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

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MAY 8 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: MANCOZEB (Dithane M-45®). Tox.
Data Submitted Under MRID No. 419818-01

ID No. 707-78

Chemical: 913 (014504)
RD Record: S401532
HED Project: 1-2165

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I
Health Effects Division (H7509C) 04-30-92

TO: Walter Waldrop/Terri Stowe, pm 71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effects Division (H7509C) 5/4/92

Registrant: Rohm and Haas, Spring House, PA

Request: Review and evaluate the following carcinogenicity study in CD-1 mice, submitted in response to a data gap identified in the MANCOZEB REGISTRATION STANDARD:

(83-2) Oncogenicity Study --- Mancozeb: 18 Month Dietary Oncogenicity Study in Mice, performed for the registrant by Tegeris Laboratories, Inc., Temple Hills, MD, Study No. 85051; Final Report dated June 04, 1991 (EPA MRID No. 41981801).

Doses tested: 0, 30, 100, 1000 ppm, administered in the diet for 18 months.

TB-I Conclusions: This study is graded CORE-MINIMUM DATA, demonstrating no evidence of carcinogenicity at the HDT, 1000 ppm, which is considered the MTD.

ATTACHMENT (DER)

mARY/dISK 4/Mancozeb

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FINAL

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DATA EVALUATION REPORT
MANCOZEB
ONCOGENICITY STUDY – MANCOZEB: 18 MONTH DIETARY ONCOGENICITY STUDY IN MICE

Prepared for:

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

Prepared by:

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

Principal Reviewer	Annette M. Hugo	Date 4-17-92
Independent Reviewer		Date 4-23-92
QA/QC Manager	William A. Vigil	Date 4-27-92

Contract Number: 68D10075
Work Assignment Number: 1-29
Project Officer: Mr. Jim Scott
EPA Work Assignment Manager: Dr. Irving Mauer

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Primary Review by: Irving Mauer, Ph.D.
{title}, Review Section {#}, Toxicology Branch I/HED
Secondary Review by: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I/HED

04-30-92
5/4/92

DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity study - Mice (§ 83-2)

TEST MATERIAL: Mancozeb

TOX. CHEM. NO.:

SYNONYMS: Dithane M-45

MRID NO.:

STUDY NUMBER: 85051

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, Pennsylvania 19477

TESTING FACILITY: Tegeris Laboratories, Inc.
5050 Beech Place
Temple Hills, Maryland 20748

TITLE OF REPORT: Mancozeb: 18 Month Dietary Oncogenicity Study in Mice

AUTHORS: Thomas E. Shellenberger, Ph.D.

REPORT ISSUED: June 4, 1991

CONCLUSIONS:

Mancozeb, administered in the diet to CD-1 mice for eighteen months at concentrations of 0, 30, 100, and 1000 ppm had the following effects:

1000 ppm - (131 mg/kg/day in males, 180 mg/kg/day in females) - Effects on thyroid function in both sexes: decreased T3 and T4 levels in females, increased T3 in males; 13% decrease in body weight in males (9% decrease in females), accompanied by an increase in relative liver-to-body weight ratio in females.

100 ppm - (13 mg/kg/day in males, 18 mg/kg/day in females) - no effects.

30 ppm - (4 mg/kg/day in males, 5 mg/kg/day in females) - no effects.

No carcinogenic effects attributable to compound intake.

This study is classified Core-Minimum for carcinogenicity.

I. Materials, Methods and Results

A. Test Article Description

Name: Mancozeb

Formula:

Lot Number: 56528, TD Number 85-323
Purity: 82.8%
Physical Property: yellow fine powder
Stability: stable at room temperature

B. Test Article Analyses for Purity and Stability

The purity of mancozeb was reported to be 82.8% active ingredient (AI) at the beginning of the study, and was 73% AI at the end of the study (see Report page 23, 3.0 MATERIAL AND METHODS, 3.1 Test Material).

Batches of diets were reported to be mixed weekly at dietary concentrations of 0, 30, 100 and 1000 ppm by Tegeris Labs. After final mixing, samples from the top, middle, and bottom strata of these four batches were frozen until analysis could be conducted for homogeneity. Samples were sent weekly for analysis the first four weeks of the study and once monthly thereafter. Adequate mixing was indicated if the variation was less than $\pm$10% of the target concentration for the top, middle, and bottom strata of each dose level. The results indicated homogeneity of the mixture (see Table 1) (see Appendix #A-4.9 of the Report).

Mancozeb degrades to ethylene thiourea (ETU) over time. Therefore, selected samples of diet batches (weeks 1, 2, 3, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 50, 51, 52, 56, 62, 66, 70, 74, 78, and 80 of the study) were analyzed for mancozeb and ETU to ensure that the specified dietary concentrations during the 18-month study were being achieved. A second analysis of the diet samples from weeks 52 and 56 was also conducted approximately one year after the initial analysis to confirm the results of these tests. The results for the stability tests are

Table 1

RESULTS OF HOMOGENEITY ANALYSES FOR MANCOZEB

Target Dose Level (ppm)	Feed Component	% of Target	Average % of Target
30	Top	99	
	Middle	102	101
	Bottom	103	
100	Top	103	
	Middle	110	108
	Bottom	110	
1000	Top	102	
	Middle	101	103
	Bottom	105	

Data extracted from a table located on Report page 1288.

