[b]	135 ± 12.4	128 ± 14.9	134 ± 10.4	134 ± 10.1
Hematocrit (%)				
53 weeks	0.42 ± 0.021	0.42 ± 0.020	0.42 ± 0.021	0.41 ± 0.022
91 weeks	0.42 ± 0.038	0.39 ± 0.039**	0.40 ± 0.024*	0.40 ± 0.027*
Platelets ($10^3/cm^2$)				
53 weeks	899 ± 220.4	1036 ± 208.8	939 ± 103.3	1111 ± 159.6*
91 weeks	1074 ± 173.1	1077 ± 133.8	963 ± 330.0	1195 ± 194.2

[a]Means ± standard deviations were based on results from 10 females/group.

[b]Means ± standard deviations were based on results from 17 females in the control group, 19 females in the mid-dose group, and 20 females in the low- and high-dose groups.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

Results: Table 6 summarizes data on serum enzyme activities. Alkaline phosphatase activity was significantly increased ($p < 0.01$) in high-dose males at weeks 53 and 91 and in high-dose females at week 91 ($p < 0.05$). Alanine aminotransferase (SGPT) and aspartate aminotransferase (SGOT) were significantly increased in high-dose males at week 91 and in high-dose females at weeks 53 and 91. The activity of SGPT was nonsignificantly increased in high-dose males at week 51 when compared to controls but the concurrent control value was substantially higher than the value for the mid- and low-dose groups and higher than that the historical laboratory controls (47.8 U/L). The activity of SGPT was significantly increased ($p < 0.05$) at week 53 in mid-dose females.

Representative results for other clinical chemistry parameters are presented in Table 7. Mean cholesterol levels for males and females receiving 1800 ppm were significantly lower than controls at week 53 and 91 and total bilirubin levels were decreased in high-dose females at both intervals. Inorganic phosphorus levels were significantly increased in high-dose males ($p < 0.01$) and mid- and high-dose females ($p < 0.05$). The creatinine level was significantly decreased in dosed males at 53 weeks compared to control; however, the concurrent control value (88 µmol/L) was high compared to the historical mean (59 µmol/L). The authors did not consider the changes in cholesterol, bilirubin, creatinine, and phosphorus to be of toxicologic importance since all values fell within the variation of historical data.

6. Urinalysis: Urinalysis was not performed.

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TABLE 6. Serum Enzyme Activities (Mean ± S.D.) in Mice Fed MEB 6447 for 21 Months[a]

Parameter/Interval	Dietary Level (ppm) 0	50	300	1800
		Males		
Alkaline phosphatase (U/L)				
53 weeks	77 ± 16.9	76 ± 13.1	80 ± 15.8	120 ± 26.3**
91 weeks	104 ± 33.6	122 ± 58.7	109 ± 13.6	214 ± 52.2**
Aspartate aminotransferase (U/L)				
53 weeks	86.3 ± 53.1	46.5 ± 15.2*	61.5 ± 26.0	67.5 ± 27.2
91 weeks	59.2 ± 28.3	58.2 ± 27.3	55.9 ± 18.5	99.4 ± 26.7**
Alanine aminotransferase (U/L)				
53 weeks	72.2 ± 37.3	40.5 ± 15.5	49.5 ± 10.9	113.0 ± 64.5
91 weeks	63.7 ± 24.0	106.2 ± 104.4	80.8 ± 40.5	216.5 ± 72.4**
		Females		
Alkaline phosphatase (U/L)				
53 weeks	176 ± 102.6	138 ± 55.4	151 ± 56.1	179 ± 51.6
91 weeks	267 ± 135	208 ± 89.5	299 ± 211	296 ± 132*
Aspartate aminotransferase (U/L)				
53 weeks	54.6 ± 30.9	66.7 ± 38.6	71.0 ± 20.0*	108 ± 29.0**
91 weeks	86.7 ± 58.8	85.2 ± 56.7	72.8 ± 20.2	136 ± 57.0*
Alanine aminotransferase (U/L)				
53 weeks	41.4 ± 23.3	45.2 ± 19.1	60.3 ± 19.2*	184 ± 42.6**
91 weeks	81.4 ± 34.3	102 ± 109	74.4 ± 19.1	227 ± 107**

[a]Means and standard deviations were based on results from 10 animals/sex/group interval.

