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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: 6(a)(2) Data: Toxicological Data Review of a Chronic Feeding/Oncogenicity Study (83-5) in Rats with Bayleton.

HED Project No: 2-1022
ID No: 003125-00319
MRID No: 421539-01
PC No.: 109901
Record No: S409658
Caswell No.: 862AA
DP Barcode No: 173169

TO: Cynthia Giles-Parker/James Stone, PM 22
Registration Division (H7505C)

THRU: Roger Gardner, Section Head
Review Section 1
Toxicology Branch
Health Effects Division (H7509C)
Roger Gardner 9/2/92

FROM: Nguyen Bich Thoa, Ph.D
Review Section 1
Toxicology Branch I
Health Effects Division (H7509C)
NBT 08/18/92
Karl Baetcke 9/4/92

Registrant: Mobay Corporation

ACTIONS REQUESTED:

Review a Chronic Feeding/Oncogenicity Study (83-5) in Rats with Bayleton containing 6 (a)(2) data.

CONCLUSIONS:

The study in Rats entittled "MEB 6447 - Chronic Toxicity and Cancerogenicity Studies on Wistar Rats with Administration in Diet Over a Period of 105 Weeks" satisfies the toxicological requirements for a chronic feeding/oncogenicity study in rats (83-5) and is classified Core Minimum.

The NOEL for systemic effect was 300 ppm (males = 16.4 mg/kg/day; females = 22.5 mg/kg/day). The systemic LOEL was 1800 ppm (males

1 of 19

= 114.0 mg/kg/day; females = 199.0 mg/kg/day), based on the following neoplastic and systemic effects:

- A positive dose-related trend for incidence of thyroid follicular cell (TFC) adenomas/adenomas multiple in males with no statistically significant pair-wise difference, and a positive dose-related trend for combined incidence of TFC cystic hyperplasia and adenomas/adenomas multiple in both sexes with no statistically significant pair-wise difference.

- Decreased body weight and body weight gain (compatible with an MTD) in both sexes, decreased RBC count, HGB, HCT, and MCHC, and increased liver weight (abs and rel) and plasma cholesterol in females, increased lipopexia in hepatocytic plasma in both sexes, and observation of thyroid follicular cell (TFC) cystic hyperplasia.

Several deviations from acceptance criteria were noted in the conduct and/or reporting of the study including the use of analytical results from a previously conducted 2-year study to demonstrate test material stability/homogeneity of blending for this study, and lack of summary tables for body weight and body weight gain. Historical control data for thyroid and other tumors were also not provided. A submission of these data is requested.

Special Review Criteria (40 CFR 154.34) This study meets criteria No. 1 (An incidence of neoplasms in male or female animals which increases with dose) for flagging of a chronic feeding/oncogenicity study for potential adverse effect. Toxicology Branch recommends that bayleton be referred to the Peer Review Committee for an assessment of the weight of evidence and a determination of the risks characterization method most appropriate to the available data.

2

Primary Review by: Nguyen B. Thoa, Ph.D. 12 05/14/92
Review Section I, Tox. Branch I/HED
Secondary Review by: Roger Gardner Roger Gardner 9/2/92
Section Head, Review Section I, Tox. Branch I/HED

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding/Oncogenicity Study in Rats (83-5); 6(a)(2) Data.

EPA IDENTIFICATION Nos.:

HED Project No:	2-1022
ID No:	003125-00319
MRID No:	421539-01
PC No.:	109901
Record No:	S409658
Caswell No.:	862AA
DP Barcode No:	173169

TEST MATERIAL: MEB 6447 (Bayleton®) technical Grade (94.4% a.i.)

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

STUDY NUMBER: T-3027626

REPORT No: 101922

SPONSOR: Mobay Corporation

TESTING FACILITY: Bayer AG; Department of Toxicology; Germany.

TITLE OF REPORT: MEB 6447 - Chronic Toxicity and Cancerogenicity Studies on Wistar Rats with Administration in Diet Over a Period of 105 Weeks.