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presented in Table 2. Stability in the diets was assumed by a variation of less than ±10% of the target dietary concentration for feed samples of each dose level. For weeks 0-48, a small amount of ETU (0.02 ppm) was reported in the control diet at the method sensitivity of 0.01 ppm. For weeks 0-48, approximately 5-7% of the degradation of mancozeb could be attributed to the conversion to ETU. For weeks 50-80, approximately 28-46% of this degradation was attributed to conversion to ETU. Degradation to any product other than ETU was not reported. A suspected problem in mixing or storage of the feed prior to freezing of the samples for shipment to be analyzed was suspected by the Laboratory which conducted the analyses (see Appendix #A-4.9 of the Report). However, no explanation for this stability problem was provided by Registrant.

C. Animals

Five hundred CD-1 mice of each sex, aged approximately three weeks, were purchased from Charles River Labs, Inc., Portage, Michigan. The animals were examined upon receipt for good health and given temporary numbers. The mice were gang-housed for one week, three per cage for another week of acclimation, and individually for one week prior to the initiation of mancozeb administration and throughout the treatment period. Animals were singly housed

Table 2

RESULTS OF STABILITY TESTS FOR MANCOZEB

Target Dose Level (ppm)	Stability (0-48 weeks) Average % of Target	Percent Conversion of Mancozeb to ETU
30	96.0	6.5
100	99.0	7.4
1000	98.0	5.9
Target Dose Level (ppm)	**Stability (50-80 weeks) Average % of Target**	**Percent Conversion of Mancozeb to ETU**
30	61.0	46.0
100	63.0	39.0
1000	81.0	28.0

Data extracted from tables located on Report page 1283.

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in stainless steel cages in an environmentally controlled room to 79 + 3°F with a relative humidity of 40-60%. The light cycle was maintained at 12 hours on/12 hours off. The cages were placed on racks in order that each dosage group had the same number of cages in each location on the racks. Every other week the rack in the front of the room was moved to the back and all other racks were moved forward one space. Clean cages were provided every 2-3 weeks and paper under the cage was changed three times a week (see Report pages 24 and 25, 3.0 MATERIALS AND METHODS, 3.2 Test Animals and 3.4 Animal Husbandry).

The feed used was Agway Certified Meal RHM 3200 which was analyzed by the supplier and certified to be free of contaminants. However, ETU was found in the control diet at a concentration of 0.02 ppm. A weighed quantity of feed was placed in each cage on a weekly basis throughout the study and unconsumed feed at the end of the one-week feeding period was weighed and discarded. Feed consumption was calculated as the difference in weights. Feed was available ad libitum. Tap water was provided ad libitum. Water was analyzed every 6 months. There were no known contaminants in either the food or water that were expected to interfere with the study, except for the degradation to ETU (see Report page 26, 3.0 MATERIALS AND METHODS, 3.4 Animal Husbandry).

A total of 376 male and 376 female mice were selected for treatment with mancozeb. Animals were randomly assigned to dose groups (approximately 94 animals/dose group) (see Table 3) using the computerized weight randomization program. The weight variation of the animals of each sex used did not exceed ± 2 standard deviations of the mean weight, and the mean body weights for each group and sex were not statistically different. Twenty-four animals/group/sex were selected for interim sacrifice at 12 months. An additional 66 mice (33/sex) were assigned to be concurrently used as sentinel animals for monitoring intercurrent diseases and for monitoring the health status of mice received for the study. Five mice of each sex in the sentinel group were necropsied at the start of the study and at 3, 6, 12, 15, and 18 months. The remaining animals were necropsied at study termination (18 months) (see Report pages 24, 25, 29, and 32, 3.0 MATERIALS AND METHODS, 3.2 Test Animals, 3.3 Group Assignments and Dose Levels, 3.6.4 Sacrifice and Gross Pathology, and 3.8 Sentinel Animals).

Table 3

NUMBER OF ANIMALS AND THEIR TREATMENT
DURING AN EIGHTEEN-MONTH STUDY WITH MANCOZEB

Concentration of test substance in diet (ppm)	Females Number of animals*	Males Number of animals*
0	93**	95**
30	94	94
100	94	94
1000	94	94

* Includes a satellite group of 24 animals/group/sex for interim sacrifice at 12 months.
** One mouse originally assigned to this group was mis-sexed. All data collected for this animal were deleted from the male group and added to the female control group.

Data extracted from a table located on Report page 25.