*Significantly different from the control value at $p < 0.05$.

**Significantly different from the control value at $p < 0.01$.

TABLE 7. Representative Results from Other Clinical Chemistry Parameters (Mean ± S.D.) in Mice Fed MEB 6447 for 21 Months[a]

Parameter/Interval	Dietary Level (ppm) 0	50	300	1800
		Males		
Total Bilirubin (μmol/L)				
53 weeks	2.0 ± 1.0 (8)	1.8 ± 0.5	1.6 ± 0.5 (8)	1.5 ± 0.5
91 weeks	2.4 ± 0.4 (6)	2.3 ± 0.4 (7)	2.7 ± 1.0 (5)	2.0 ± 0.4 (3)
Cholesterol (μmol/L)				
53 weeks	4.49 ± 0.4	4.52 ± 0.8	4.48 ± 1.0	3.00 ± 0.7**
91 weeks	3.49 ± 0.4	3.93 ± 2.0	3.27 ± 0.8	2.60 ± 1.0*
Inorganic Phosphorus (μmol/L)				
53 weeks	--	--	--	--
91 weeks	1.57 ± 0.3	1.78 ± 0.2	1.70 ± 0.3	2.06 ± 0.5**
		Females		
Total Bilirubin (μmol/L)				
53 weeks	2.5 ± 0.3	2.8 ± 0.5	2.2 ± 0.8 (9)	2.1 ± 0.3*
91 weeks	2.7 ± 0.7 (7)	3.0 ± 0.9 (7)	2.6 ± 0.8 (6)	1.7 ± 0.5* (5)
Cholesterol (μmol/L)				
53 weeks	3.18 ± 0.7	2.84 ± 0.9	2.94 ± 0.7	2.55 ± 1.3**
91 weeks	3.38 ± 0.9	2.95 ± 0.5	2.70 ± 1.2 (8)	2.65 ± 1.7* (9)
Inorganic Phosphorus (μmol/L)				
53 weeks	--	--	--	--
91 weeks	1.59 ± 0.4	1.76 ± 0.4	1.84 ± 0.2* (8)	1.91 ± 0.2*

[a]Means and standard deviations were based on the results from 10 animals/group/sex/interval unless indicated by numbers in parentheses.

Note: Historical reference means were as follows:

Total bilirubin	--	2.8 μmol/L, males; 3.2 μmol/L, females
Cholesterol	--	4.11 mmol/L, males; 3.17 mmol/L, females
Inorganic phosphorus	--	2.61 mmol/L, males; 2.59 mmol/L, females

[b]--indicates inorganic phosphorus not measured at 53 weeks.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta+	XX Brain+
X Salivary glands+	XX Heart+	X Peripheral nerve (sciatic nerve)+
X Esophagus+	X Bone marrow+	X Spinal cord (3 levels)
X Stomach+	X Lymph nodes+	X Pituitary+
X Duodenum+	XX Spleen	X Eyes
X Jejunum+	Thymus	X Optic nerve+
X Ileum+		
X Cecum+		
X Colon+		
X Rectum	Urogenital	Glandular
XX Liver+	XX Kidneys+	X Adrenals+
X Gallbladder+	X Urinary bladder+	Lacrimal gland
X Pancreas+	XX Testes+	X Mammary gland+
	X Epididymides	X Thyroids+
	X Prostate	Parathyroids+
	X Seminal vesicle	X Harderian glands
Respiratory	X Ovaries	X Extraorbital glands
X Trachea+	X Uterus	
XX Lung+		
		Other
		X Bone (sternum and femur)+
		X Skeletal muscle+
		X Skin
		X All gross lesions and masses

Results:

a. Organ Weights: As shown in Table 8, dose-related increases in absolute and relative (to body) liver weights of dosed males and females were observed at both sacrifice intervals. Increases were significant for high-dose males and females at both sacrifice times and for mid-dose males at week 91. There was an apparent dose-related trend. In high-dose males, absolute but not relative kidney weights were significantly lower than the control at 53 weeks; by week 91, both parameters were significantly affected. No significant differences were seen in female kidney weights.