AUTHOR(S): E. Bomhard and B. Schilde

REPORT ISSUED: October 25, 1991

CONCLUSION: The oncogenic potential of MEB 6447 (Bayleton®) was investigated by feeding 50 male and 50 female Wistar rats with diets containing 0, 50, 300, or 1800 ppm (0, 2.7, 16.4, or 114.0 mg a.i./kg/day/male rat; 0, 3.6, 22.5, or 199.0 mg a.i./kg/day/female rat) for 104 consecutive weeks. Additional groups of 10 rats/sex/group were assigned to the 12-month interim sacrifice.

Bayleton® was oncogenic in the 24-month phase, inducing a positive dose-related trend in incidence of thyroid follicular cell adenomas/adenomas multiple in males. There was however no statistically significant pair-wise difference. Increased incidences of thyroid follicular cell cystic hyperplasia, which did not attain statistical significance, were also observed in high

dose males and females, but positive dose-related trends were achieved in both sexes for combined incidences of thyroid follicular cell cystic hyperplasia and adenomas/adenomas multiple.

Increased lipopexia of the hepatocytic plasma was observed in high dose males at the interim sacrifice, and in both high dose males and females at the 24-month necropsy. The liver histopathology was accompanied by increased liver weight and plasma cholesterol in high dose females but not in high dose males. Slight reductions in RBC count, hemoglobin, hematocrit, and MCHC were observed in high dose females.

Mean group body weight and body weight gains were depressed in high dose males and females by amounts compatible with an MTD. Survival rates were comparable between groups. No toxic effects were observed at ≤300 ppm.

Based on these results, the systemic NOEL is 300 ppm and the LOEL is 1800 ppm.

Classification: core MINIMUM (See Reviewer'Discussion Section for deviations from acceptance criteria). This study has minimally satisfied the toxicological data requirements for a chronic feeding/oncogenicity study (83-5) in rats.

Special Review Criteria (40 CFR 154.34) This study meets criteria No. 1 (An incidence of neoplasms in male or female animals which increases with dose) for flagging of a chronic feeding/oncogenicity study for potential adverse effect. Toxicology Branch recommends that bayleton be referred to the Peer Review Committee for an assessment of the weight of evidence and a determination of the risks characterization method most appropriate to the available data.

A. MATERIALS:

1. Test compound: Bayleton® Technical; Description: A grayish-white powder, Batch # 203780190, Purity 94.4%.

2. Test animals: Species: SPF-bred Wistar Rats; Strain: Bor:WISW; Age: 6-7 wks; Weight at week 0: 117-184 g (males); 114-144 g (females); Source: Winkelman Experimental Animals Breeder, Borchen, Germany.

3. Environment: Individual housing; 22°C ± 2; about 50% rel. humidity; 12-hr light/12-hr dark (6am- 6pm); 12 days acclimation.

B. STUDY DESIGN:

1. Animal assignment

Four hundred eighty healthy animals were randomly assigned to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 24 months male	Main Study 24 months female	Interim Sacrifice 12 months male	Interim Sacrifice 12 months female
1 Control	0	50	50	10	10
2 Low dose	50	50	50	10	10
3 Mid dose	300	50	50	10	10
4 High dose	1800	50	50	10	10

In life phase: 01/27/88 to 01/30/90.

2. Diet preparation: Diet was prepared weekly (mechanical blending of test material into feed), stored at room temperature, and fed to the rats the following week. Dietary samples were retained every three months for analysis of a.i. concentration (Bayer AG laboratories; acetonitrile extraction and HPLC with U-V detection). Stability and homogeneity of blending were not tested with samples from this study . The method was validated.

Results: The extraction and quantification results indicated respective groups (mean ± SD) a.i. recoveries of 104% ± 21.7, 94% ± 9.3, and 102% ± 9.1 for diets containing 50, 300, and 1800 ppm. Homogeneity and stability tests, previously conducted with samples from a different study (Study No. T9017407), indicated that diets prepared and stored in similar conditions were stable for 12 days at room temperature. and showed acceptable homogeneity of blending (within 10% of target).

3. Animals received standard diet (Altromin® 1321 flour; Altromin GmbH in Lage) and water (tap water) ad libitum.

4. Statistics - Body weight, feed intake, organ weight, clinical chemistry, urinalysis, and hematology results were expressed as group mean (± SD). Statistical comparison between treated and control values were made by two-tailed "U Test" (Mann and Whitney) at significance levels ≤0.05 (Wilcoxon). Macroscopic and microscopic pathology data were entered into a pathdata computer system for statistical analysis (Cochran and Armitage trend testing for non-neoplastic changes and Peto et al. trend testing for neoplastic changes).