D. Dosing

Mancozeb was administered to male and female CD-1 mice in the diet at nominal concentrations of 0, 30, 100, or 1000 ppm. The test article was administered at a constant concentration (ppm) in the diets. Formulated feeds containing the test material were prepared weekly within 1 week of the day on which the prepared feed was given to the animals. Dietary concentrations were expressed as 100% AI (active ingredient). For weeks 0 to 59, the initial purity of 82.8% AI was used to calculate the amounts of the test article needed for diet preparation. The purity decreased to 77.0% AI by week 59 and effective week 60.

the new purity was used to re-calculate the amounts of test material needed to attain the nominal dietary levels. The actual analyzed mean concentrations at each dietary level over the total 18 month study were 83.0% (30 ppm), 76.4% (100 ppm), and 91.2% (1000 ppm) of nominal.

E. General Observations

1. Mortality/Moribundity/Survival - The animals were observed at least twice daily, at least six hours apart.

The survival of the animals at the termination of the study is reported in Table 4. According to the authors, a statistically significant difference in survival among the four male dose groups was observed and could be attributed to an unusually high survival rate among mid-dose males (100 ppm). The remaining dose groups had comparable survival patterns. For female animals, the survival distributions among the four dose groups were not significantly different. Therefore, no relevant increases in mortality incidences could be associated with exposure to mancozeb based on analyses using SAS PROC LIFETEST, the Wilcoxon test, or the log rank test (see Report pages 33-34, 4.0 RESULTS, 4.1 Clinical Signs and Mortality).

2. Clinical Observations - All animals were observed twice daily, at least six hours apart, for clinical, pharmacological, or toxicological signs (appetite, appearance, behavior, excretory functions and discharges). Detailed examinations of each mouse were conducted weekly at the time of body weight measurements. These examinations included behavior, gait posture, righting reflex, urine and feces, palpation for masses, as well as respiration and gross abnormalities in body temperature.

No treatment-related increases in the incidence of any clinical signs were observed in male or female mice following exposure to mancozeb. The authors reported that the predominant clinical sign observed in male mice, both treated and control, was alopecia and rough skin associated in some animals with skin lesions, rashes, and edema. These findings appeared to be more prevalent in control and low-dose male mice. Palpable masses were observed in all dose groups and control male mice. The authors reported that these masses appeared to be preputial gland abscesses, not tumors. Other signs which were observed among all male dose groups were opaque and recessed eyes, convulsions, labored breathing, inactivity, and emaciation. In females as in males, the prevalent clinical signs observed among all groups were alopecia along with rough skin, skin lesions, edema, and rashes. Other clinical signs in females included inactivity, emaciation, recessed and opaque eyes, convulsions, spinning, labored breathing, and the occurrence of palpable masses.

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Table 4

INCIDENCE OF MORTALITY FOR MICE IN AN EIGHTEEN-MONTH TOXICITY/ONCOGENICITY STUDY WITH MANCOZEB

Treatment Weeks	0 ppm per group (%)	30 ppm per group (%)	100 ppm per group (%)	1000 ppm per group (%)
MALES				
1-52	9/93* (9.6)	9/94 (9.6)	3/94 (3.2)	4/94 (4.3)
53-78**	12/60 (20.0)	13/61 (21.3)	6/67 (9.0)	13/66 (19.7)
Total unscheduled mortality	21/69 (30.4)	22/70 (31.4)	9/70 (12.9)	17/70 (24.3)
FEMALES				
1-52	9/95* (9.5)	5/94 (5.3)	4/94 (4.3)	10/94 (10.6)
53-78**	12/62 (19.4)	12/60 (18.5)	10/70 (14.3)	9/60 (15.0)
Total unscheduled mortality	21/71 (29.6)	17/70 (24.3)	14/70 (20.0)	19/70 (27.1)

* One animal was initially mis-sexed.
** Total animals in each group excludes deaths through week 52 and the 24 sex/group sacrificed at week 53.
Data extracted from Report Tables #T-4.1.5. and #T-4.1.6, pages 149 and 150.

The authors did not report any details about the masses observed in female mice.

3. Body Weights/Food Consumption/Test Material Intake - Body weights were measured prior to the initiation of the study for randomization purposes on Day 0 (day of initiation), weekly for the first 14 weeks, and once every other week thereafter. Terminal body weights were recorded prior to the necropsy (see Report page 28-29, 3.0 MATERIALS AND METHODS, 3.6.1 Observations, Body Weight, and Feed Consumption, and 3.6.4. Sacrifice and Gross Pathology).

As noted in Table 5, in males, significant differences in mean body weights were noted at various times in the 100 and 1000 ppm dose groups. These significant changes in body weight were not sustained throughout the study. Significant decreases in body weight were also noted in female mice in the 1000 ppm dose group. However, at terminal sacrifice, body weights were not significantly

different for any of the treated groups for both males and females. No significant changes in body weight gains were observed in any dose group for both males and females at either 52 or 78 weeks according to the results of the authors' statistical tests; however, body weight gains in high-dose males and females were decreased approximately 13 and 9%, respectively, at terminal sacrifice.