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 8. Mean Organ Weights (± S.D.) and Organ-to-Body Weight Ratios for Liver and Kidney of Mice Fed MEB 6447 for 21 Months

Organ/ Interval	Dietary Level (ppm) 0	50	300	1,000
		Males		
Liver				
Week 53 (g)	2.37 ± 0.58	2.41 ± 0.33	2.75 ± 0.54	2.94 ± 0.26**
(% b. wt)	4.80 ± 0.62	4.72 ± 0.32	5.24 ± 0.55	7.44 ± 0.24**
Week 91 (g)	2.10 ± 0.38	2.28 ± 0.78	2.49 ± 0.51**	3.45 ± 0.83**
(% b. wt)	4.62 ± 0.54	5.06 ± 1.70	5.23 ± 0.94**	8.44 ± 1.72**
Kidneys				
Week 53 (g)	0.79 ± 0.09	0.81 ± 0.15	0.84 ± 0.16	0.64 ± 0.09**
(% b. wt)	1.64 ± 0.19	1.59 ± 0.26	1.60 ± 0.22	1.62 ± 0.16
Week 91 (g)	0.80 ± 0.12	0.78 ± 0.13	0.88 ± 0.18	0.67 ± 0.08**
(% b. wt)	1.76 ± 0.20	1.74 ± 0.26	1.84 ± 0.27	1.65 ± 0.17*
		Females		
Liver				
Week 53 (g)	1.78 ± 0.24	1.91 ± 0.21	1.96 ± 0.39	2.73 ± 0.64**
(% b. wt)	4.70 ± 0.43	5.23 ± 0.37	5.00 ± 0.78	7.51 ± 1.26**
Week 91 (g)	2.09 ± 0.39	2.26 ± 0.87	2.34 ± 0.68	3.54 ± 1.33**
(% b. wt)	5.56 ± 0.77	5.83 ± 1.77	5.95 ± 1.19	9.44 ± 2.46**
Kidneys				
Week 53 (g)	0.51 ± 0.06	0.50 ± 0.05	0.50 ± 0.08	0.46 ± 0.07
(% b. wt)	1.36 ± 0.12	1.36 ± 0.14	1.28 ± 0.15	1.26 ± 0.17
Week 91 (g)	0.59 ± 0.23	0.52 ± 0.08	0.58 ± 0.36	0.52 ± 0.13
(% b. wt)	1.57 ± 0.54	1.36 ± 0.19	1.49 ± 0.92	1.41 ± 0.29

*Significantly different from the control value at $p < 0.05$.

**Significantly different from the control value at $p < 0.01$.

Significant differences in other organ weight data for the high-dose group included a decrease in lung ($p < 0.01$) and spleen ($p < 0.05$) weights in high-dose males at week 53 but not week 91, a slight decrease in brain weight in high-dose females (week 91, $p < 0.05$), an increase in brain-to-body weight raio in high-dose males (weeks 53 and 91, $p < 0.05$), and an increase in testes-to-body weight ratio in high-dose males (91 weeks). These differences were reported by the study authors to be associated with terminal body weights and were, therefore, not considered to be treatment related.

b. Gross Pathology: Table 9 summarizes frequently occurring gross lesions. With the exception of the liver, there were no increases in findings in dosed groups as compared to controls. Several findings were less frequent in high-dose groups as compared to controls. In the livers, there was an increase in surface areas with changed appearance in the high-dose groups in both sexes. The incidence of nodules on the liver was increased in high-dose females and mid- and high-dose males.

c. Microscopic Pathology:

1) Nonneoplastic: There were several nonneoplastic changes in the livers of dosed mice and some were apparent in high-dose groups at the 12-month interim sacrifice (Table 10). No hyperplastic hepatocellular nodules or altered cell foci were noted at 12 months. Table 11 summarizes frequent nonneoplastic findings in the main study. The liver was the only organ with any compound-related increase in lesions. There was an increase in altered cell foci and in hepatocellular hyperplastic nodules in males and females fed 1800 ppm. Hepatocellular hypertrophy was increased in both dosed males and females; in the low- and mid-dose groups, this change was confined to the centrilobular area of the liver and was predominantly grade 2 (slight to moderate). In high-dose males and females, the hypertrophy was not only increased but was more diffuse and of increased severity. There was an increase in slight to moderate Kupffer cell proliferation predominantly in high-dose males and mid- and high-dose females; the degree of severity was also increased in the above groups. Accumulation of lipofuscin pigment in liver macrophages was increased in high-dose males and