5. A signed quality assurance statement was attached.

C. METHODS:

1. Observations: Animals were inspected twice daily (once daily during weekends and holidays) for signs of toxicity and mortality. They were also inspected weekly for anomalies of body surface/openings, posture, general behavior, respiration, and excrements.

Observations Results: Various clinical signs were observed including rough coat, poor general condition, hair loss, emaciation, tilted head, eye opacity, bloody eyes/muzzles, red tears, accelerated breathing, discolored urine, distended abdomen, diarrhea, palpable mass, and apathy but the incidences were either comparable between groups or were not dose-related.

In the main study, survival rates (Table 1) appeared to be slightly decreased in all treated female groups. A global comparison of the survival curves, using the generalized Wilcoxon test, indicated however that these decreases were not statistically significant. One single mid dose female died in the 12-month phase of the study.

2. Body weight: Animals were weighed weekly from weeks 0 to 13, then biweekly from weeks 14 to 104.

Results: Mean body weight (BW) of the low- and mid dose groups were not affected by treatment with bayleton for 24 months. The high dose (1800 ppm) consistently decreased body weight and body weight gains (BWG) in both sexes [BW ↓: 7-9%, wks 1-103 in males; 5-10%, wks 1- 53 and 10-12%, wks 53-103, in females. BWG ↓: 12%, wks 1-53 and 9%, wks 0-103 in males; 15%, wks 1-53 and 23%, wks 0-103 in females] (Table 2)].

Table 1. Cumulative Mortality and Percent (%) Survival in Rats fed Bayleton for 24 Months

ppm in Diet	Mortality (% Survival) at Week 26	40	52	78	90	104
			MALES			
0	0(100%)	0(100%)	0(100%)	1(98%)	2(96%)	6(88%)
50	0(100%)	0(100%)	0(100%)	2(96%)	5(90%)	7(86%)
300	0(100%)	0(100%)	0(100%)	1(98%)	6(88%)	8(84%)
1800	0(100%)	0(100%)	0(100%0	2(96%)	2(96%)	5(90%)
			FEMALES			
0	1*(98%)	1(98%)	1(98%)	3(94%)	5(90%)	8(84%)
50	0(100%)	0(100%)	0(100%)	3(94%)	4(92%)	12(76%)
300	0(100%)	0(100%)	1*(98%)	3(94%)	5(90%)	13(74%)
1800	1*(98%)	1(98%)	2(96%)	7(86%)	9(82%)	14(72%)

*: Rats found dead after blood sampling.
Data excerpted from part 2 of report (Surviving Animals; pp 176-183).
Global comparison of survival rates conducted according to the generalized Wilcoxon test.

Table 2. Body Weight and Body Weight Gain (BWG) of Rats treated with Bayleton for 24 Months.

ppm in Diet		BODY WEIGHT (g) Week 0	1	8	13	27	53	79	103	BWG of High Dose Group (% Below Control)		
						MALES						
0	MEAN	168	201	330	362	392	420	430	418			
	SD	9	10	19	23	28	32	36	43			
50	MEAN	165	201	331	363	393	422	434	426			
	SD	9	10	21	25	30	34	42	43			
300	MEAN	166	199	336	367	397	422	432	420			
	SD	9	10	23	28	36	40	40	47			
1800	MEAN	163*	183*	306*	334*	359*	384*	401*	390*	Weeks	0-53	12%
	SD	9	10	19	23	28	32	36	43		0-104	9%
	(%)	(3)	(7)	(9)	(7)	(8)	(8)	(7)	(7)			
						FEMALES						
0	MEAN	128	140	190	205	219	237	254	258			
	SD	7	8	14	15	18	23	29	33			
50	MEAN	127	141	189	203	217	237	255	261			
	SD	8	10	14	14	17	19	22	29			
300	MEAN	126	139	192	204	217	233	256	261			
	SD	9	10	14	14	18	22	28	29			
1800	MEAN	127	133*	177*	188*	204*	213*	223*	227*	Weeks	0-53	15%
	SD	7	7	14	14	16	19	19	19		0-104	23%
	(%)		(5)	(7)	(8)	(7)	(10)	(12)	(12)			

Body weight data excerpted from part 2 of report (Tables of means and statistics; pp 130-139).
Numerical values are rounded to the next decimal.
*: $p \leq 0.05$; U-Test.
Nos. males/group: 60, wks 0-27; 50, wk 53; 48-49, wk 79; and 42-45, wk 103.
Nos./female group: 58-60, wks 0-27; 47-50, wk 53; 43-47, wk 79; and 35-42, wk 103.