Food consumption was determined at the same intervals as body weight. A weighed quantity of feed was offered to each mouse for one week; unconsumed feed at the end of this period was weighed and the feed consumption calculated by the difference in these weights (see Report page 28, 3.0 MATERIALS AND METHODS, 3.6.1 Observations, Body Weight, and Feed Consumption).

Mean food consumption was evaluated using the one-way analysis of variance (ANOVA). When this analysis suggested group differences, comparisons between control and treated animals were compared using Dunnett's t-test at the 95% confidence level. Male weekly feed consumption was significantly decreased in all treated groups at week 1 and week 58. Feed consumptions of all groups of mancozeb-treated male mice otherwise were either similar to or exceeded the feed consumption of the control group, except in the 1000 ppm group at week 16 and in the 100 ppm group at week 46. In females, statistically significant decreases in feed consumption were observed in the low-dose group at weeks 2 and 68 and in the mid- and high-dose group at weeks 12 and 58. No consistent differences in feed consumption were noted among any of the treated groups, male or female (see Report page 35, 4.0 RESULTS, 4.3 Feed Consumption and Feed Efficiency).

The estimated compound intake of mancozeb in mg/kg/day over 18 months was 3.75 (30 ppm), 12.52 (100 ppm), and 130.60 (1000 ppm) for male mice and 5.18 (30 ppm), 17.81 (100 ppm), and 179.74 (1000 ppm) for female mice. No details were reported on the determination of these compound intakes. To determine if the change in compound consumed for a specific group was proportional to the other dose levels, the percent change in mean intake was calculated at weeks 1-12, 12-26, 26-52, and 53-78. The percent change in compound intake was similar at each dose level within a specific time interval and sex, except for weeks 53-78 (see Report page 36, 4.0 RESULTS, Compound Intake). No explanation for this difference was reported by the authors of the study and, based on the reported information, the reviewer could find no explanation (see Table 6).

Table 5

GROUP MEAN BODY WEIGHTS AND BODY WEIGHT GAINS - EIGHTEEN-MONTH MOUSE ONCOGENICITY STUDY WITH MANCOZEB

Week	Males				Females			
	0 ppm	30 ppm	100 ppm	1000 ppm	0 ppm	30 ppm	100 ppm	1000 ppm
	BODY WEIGHTS - g							
0	28.15	28.16	27.58	28.06	22.99	22.63	22.39*	22.41*
1	28.95	28.73	28.28*	27.87*	23.21	22.96	23.24	22.84
2	31.40	30.61*	30.35*	30.00*	23.83	23.63	23.61	24.28
3	31.63	31.23	30.97	30.07*	24.43	24.71	24.35	24.29
4	32.16	31.86	32.26	31.52	25.46	25.42	25.83	25.19
8	35.05	35.33	34.70	33.84*	26.72	26.76	27.42	26.70
12	35.79	36.19	36.12	34.80	28.56	28.32	29.02	27.64*
26	37.70	37.61	36.82	36.02*	32.00	31.84	32.02	30.38*
38	40.61	40.13	40.19	39.02	34.27	33.47	33.56	32.80
50	40.69	40.15	40.10	38.90	35.07	34.13	34.12	32.68
52	41.49	41.14	40.89	39.02*	35.53	34.23	34.29	33.16*
66	40.78	41.70	40.43	38.85	35.98	35.05	35.25	33.12*
78	39.73	40.86	39.33	38.11	36.10	35.37	35.93	34.28
	BODY WEIGHT GAINS - g							
0-52	13.34	12.98	12.31	10.96	12.54	11.60	11.90	10.75
0-78	11.58	12.46	11.75	10.05	13.11	12.74	13.54	11.87

Statistical Significance: $p<0.05$ = *
Data extracted from Report Tables #T-4.2.1, #T-4.2.2, and #T-4.2.3, pages 152-169.

4. Ophthalmoscopic examination - No details about ophthalmoscopic examinations were included in the Materials and Methods section of this study. However, ophthalmic endpoints, such as opaque eyes and hemodacryorrhea, were included in the evaluation of clinical signs. The incidences of ophthalmic endpoints were not significantly increased compared to untreated controls.

Table 6

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PERCENT CHANGE IN MEAN COMPOUND INTAKE FOR MALE AND FEMALE MICE

	Percent Change in Compound Intake[a]							
	1-12 weeks		12-26 weeks		26-52 weeks		52-78 weeks	
Dose (ppm)	M	F	M	F	M	F	M	F
30	-35	-25	-4	-8	-10	-18	+11	+8
100	-38	-35	+5	-1	-17	-15	+18	+1
1000	-33	-35	-1	+4	-16	-14	+20	-2

[a] Calculated as the percent change between the initial and final compound intake for the specified interval.

Data extracted from Report Table #T-4.4.3, page 207.

F. Clinical Pathology

1. Hematology - White blood cell (WBC) counts, red blood cell (RBC) counts, and WBC differential counts were conducted for 15 mice/sex/dose at 12 and 18 months. The CHECKED (x) parameters were examined.