TABLE 9. Frequently Occurring Gross Lesions in Mice Fed MEB 6447 for 21 Months[a]

Organ/Finding	Dietary Level (ppm)							
	Males				Females			
	0	50	300	1800	0	50	300	1800
Liver								
Nodules	2	4	7	11	3	1	2	10
Swollen	1	1	1	3	6	1	1	7
Changed areas	0	0	1	21	1	3	0	10
Lungs								
Nodules	19	18	20	12	8	7	7	7
Firm	11	12	15	10	6	7	5	3
Lymph nodes								
Enlarged	3	1	3	2	10	4	7	4
Spleen								
Enlarged	6	1	7	3	19	12	17	8
Kidney								
Cysts	19	14	17	5	0	3	1	2
Ovaries								
Enlarged	-	-	-	-	2	4	6	3
Cysts	-	-	-	-	5	3	2	2
Uterus								
Nodules	-	-	-	-	6	2	1	3
Urinary bladder								
Thickened mucosa	3	1	1	6	0	0	2	1
Seminal vesicles								
Enlarged	5	7	5	2	-	-	-	-

[a]Based on 60 animals/group (includes mice for interim sacrifice).

TABLE 10. Histologic Findings at 12 Months in Livers of Mice Fed MEB 6447

Histologic Finding	Dietary Level (ppm)							
	Males				Females			
	10	50	300	1800	0	50	300	1800
No. tissues examined	(10)	(10)	(10)	(10)	(10)	(9)	(10)	(10)
Liver								
Hepatocellular hypertrophy	1	1	6	10	0	0	1	8
Fatty change	4	6	4	10	0	1	1	9
Single cell necrosis	0	0	2	7	0	0	2	4
Kupffer cell proliferation	0	0	1	6	0	0	2	5
Pigment accumulation	0	0	1	6	0	0	0	2
Altered cell foci	0	0	0	1	0	0	0	1

TABLE 11. Frequent Nonneoplastic Lesions in Mice Fed MEB 6447 for 21 Months

Histologic Finding	Dietary Level (ppm) Males 10	50	300	1800	Females 0	50	300	1800
Liver	(50)[a]	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Hepatocellular hypertrophy	16^{TT}	19	33**	49**	3	24**	42**	47**
Hyperplastic nodules	2^{TT}	1	3	23**	1	1	1	23**
Altered cell foci	0^{TT}	0	2	36**	0	0	1	28**
Bile duct proliferation	0	1	1	1	0	0	0	5*
Kupffer cell proliferation	12^{TT}	6	3	46**	5^{TT}	9	19**	37**
Pigment accumulation	3^{TT}	4	3	35**	6^{TT}	9	19**	37**
Single cell necrosis	10^{TT}	7	16	49**	10^{TT}	12	22**	42**
Fatty change	12^{T}	10	13	18	12^{TT}	12	9	28**
Microvesicular fatty change	0	1	2	14**	6	1	1	18**
Round cell infiltration	23	9	18	29	9	14	23	30**
Kidneys	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Tubular atrophy	25	22	29	17	7	6	5	7
Cortical cysts	37	33	35	22	3	8	3	4
Chronic nephropathy	7	3	1	2	14	7	10	11
Tubular dilation	1	8	2	4	7	9	5	1
Stomach	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Adenomatous hyperplasia	19	17	14	16	22	18	21	18
Adrenal	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Medullary hyperplasia	3	1	0	5	7	2	1	2

[a]The number in parentheses is the number of animals with tissue examined histologically; includes animals that died after 12 months, were sacrificed moribund or were sacrificed at study termination.

**Significantly different from control incidence, $p < 0.01$ (Fisher exact test); analyses by reviewers.

^{TT}Significant trend by Cochran-Armitage test, $p < 0.01$; ^{T}=$p < 0.05$.

mid- and high-dose females. The severity of the finding was increased in male but not female dose groups. The incidences of single cell necrosis was relatively high in control males and females, the incidence was increased in the mid- and high-dosegroups, and the severity increased in the high-dose groups. There was an increase in fine droplet fatty changes of the liver in high-dose groups but no increase in macrovesicular fatty changes.