3. Food consumption and compound intake: Feed consumption was determined weekly (wk 1-13) then every 4th week (wk 14-103). Compound intake were calculated from the consumption and body weight gain data. Feed efficiency was not calculated.

Results: Feed consumption was not significantly affected at 50 and 300 ppm; the changes were only occasional and minimal (<5% at 50 ppm and ≤10% at 300 ppm). The high dose significantly ($p \leq 0.05$) increased feed consumption (Table 3), that of the females markedly and throughout the study and that of the males moderately and at all observation time points except for one (wk 73). Group overall average increases in daily feed consumption for the 104-week dosing period were 19% for high dose males and 53% for high dose females. Group overall averages compound intake for the 104-week dosing period were:

	COMPOUND INTAKE		
	Males	Females	
	mg/kg/day	mg/kg/day	% Over Male's Intake
ppm			
0	0	0	
50	2.7	3.6	33%
300	16.4	22.5	37%
1800	114.0	199.0	75%

Group average compound intakes were higher in females than in males, at all dose levels. Respective increases observed in the 50, 300, and 1800 female groups were 33%, 37%, and 75% over the corresponding male dose groups.

4. Ophthalmological examination: Ophthalmological examination was performed at week 0, 52, and 104 on 10 animals/sex/group.

Results: Cataract was observed in every group sacrificed at week 104 but there was no dose-effect relationship. The incidence for the 0 ppm, 50 ppm, 300 ppm, and 1800 ppm groups were respectively 3/10, 7/10, 2/10, and 7/10 for the males and 4/10, 3/10, 5/10, and 4/10 for the females.

Table 3. Feed Consumption (g/kg body weight/day) in Control and High Dose Rats treated with Bayleton for 24 Months.

Week		FEED CONSUMPTION (g/kg/day) 1	8	13	27	53	79	103	GROUP OVERALL AVERAGES Feed Consumption (g/kg/day)	% Increases over Control
						MALES				
ppm in Diet										
0	MEAN	91	58	54	47	40	42	54	53.4	
1800	MEAN	122*	71*	67*	53*	45*	47*	64*	63.3	19%
	% ↑	34	22	24	13	13	12	19		
						FEMALES				
0	MEAN	102	75	77	66	62	58	75	72.3	
1800	MEAN	163*	125*	119*	93*	83*	89*	112*	110.5	53%
	% ↑	34	22	24	13	13	12	19		

Group mean feed consumption data excerpted from part 2 of report (Tables of means and statistics; pp 125-129).
Group overall averages data (Average daily feed consumption and average % increase over control computed over the 104-week dosing period) excerpted from Table 2 of report.
Numerical values are rounded to the next decimal.
*: $p < 0.05$; U-Test.

5. Blood was collected from the retro orbital venous plexus of all animals (unfasted) for hematology and clinical analysis, at weeks 26, 52, 78, and 104 (exception: blood drawn from caudal vein of fasted animals for glucose). The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements (Thromboplastin time)		

* Required for subchronic and chronic studies

Results: Treatment with bayleton 50 to 1800 ppm did not affect any of the hematology parameters examined in males. Females treated with the low dose (50 ppm) were also unaffected. High dose females showed concomitant minor decreases ($p < 0.05$) in RBC count and hemoglobin (6-7%; wks 26, 78, and 104), hematocrit (4-5%; wks 26 and 104), and MCHC (3%; wks 26 and 104). Slight decreases ($p < 0.05$) in leucocyte counts were also observed in mid- and high dose females at weeks 26 (23%), and 104 (39 and 32% respectively).