X		X	
x	Hematocrit (HCT)*	-	Total plasma protein (TP)
x	Hemoglobin (HGB)*	x	Leukocyte differential count
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB (MCH)
x	Erythrocyte count (RBC)*	x	Mean corpuscular HGB conc. (MCHC)
x	Platelet count*	x	Mean corpuscular volume (MCV)

* = EPA Guideline §82-5 Requirement "-" = Not examined

Statistically significant changes in parameters were noted at certain time points, but were not consistent throughout the experiment. Significant alterations in the following parameters were observed:

- Males - decreases in RBC (1000 ppm). Females - decreases in RBC (1000 ppm), increases in MCH (100 and 1000 ppm), increases in MCV (1000 ppm) at 12 months.

- Males - no significant changes. Females - increases in MCHC (1000 ppm) at 18 months.

The only hematological parameter that may possibly have been altered by mancozeb administration was an increase in the mean corpuscular hemoglobin concentration (MCHC) in female mice at terminal sacrifice (18 months) (see Table 7). There appeared to a dose-related increase in MCHC.

Table 7

HEMATOLOGICAL PARAMETERS EVALUATED FOR RELATIONSHIP TO MANCOZEB TREATMENT IN MICE IN AN EIGHTEEN-MONTH STUDY

	Males				Females			
	0	30	100	1000	0	30	100	1000
Time	Red Blood Cells - $x10^6/mm^3$							
12 months	8.978	8.696	9.003	8.428*	9.127	8.945	8.930	8.379*
18 months	7.908	8.261	8.331	7.639	8.484	8.683	8.352	8.125
	Mean Corpuscular Hemoglobin - uug							
12 months	16.047	16.167	16.233	16.493	16.000	16.427	16.747*	17.173*
18 months	17.000	16.940	16.473	16.967	16.519	16.480	16.927	17.133
	Mean Corpuscular Volume - u^3							
12 months	45.733	46.667	47.000	46.867	46.400	47.333	47.467	49.267*
18 months	47.500	46.933	46.933	48.400	47.063	46.933	47.467	47.400
	Mean Corpuscular Hemoglobin Concentration - %							
12 months	35.153	34.673	34.627	35.067	34.533	34.787	35.293	34.913
18 months	35.843	36.153	35.053	35.153	35.056	35.207	35.740	36.093*

Statistical Significance: $p<0.05$ = *
Data extracted from Report Tables #T-4.5.1 and #T-4.5.2, pages 209-211.

2. Thyroid Function - Special biochemical assays for serum levels of 3',3,5-triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) were conducted on a minimum of eight animals/sex/group after 12 and 18 months. Blood samples were obtained via orbital bleeding after light ether anesthesia. Statistical significance was indicated when a p-value of 0.05 or less was obtained using Dunnett's t-test.

At 12 months, levels of T4 were significantly decreased in high-dose male mice. At terminal sacrifice (18 months), T4 levels were not

significantly decreased. However, T3 levels were significantly increased in high-dose males. In female mice, levels of T4 were significantly decreased at 12 months, while at terminal sacrifice, levels of both T3 and T4 were significantly decreased. No significant changes in thyroid stimulating hormone (TSH) levels were reported (see Table 8).

Table 8

MEAN THYROID FUNCTION DATA FOR MICE IN AN EIGHTEEN-MONTH ONCOGENICITY STUDY WITH MANCOZEB

	Males				Females			
	0	30	100	1000	0	30	100	1000
Time	T3 levels - ng/dl							
12 months	75.580	70.020	78.263	65.144	66.919	64.869	63.850	68.206
18 months	66.470	58.300	77.000	96.360*	79.700	75.000	78.900	54.780*
	T4 levels - µg/dl							
12 months	3.325	3.375	3.213	1.463*	2.738	2.125	2.156	0.650*
18 months	5.140	4.470	5.100	3.860	4.390	4.480	3.720	1.690*
	Thyroid Stimulating Hormone levels - ng/dl							
12 months	0.700	0.631	0.556	0.563	0.281	0.107	0.150	0.369
18 months	0.486	0.578	0.470	0.617	0.175	0.090	0.113	0.057

Statistical Significance: $p<0.05$ = *
Data extracted from Report Tables #T-4.6.1 and #T-4.6.2, pages 213-214.

G. Sacrifice and Pathology

Necropsies were performed on all unscheduled deaths, on 24 animals/sex/group at the interim sacrifice (12 months) and on all surviving animals at terminal sacrifice. The animals scheduled for necropsy at 12 months and at study termination were randomized via the computer prior to the scheduled necropsies. Reviewer could not find an explanation for the randomization of animals for necropsy at terminal sacrifice. Animals were sacrificed by exsanguination under CO_2 anesthesia prior to scheduled sacrifice. Necropsies were performed by trained personnel using procedures approved by board-certified pathologists and were performed under the direct supervision of a board-certified pathologist. The animals scheduled to be necropsied were examined on a "blind" basis (see Report page 29, 3.0 MATERIALS AND METHODS, 3.6.4 Sacrifice and Gross Pathology).