There were no increases in nonneoplastic findings in other organs in dosed groups; the frequency and severity of lesions appeared slightly less in dosed groups than in controls for several sites.

2) Neoplastic: Table 12 summarizes neoplastic incidence data. The incidence of hepatocellular adenomas was significantly increased in both males and females receiving 1800 ppm. Trend analysis (by the authors) using the Peto method indicated a significant positive trend in males ($p = 0.037$) and females ($p < 0.001$). In high-dose males, 8 of the 11 adenomas were found at final sacrifice (days 630 to 634) and the first tumor was found at 592 days; in high-dose females, 5 of the 9 adenomas were at final sacrifice and the first tumor was found at 570 days. There were no hepatocellular carcinomas in females and no dose-related increase in males. The incidence of mice with adenomas or hyperplastic nodules in the liver is analyzed in Table 13. Four high-dose males and six high-dose females had both findings. Nodules generally occurred late in the study; in high-dose males and females, 17/23 and 15/23, respectively, of mice with liver nodules were those sacrificed at study termination.

There were no increases in neoplasms at other sites that were related to dosing and no unusual neoplasms were found. The incidence of all neoplasms in all groups is consistent with that found incidentally in aging mice.

D. STUDY AUTHORS' CONCLUSIONS:

There were no effects of dosing on behavior, toxic signs, survival, or food consumption. Weight gain was delayed in males and females receiving 1800 ppm and the effect was markedly more severe in males than in females. Effects on erythrocyte parameters were not of toxicologic importance. It is possible that the increases in platelet counts observed

TABLE 12. Neoplastic Findings in Mice Fed MEB 6447 for 21 Months

Histologic Finding	Dietary Level (ppm)							
	Males				Females			
	10	50	300	1800	0	50	300	1800
Lungs	(50)[a]	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Bronchioalveolar tumor (M)[b]	15	18	19	11	9	12	13	7
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Hepatocellular adenoma[c]	3	3	4	11*	2	1	0	9*
Hepatocellular carcinoma	1	2	1	2	0	0	0	0
Lymphoreticular	(50)	(50)	(50)	(50)	(50)	(50)	(49)	(49)
Malignant lymphoma	5	8	4	4	27	22	23	20
Harderian glands	(48)	(50)	(50)	(50)	(50)	(48)	(46)	(49)
Adenoma	4	4	3	0	3	1	1	0
Adrenal medulla	(50)	(50)	(49)	(50)	(50)	(50)	(49)	(49)
Benign tumor	0	0	0	1	1	0	1	1
Malignant tumor	0	2	0	0	0	0	0	0
Testes	(50)	(50)	(50)	(50)				
Leydig cell tumor	2	3	0	0	-	-	-	-
Ovaries					(50)	(50)	(48)	(49)
Theca-granulosa cell tumor (M)	-	-	-	-	0	1	1	1
Theca-granulosa cell tumor (B)	-	-	-	-	2	4	5	0
Pituitary	(49)	(46)	(49)	(48)	(49)	(49)	(49)	(48)
Adenoma	0	0	0	0	5	5	4	0
Mammary glands					(50)	(50)	(49)	(49)
Malignant tumor[d]	-	-	-	-	3	4	2	1

[a]The numbers in parentheses are the number of animals with tissue examined histologically; includes animals that died after 12 months where sacrificed moribund or sacrificed at study termination. Does not include animals in the satellite groups sacrificed at 51 weeks.

[b]M = Malignant, B = Benign.

[c]Significant trend in each sex, $p < 0.05$; analysis by the study authors using the Peto method.

[d]Adenoacanthoma, carcinosarcoma, or adenocarcinoma.

*Significantly different from control incidence, $p < 0.05$ (Fisher exact test); analysis by our reviewers.