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum Protein (TP)*
	Cholinesterase (ChE)#		Triglycerides
	Creatinine phosphokinase*^		Serum protein electrophores
	Lactic acid dehydrogenase (LAD)		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		

* Required for subchronic and chronic studies

Results: Treatment with bayleton 50 to 300 ppm did not affect any of the clinical chemistry parameters examined. High dose males showed minor increases in SGPT at weeks 26 (22%) and 104

(37%) which were statistically significant ($p \leq 0.05$). High dose females showed significant ($p \leq 0.05$) increases in blood cholesterol (24%, wk 26; 32%, wk 53; and 27%, wk 78), and urea (22%, wk 26; 17%, wk 53; and 10%, wk 78).

6. Urinalysis: Urine was collected from unfasted animals at weeks 26, 52, 78, and 104. The CHECKED (X) parameters were examined.

X		X	
	Appearance*	x	Glucose*
x	Volume*	x	Ketones*
x	Specific gravity*	x	Bilirubin*
x	pH	x	Blood*
x	Sediment (microscopic)*		Nitrate
x	Protein*	x	Urobilinogen

* Required for chronic studies

Results: Decreases ($p \leq 0.05$) in urinary protein (31%) and total protein excretion (51%) were observed once in high dose males (wk 103). No other changes were observed.

7. Sacrifice and Pathology: All animals that died and/or were sacrificed on schedule were subjected to gross pathological examination. The CHECKED (X) tissues of animals that were sacrificed on schedule were collected for histological examination. The (XX) organs, in addition, were weighed.

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
x	Tongue	x	Aorta*	xx	Brain*•
x	Salivary glands*	x	Heart*	x	Periph. nerve*#
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*#
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen	x	Eyes (optic n.)*#
x	Jejunum*	x	Thymus*	Glandular	
x	Ileum*	Urogenital		xx	Adrenal gland*
x	Cecum*	xx	Kidneys*+	x	Lacrimal gland#
x	Colon*	x	Urinary bladder*	x	Mammary gland*#
x	Rectum*	xx	Testes*•	x	Parathyroids*••
xx	Liver *•	x	Epididymides	x	Thyroids*••
	Gall bladder*	x	Prostate	Other	
x	Pancreas*	x	Seminal ves.	x	Bone*#
Respiratory		x	Ovaries*•	x	Skeletal muscle*#
x	Trachea*	x	Uterus*	x	Skin*#
xx	Lung*			x	All gross lesions and masses*
x	Larynx^				

* Required for subchronic and chronic studies.
• Organ weight required in subchronic and chronic studies.

Results:

a. Organ weight:

At the interim sacrifice, body weight of high dose males were reduced ($p \leq 0.05$) by 11% and that of the females by 9%. Some significant ($p \leq 0.05$) alterations in organ weight were observed, including an increase in relative (to body) weight of the testes in high dose males (23%) and an increase in liver weight in high dose females (absolute increase of 15% and relative increase of 27%).

In the main study, body weight of high dose males were reduced ($p \leq 0.05$) by 7% and that of the females by 13%. Liver weight was increased in a dose-related manner in females treated with 300 and 1800 ppm (absolute increases were 11% at 300 ppm and 17% at 1800 ppm; relative increases were 8% at 300 ppm and 32% at 1800 ppm). Other alterations in organ weight were also observed at the high dose but they were probably related directly to the observed decreases in terminal body weight [Increased relative weight of liver (5% in males), testes (5%), spleen (9% in females) and brain (5% in males; 14% in females). Decreased absolute weight of heart (11% in males and 6% in females), lungs (10% in males), and spleen (18% in males and 16% in females), (Table 4)]. The thyroid was not weighed.

b. Gross pathology:

No treatment related gross pathology changes were observed.

c. Microscopic pathology:

1) Non-neoplastic lesions: Three of the 10 high dose males sacrificed at the interim phase showed slightly increased lipopexia of hepatocytic plasma (fat in hepatocytes).

In the main study (Table 5), the incidence of lipopexia of hepatocytic plasma was also increased at the high dose (male incidence = 32/49; female incidence = 22/50). Both male and female increases were statistically significant. In addition, a positive trend (Cochran Armitage trend test) was observed with the females. The incidence of thyroid follicular cell cystic hyperplasia was increased in high dose males (3/49) and females (4/50) but the increases were not statistically significant.