The CHECKED (x) parameters were examined histopathologically and the (xx) organs were weighed.

x	Digestive System	x	Respiratory	x	Urogenital
x	Salivary glands*	x	Trachea*	xx	Kidneys*
x	Esophagus*	x	Lungs*	x	Urinary bladder*
x	Stomach*			xx	Testes*
x	Duodenum*		Cardiovasc./Hemat.	x	Epididymides
x	Jejunum*	x	Aorta*	x	Prostrate
x	Ileum*	xx	Heart*	x	Seminal vesicle
x	Cecum*	x	Bone Marrow*	x	Ovaries
x	Colon*	x	Lymph Nodes*	x	Uterus*
x	Rectum*	xx	Spleen*		
xx	Liver*	x	Thymus*		
x	Pancreas*				

x	Neurologic	x	Glandular	x	Other
xx	Brain*	xx	Adrenals*a	x	Bone*
x	Peripheral Nerve* (sciatic)	x	Mammary gland*	x	Muscle*
x	Spinal Cord*	xx	Parathyroid*a	x	Skin*
x	Pituitary*	xx	Thyroid*a	x	All gross lesions and masses*
x	Eyes*				

x Vagina
x Gall bladder*
* = EPA Guideline §83-2 Requirement "-" = Not examined
[a] = weights obtained post fixation

1. Macroscopic -

Interim (12 month) Sacrifice: Various gross lesions were observed in male and female mice at the interim sacrifice. However, no incidence of gross lesions was significantly increased in male or female mice compared to untreated control mice. The incidence

of lesions observed did not appear to increase in a dose-related fashion.

Terminal (18 month) Sacrifice: When all animals found dead or sacrificed moribund and all animals sacrificed at 18 months (terminal sacrifice) were considered, a significant increase in unilateral anatomic variation of the eyes (30 ppm) and diffuse discoloration of the lymph nodes (1000 ppm) were observed in male mice, using Fisher's exact test (see Table 9). No significant increase in the incidence of any gross lesion was observed in female mice.

Table 9

GROSS PATHOLOGICAL FINDINGS IN MALE MICE AT TERMINAL SACRIFICE IN AN EIGHTEEN-MONTH MOUSE STUDY WITH MANCOZEB[a]

	0	30	100	1000
Unilateral anatomic variation of the eyes	1/60	8/61*	4/67	4/66
Diffuse discoloration of the lymph nodes	0/60	2/61	1/67	6/66*

[a] Includes all animals found dead/moribund sacrificed.

Statistical significance: $p<0.05$ = *
Data extracted from Report Table #T-4.8.5, pp. 238-244.

2. Organ weights and organ-to-body weight ratios -

At each scheduled sacrifice, selected organs from 14 to 15 animals/sex/group were weighed following careful dissection and trimming to remove fat and other contiguous tissue in a uniform manner. The organ and organ-to-body weights are reported in Table 10.

Interim (12 month) Sacrifice:

Males
30 ppm - no effects
100 ppm - no effects
1000 ppm - no effects

Females
30 ppm - no effects
100 ppm - no effects
1000 ppm - significant increase in relative liver to body weight ratio

Terminal (18 month) Sacrifice:

Males
30 ppm - no effects
100 ppm - significant increase in relative kidney to body weight ratio
1000 ppm - no effects

Females
30 ppm - no effects
100 ppm - no effects
1000 ppm - no effects

3. Microscopic

Non-Neoplastic

Statistically significant increases in the incidence of calculus of the urinary bladder (100 ppm) were observed in male mice (see Table 11). In female mice, statistically significant increases in the incidence of cystic follicles of the thyroid (100 ppm) and lymphocytosis of the urinary bladder (1000 ppm) were observed. However, no dose-related patterns or trends were observed in these responses; therefore, the incidence of these lesions did not appear to be related to mancozeb treatment. The incidences of other microscopic non-neoplastic lesions were not significantly increased compared to untreated controls.

The authors reported that the most consistent non-neoplastic finding in mice of both sexes was systemic amyloidosis. The organs most frequently affected were the adrenal glands, heart, kidneys, liver, mesenteric lymph node, parathyroid gland, salivary glands, spleen, small intestine, stomach and thyroid glands in both sexes. Amyloidosis was noted in all groups of mice, including the controls, and when the incidence of amyloidosis was compared systemically by animals rather than by individual organ, there were no significant increases in treated compared to control animals (males - 31/60, 26/61, 22/67, 18/66, females - 30/62, 32/65, 38/66, 28/60) (see Report page 1877, Appendix #A-4.10.2, Gross and Histopathology Report).