TABLE 13. Incidence of Mice Fed MEB 6447 with Liver Nodules and/or Hepatocellular Adenoma

	0	50	300	1800
Males	5/50	4/50	6/50	30/50**
Females	3/50	2/50	1/49	26/49**

**Significantly different from control incidence, $p < 0.01$ (Fisher exact test); also a significant ($p < 0.01$) trend using the Cochran-Armitage trend test (analysis by our reviewers).

in males receiving 1800 ppm at 12 months and in high-dose females at 53 and 91 weeks was compound related but, this is of secondary importance. Increases in serum aspartate and alanine aminotransferases in males receiving 1800 ppm and females receiving 300 and 1800 ppm correlated with pathologic liver changes. At 300 ppm and higher, there were increases in single cell necrosis, Kupffer cell proliferation, and accumulation of lipofuscin pigment in macrophages in the liver. Fatty changes in the liver, altered cell foci, and hyperplastic nodules of the liver were also seen in both sexes receiving 1800 ppm. There were increases in hepatocellular adenomas in high-dose males and females. These benign tumors were considered an effect secondary to liver damage and thus not the result of primary carcinogenic effects. There were no increases in tumors at other sites. Under the conditions of the study, the NOEL was 50 ppm MEB 6447 for male and female mice.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were adequate. In addition to the required parameters for an oncogenic study, several hematology and clinical chemistry parameters relevant to organ site toxicity were examined and organ weights were recorded. The histopathologic examination was complete and all groups were routinely examined. Individual animal data were present and supported the summary tabulations. We agree with the study authors' conclusions that there was a toxicologically important effect on body weights in high-dose males; however, the decrease in body weights in high-dose females were marginal. There were no effects on survival, on the incidence of toxic signs, or on food or water consumption. We assess that there were no effects of toxicologic importance on hematology parameters; all mean values that differed from controls were within the normal historical range and close to the historical means provided by the testing laboratory. The liver is the target organ of the test compound. This was evidenced by clinical chemistry changes, gross changes, liver weight increases, and histopathologic changes that were predominantly found in mice of both sexes receiving 1800 ppm MEB 6447, but there were also some changes in mice receiving 300 ppm. Some of the effects were apparent at the 12-month interim sacrifice.

The study authors considered hepatocellular hypertrophy to be an adaptive effect compatible with induction of drug-metabolizing enzymes; since it is expected to be a reversible effect, they did not consider it to be adverse. Hence, they set the NOEL for the study at 50 ppm MEB 6447 despite a significant increase ($p < 0.01$) in the incidence of hepato-cellular hypertrophy in females (24/50) compared to controls

(3/50). Hepatocellular hypertrophy was not significantly increased in males receiving 50 ppm. We assess that setting the NOEL at 50 ppm was not the most conservative approach. The spontaneous incidence of hepatocellular hypertrophy is much higher in control males (32%) than control females (6%). Historical incidence data for the finding may be useful for further evaluation but was not available. Hyperplastic liver nodules, Kupffer cell proliferation, lipofuscin accumulation in macrophages, and round cell infiltration were considered to be a response to increased hepatic cell death (single cell necrosis). Hyperplastic nodules were not found at the 12-month sacrifice whereas Kupffer cell proliferation and macrophage pigment accumulation was increased in high-dose males and females and correlated with an increased in single cell necrosis (Table 10). Hyperplastic nodules were differentiated from adenomas as proliferative lesions with normal hepatic architecture with a diameter of less than 3 mm, whereas adenomas had changes in hepatic lobular architecture and usually loss of central veins or portal triads. The authors stated that the hepatocellular adenomas occurring in high-dose males and females coincided with an increase in single cell necrosis in the liver. They interpreted that their occurrence was an "excessive or uncontrolled" regenerative response to tissue injury rather than a result of a primary oncogenic effect of the test compound.

It is our assessment that there is sufficient evidence of an oncogenic response in the liver, even in the absence of an increase in hepatocellular carcinomas. Since the NMRI strain of mice does not have a high spontaneous incidence of hepatocellular adenoma, significance should be evaluated at a p level of 0.05 rather than 0.01. In addition to a significant increase in incidence in both sexes at 1800 ppm using pairwise comparison with controls, there was a highly significant positive trend with the Peto analysis (life-table analysis) and with the Cochran-Armitage test uncorrected by life-table analysis. In addition, the combined incidence of liver hyperplastic nodules and hepatocellular adenoma was highly significant ($p < 0.01$) with either the Fisher exact test or the Cochran-Armitage trend test. Based on hepatocellular hypertrophy, the NOEL in males is 50 ppm and the LOEL in females is 50 ppm, the lowest dose tested.