Table 4. Terminal Body weight (BW) and Organ Absolute (Abs)/Relative (Rel) Weight

GROUP		BW (g)	ORGAN WEIGHT								
			Liver		Testes	Heart	Lung	Spleen		Brain	Adrenal
			Abs	Rel	Rel	Abs	Abs	Abs	Rel	Rel	Rel
						MALES					
0	MEAN	421		3784	908	1.65	2.03	0.91	219	518	14
	SD	42		538	156	0.27	0.49	0.18	49	44	6
50	MEAN	NS		NS	NS	NS	NS	NS	NS	NS	NS
300	MEAN	NS		NS	NS	NS	NS	NS	NS	NS	NS
1800	MEAN	393*		3990*	957*	1.48*	1.83*	0.75*	190*	546*	12
	SD	37		517	130	0.20	0.29	0.17	37	51	3
	% change	-7%		+5%	+5%	-11%	-10%	-18%	-13%	+5%	-14%
						FEMALES					
0	MEAN	263	9.62	3685		1.13		0.58	500	754	
	SD	32	2.17	732		0.18		0.14	57	84	
50	MEAN	NS	NS	NS		NS		NS	NS	NS	
300	MEAN	NS	10.65*	3975*		NS		NS	NS	NS	
	SD		1.45	409							
1800	MEAN	230*	11.22*	4879*		1.06*		0.49*	549*	859*	
	SD	37	1.36	470		0.20		0.10	106	72	
	% change	-13%	+17%	+32%		-6%		-16%	+9%	+14	

*: p ≤0.05; treated vs. control (2-tailed U-TEST)
NS: Not significantly different from control.
% change: % increase (+) over control or decrease (-) below control.

Table 5. Non-Neoplastic Lesions in Rats treated with Bayleton for 24 Months

ppm in Diet	0	50	300	1800
LIVER LESIONS				
Fat in Hepatocytes				
Male Incidence (%)	8/50(16)	10/50(20)	15/50(30)	32/49*(60)
Female Incidence (%)	3/49*(6)	2/49(4)	4/50(8)	22/50*(44)
Focal Necrosis				
Male Incidence (%)	0/50(16)	1/50(2)	2/50(4)	4/49(8)
Female Incidence (%)	2/49(4)	2/49(4)	5/50(10)	2/50(4)
Clear cell foci				
Male Incidence (%)	15/50(30)	25/50(50)	9/50(18)	8/49(16)
Female Incidence (%)	2/49(4)	2/49(4)	4/50(8)	1/50(2)
THYROID FOLLICULAR CELL LESIONS				
Cystic Hyperplasia				
Male Incidence (%)	2/50(4)	3/50(6)	1/50(2)	3/49(6)
Female Incidence (%)	2/50(4)	0/50	1/50(2)	4/50(8)

Data excerpted from part 2 of report (Number of animals with microscopic findings by organ/group/sex; pp 407-431).
* at control: Positive trend (Cochran Armitage trend test).
* at treated group: $p \leq 0.05$; treated vs. control.

2) Neoplastic Lesions: Neoplastic lesions were not observed at the interim sacrifice.

In the main study (Table 6), the incidence of thyroid follicular cell adenomas/adenomas multiple was slightly increased in high dose males (4/49) and females (2/50). The increases were not statistically significant but a significant slope was observed in males (Peto "combined prevalence and death rate" method). A positive trend was also observed in both sexes for combined incidences of thyroid follicular cell adenomas/multiple adenomas and cystic hyperplasia. No historical control data on thyroid tumor incidence were submitted by the registrant.