Neoplastic

No statistically significant increase in the incidence of any neoplastic lesion was observed in this study. An extensive analysis of liver and lung tumors (see Table 12 for incidence information) was conducted, adjusting for survival in both male and female mice. The authors concluded that there were no statistically significant differences in tumor incidence between any dose level and control for any of the tumor types observed in males and females (see Appendix #A-4.10.4 of the Report).

Table 10

GROUP MEAN ORGAN WEIGHTS OF MICE ADMINISTERED MANCOZEB FOR EIGHTEEN-MONTHS

	Males				Females			
	0 ppm	30 ppm	100 ppm	1000 ppm	0 ppm	30 ppm	100 ppm	1000 ppm
12 MONTH SACRIFICE								
No. of organs weighed	15	15	15	15	15	15	15	15
Body weight g	40.373	37.860	39.420	37.753	34.407	32.375	31.880	31.653
Brain g	0.581	0.583	0.594	0.581	0.623	0.602	0.610	0.580
rel. to BW g/1000g	14.707	15.482	15.242	15.485	18.261	18.735	19.172	18.547
Adrenals g	0.009	0.010	0.010	0.009	0.014	0.016	0.016	0.014
rel. to BW g/1000g	0.225	0.272	0.251	0.247	0.415	0.510	0.494	0.441
rel. to brain g/g	0.015	0.018	0.017	0.016	0.023	0.027	0.026	0.027
Heart g	0.198	0.199	0.210	0.207	0.184	0.179	0.171	0.185
rel. to BW g/1000g	5.015	5.287	5.330	5.481	5.398	5.560	5.362	5.839
rel. to brain g/g	0.342	0.342	0.354	0.356	0.296	0.299	0.281	0.357
Kidneys g	0.719	0.746	0.741	0.851	0.510	0.496	0.502	0.532
rel. to BW g/1000g	18.163	19.824	18.911	22.878	14.882	15.441	15.748	16.790
rel. to brain g/g	1.235	1.283	1.252	1.480	0.826	0.825	0.828	1.063
Liver g	2.281	2.037	1.997	1.957	1.773	1.623	1.730	1.941
rel. to BW g/1000g	57.180	53.988	50.831	51.613	52.015	50.669	54.115	60.871*
rel. to brain g/g	3.942	3.501	3.370	3.362	2.863	2.705	2.843	3.868
Testes g	0.272	0.286	0.270	0.252	NA	NA	NA	NA
rel. to BW g/1000g	6.914	7.588	6.943	6.701	NA	NA	NA	NA
rel. to brain g/g	0.470	0.490	0.454	0.434	NA	NA	NA	NA
Thyroid/parathyroid g	0.015	0.014[a]	0.014	0.013	0.013	0.013	0.010	0.014
rel. to BW g/1000g	0.393	0.374[a]	0.350	0.359	0.379	0.406	0.328	0.452
rel. to brain g/g	0.026	0.024[a]	0.023	0.023	0.021	0.022	0.017	0.026
18 MONTH SACRIFICE								
No. of organs weighed	14	15	15	15	16	15	15	15
Body weight g	41.393	41.300	36.587*	37.400	36.731	35.187	34.160	35.267
Brain g	0.566	0.559	0.515	0.544[a]	0.561	0.569	0.570	0.560
rel. to BW g/1000g	13.604	13.783	14.241	14.742[a]	15.571	16.499	16.777	15.961
Adrenals g	0.006	0.005[a]	0.005	0.005	0.011	0.010	0.011	0.010
rel. to BW g/1000g	0.134	0.129[a]	0.145	0.135	0.292	0.300	0.312	0.281
rel. to brain g/g	0.010	0.009[a]	0.010	0.009[a]	0.019	0.019	0.019	0.018
Heart g	0.253	0.244	0.253	0.252[a]	0.212	0.213	0.234	0.216
rel. to BW g/1000g	6.182	6.043	6.979	6.847[a]	5.871	6.121	6.901	6.154

	Males				Females			
	0 ppm	30 ppm	100 ppm	1000 ppm	0 ppm	30 ppm	100 ppm	1000 ppm
rel. to brain g/g	0.460	0.441	0.490	0.466[a]	0.381	0.377	0.412	0.388
Kidneys g	0.896	0.907	0.901	0.905[a]	0.654	0.702	0.625	0.661
rel. to BW g/1000g	21.832	22.197	24.550*	24.321[a]	18.035	20.192	18.370	18.793
rel. to brain g/g	1.625	1.633	1.752	1.677[a]	1.180	1.258	1.104	1.183
Liver g	2.817	2.583	2.289	2.347[a]	2.273	2.310	2.157	2.239
rel. to BW g/1000g	68.080	64.500	62.373	63.161[a]	62.596	66.108	62.970	63.596
rel. to brain g/g	5.047	4.673	4.448	4.358[a]	4.122	4.140	3.818	4.029
Testes g	0.242	0.274	0.248[a]	0.254[a]	NA	NA	NA	NA
rel. to BW g/1000g	5.902	6.747	6.853[a]	6.826[a]	NA	NA	NA	NA
rel. to brain g/g	0.440	0.494	0.484[a]	0.473[a]	NA	NA	NA	NA
Thyroid/parathyroid g	0.007	0.007[a]	0.007	0.007	0.007	0.008	0.007	0.008
rel. to BW g/1000g	0.177	0.169[a]	0.200	0.192	0.196	0.226	0.205	0.228
rel. to brain g/g	0.013	0.012[a]	0.014	0.013[a]	0.013	0.014	0.012	0.014