Table 6. Neoplastic Lesions in Rats treated with Bayleton for 24 Months

ppm in Diet	0	50	300	1800
	THYROID FOLLICULAR CELL LESIONS			
Adenomas				
Male Incidence (%)	0/50	0/50	1/50(2)	3/49(6)
Female Incidence (%)	0/50	1/50(2)	0/50	2/50(4)
Adenomas Multiple				
Male Incidence (%)	0/50	0/50	0/50	1/49
Female Incidence (%)	0/50	0/49	0/50	0/50
Adenomas + Adenomas Multiple				
Male Incidence (%)	0/50*	0/50	1/50(2)	4/49(8)
Female Incidence (%)	0/50	1/50(2)	0/50	2/50(4)
Adenomas + Adenomas Multiple + Cystic Hyperplasia				
Male Incidence (%)	2/50+(4)	3/50(6)	2/50(4)	7/49(15)
Female Incidence (%)	2/50+(4)	1/50(2)	1/50(2)	6/50(12)
	PITUITARY (Pars Distalis) ADENOMAS			
Male Incidence (%)	11/50(22)	9/50(18)	9/50(18)	2/49(4)
Female Incidence (%)	15/49(30)	9/50(18)	14/50(28)	8/49(16)
	ADRENAL			
Cortical Adenomas				
Male Incidence (%)	3/50(6)	3/50(6)	2/50(4)	0/49
Female Incidence (%)	3/49(6)	2/48(4)	2/50(4)	2/50(4)
Pheochromocytomas				
Male Incidence (%)	7/50(14)	4/50(8)	7/50(14)	3/49(6)
Female Incidence (%)	1/49(2)	0/48	2/50(4)	0/50
	MAMMARY GLANDS (Female incidence)			
Adenomas	1/49(2)	0/49	1/50(2)	0/50
Fibroadenomas	6/49(12)	4/49(8)	3/50(6)	2/50(4)
Adenocarcinomas	2/49(4)	2/49(4)	1/50(2)	0/5

Data excerpted from part 2 of report (Number of animals with microscopic findings by organ/group/sex; pp 407-431).
* at control: Significant slope (Peto"combined prevalence & death rate) method.
+ at control: Positive trend (Peto Trend test).

D. DISCUSSION

1. Author's Discussion and Assessment of Results

MEB 6447 was administered in the diet of wistar rats (50/sex/group) for 104 weeks, at doses of 0, 50, 300, or 1800 ppm. Additional groups of 10 rats/sex/group were sacrificed after 12 month of dosing (interim sacrifice).

No changes in appearance and/or behavior were observed at any dose. No changes in body weight occurred at ≤ 300 ppm but significant decreases (up to about 10%) were observed at 1800 ppm during the entire study. Feed intake was unaffected at ≤ 300 ppm but was slightly increased in high dose males and markedly increased in high dose females.

Except for the female high dose group, mortality rates were comparable in every group and were within "empirical figures". Mortality rate for high dose females in the period from week 40 to 90 was twice that of the control and low dose group and exceeded known rates but a specific cause of death could not be determined on the basis of necropsy findings.

Bayleton did not cause any adverse ophthalmologic effects. The slight reductions in hemoglobin, MCHC, RBC count, and hematocrit observed in high dose females in this study were also observed in a previous 2-year study and therefore, were probably treatment related. No evidence of hepatotoxicity was observed at ≤300 ppm. The increased liver (absolute and relative) weights observed at 1800 ppm were within normal range but both the increased plasma cholesterol levels observed in high dose females (a sign of disturbance in lipid metabolism) and lipopexia observed in high dose males (interim + terminal necropsy) and females (terminal necropsy) indicated the liver as a target organ. There was no evidence of kidney damage, based on urinalysis, necropsy, and histological findings.

No adverse effects on the endocrine system was observed at ≤300 ppm. The high dose was associated with both a marginal increase in non-neoplastic (thyroid follicular cell cystic hyperplasia) and neoplastic (thyroid follicular cell adenomas/adenomas multiple) lesions of the thyroid. On the other hand, the incidences of other neoplastic lesions were reduced at the high dose [Pituitary (Pars distalis) adenomas (males and females), adrenal cortical adenomas/pheochromocytomas (males), and tumors of mamma (females)]. A shift in the spectrum of endocrine tumors represents the rule rather than the exception in rats, at the toxic dose region, especially when marked alterations of body weight are also present. Experience indicated however that the thyroid neoplasm observed in this study are not growth- or stress-related but may be related to an increase of liver metabolic process. Various fungicidal azoles, including MEB 6447, are known to induce the liver microsomal

cytochrome P-450 enzyme system. This may result in an increase in thyroxine metabolism, which may in turn lead to a compensatory increase in TSH secretion with its stimulatory sequelae on the thyroid gland. A secondary carcinogenic effect of MEB 6447 is also compatible with the chemical lack of genotoxic effects.

Under the conditions of the study, the dose 300 ppm is considered to be a NOEL.

2. Reviewer's Discussion and Assessment of Results

The oncogenic potential of MEB 6447 (Bayleton®) was investigated by feeding 50 male and 50 female Wistar rats with diet containing 0, 50, 300, or 1800 ppm (0, 2.7, 16.4, or 114.0 mg a.i./kg/day/male rat; 0, 3.6, 22.5, or 199.0 mg a.i./kg/day/female rat) for 104 consecutive weeks. Additional groups of 10 rats/sex/group were assigned to the 12-month interim sacrifice.

The dose range used was adequate (See body weight and body weight gain below).

Incidences of clinical signs and abnormal appearance/behavior were comparable between groups. Survival rates were comparable between groups. No toxic effects were observed at the low- and mid dose.

Body weight was reduced at the high dose, during most of the observation times, the males by 7-9% and the females by 5-12%. Body weight gain of the high dose group was reduced, the males only during the first 52 weeks (12%) but the females during the entire study (15%, wks 1-53; 23%, wks 1-103). The greater reduction in body weight gain observed in the female group was most probably related to a greater compound intake (compound intake of female > male by 75%) rather than to a specific sex-related effect. Feed consumption of high dose males and females were increased, the males moderately and the females markedly (group overall average increases were 19% in males and 53% in females). This sex-related differential increase may be a reflection of the greater compound intake in females. The concomitant body weight decrease and feed consumption increases are suggestive of a stimulatory effect of the test material on the metabolic function of the thyroid.

Slight reductions in RBC count, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentrations were concomitantly observed in high dose females. This slight anemia is probably a real effect, since it was also observed in a previous 2-year study with bayleton (See discussion by author; 4th paragraph) as well as in a 90-day oral toxicity study with baytan (MRID No. 421927-01), a pesticide which is also the major metabolite of bayleton. The slight changes in serum SGOT (high dose males) and leucocyte count (high dose females) were below biological significance.

The high dose was associated with non-neoplastic lesions in the

liver and thyroid. Increased lipopexia in the hepatocytes' plasma was observed in males at the interim sacrifice, and in both sexes in the main study. This finding, in conjunction with the observed increases in liver weight (absolute and relative) and plasma cholesterol in females, are suggestive of an effect of bayleton on lipid metabolism in the liver. Baytan was also observed to produce changes compatible with a disturbance of lipid metabolism in the subchronic study cited above. Both high dose males and females showed increased incidences of thyroid follicular cell cystic hyperplasia in the main study, but the increases were not statistically significant.

The high dose was associated with a positive dose-related trend in incidence of thyroid follicular cell adenomas/adenomas multiple in males. When incidences of thyroid follicular cell adenomas/adenomas multiple and cystic hyperplasia were combined, positive trends ensue for both males and females. The author theorized that Bayleton may induce thyroid neoplasm through an endocrine effect, e.g., through a disruption of the pituitary-thyroid hormonal balance, since bayleton and other bayleton-like fungicidal azoles are known to induce the liver microsomal cytochrome P-450 enzyme system. Specific evidences such as goitrogenic activity in vivo, changes in serum levels of thyroid hormone and thyroid stimulating hormone, decreased synthesis/increased metabolism of thyroid hormone, lesions progression/reversibility were however not investigated in this study to lend support to this theory.

Based on the adverse effects described above, the systemic NOEL is 300 ppm (MD) and the LOEL is 1800 ppm (HD).

Several deviations from acceptance criteria were noted and are described below:

- Tests for stability and homogeneity of blending were not conducted with samples from this study. The author used the results from a previous study to show that diets prepared and stored in similar conditions had acceptable homogeneity of blending and were stable at room temperature for 12 days.

- There were no summary tables for group mean (± SD) for body weight and body weight gain.

- Group mean feed consumption (g/kg/day) were reported without standard deviation values.

- No historical control data were provided for non- neoplastic and neoplastic lesions of the thyroid, as well as for mortality rates, and clinical chemistry.

Since the integrity of the study was not seriously compromised by these deviations, this study is core classified CORE MINIMUM.