Statistical Significance: $p<0.05$ = *
Data extracted from Report Tables #T-4.7.1 and #T-4.7.2.
[a] N = 14

Table 11

MICROSCOPIC NON-NEOPLASTIC PATHOLOGY IN MALE OR FEMALE MICE EVALUATED FOR RELATIONSHIP WITH MANCOZEB ADMINISTRATION

	0	30	100	1000
MALES				
Calculus of the urinary bladder	3/60	3/18	6/12*	6/65
FEMALES				
Cystic follicles of the thyroid	5/62	12/65	14/66*	1/60
Lymphocytosis of the urinary bladder	20/61	3/13	3/11	32/60*

Statistical Significance: $p<0.05$ = *
Data extracted from Appendix A#-4.10.2, Table 3, pp. 1897-1911.

Table 12

INCIDENCES OF LIVER AND LUNG NEOPLASMS
IN MICE ADMINISTERED MANCOZEB

	Males				Females			
	0	30	100	1000	0	30	100	1000
LIVER								
adenoma, hepatocellular	4/60	8/61	11/67	3/66	1/61	2/65	1/66	3/60
adenoma, hepatocellular, multiple	0/60	0/61	0/67	4/66	0/61	0/65	0/66	1/60
carcinoma, hepatocellular	2/60	0/61	2/67	1/66	0/61	0/65	0/66	0/60
hemangioma	1/60	1/61	0/67	0/66	3/61	0/65	0/66	0/60
hemangiosarcoma	1/60	0/61	0/67	1/66	0/61	0/65	0/66	0/60
LUNG								
adenoma, AB-cell	4/60	11/61	6/67	10/66	4/61	3/65	11/66	9/60
adenoma, AB-cell, multiple	0/60	0/61	0/67	1/66	0/61	0/65	0/66	0/60
carcinoma, AB-cell	4/60	2/61	0/67	5/66	3/61	2/65	1/66	2/60

Statistical Significance: $p<0.05$ = *
Data extracted from Appendix #A-4.10.2, Table #T-4.9.12, page 329.

The Reviewer has the following comment regarding the Materials, Methods, and Results:

This study is to be considered Core Minimum.

A brief description of the statistical analyses employed was included in the report.

A Good Laboratory Practice Compliance Statement, a Quality Assurance Statement, and a list of Quality Assurance inspections were included.

II. DISCUSSION

Body weight, feed consumption, hematological parameters, thyroid assays, and organ weights were analyzed by one-way analysis of variance (ANOVA). When this analysis suggested group differences, the Dunnett's t-test was used at a 95% confidence level. The survival distributions were compared using SAS PROC LIFETEST. The Wilcoxon test and the log rank test were used to assess differences in survival times. Statistical analyses of lesions were conducted using the Fisher's exact test to assess the significance of pairwise comparisons between the control and each of the dose groups. In all analyses, statistical significance was indicated when a p-value of 0.05 or less was obtained.

Stability problems of mancozeb in the diet were reported for weeks 50-80 of this study. Twenty-eight to forty-sex percent of the mancozeb in the diet was converted to ethylene thiourea. The authors reported that although feed samples contained less mancozeb active ingredient, the levels of ETU were elevated from weeks 50-80. Due to the toxic nature of ETU, the authors stated that these changes in test material would have resulted in expression of more toxic effects than if the purity of test material had remained constant. Therefore, the authors believe that any reduction in stability resulted in a conservative estimate of the no effect level for mancozeb.

If the potential carcinogenicity/toxicity of mancozeb is solely associated with the in situ metabolism of mancozeb to ETU, with ETU as the putative carcinogen, then the authors' statements may be valid. It should be noted that the doses of ETU that would be expected from degradation of mancozeb in the diet would be much lower than those doses at which carcinogenicity has been observed. In a recent NTP (1989) bioassay of ETU, exposure of mice to ETU resulted in carcinogenic effects in the thyroid gland and the liver. Significant increases in the incidence of follicular cell carcinomas were observed in male mice following exposure to 1000 ppm in the diet and in female mice following exposure to 330 and 1000 ppm in the diet for two years. Hepatocellular carcinomas were also significantly increased in male and female mice following exposure to 330 and 1000 ppm ETU in the diet for two years. In this study, at the highest dose tested for mancozeb (1000 ppm), with 19% degradation of mancozeb and 28% of that degradation attributed to ETU conversion, the maximum exposure to ETU in the diet would be approximately 50 ppm, much lower than the doses at which carcinogenicity was observed in the NTP (1989) bioassay.

In analyzing the survival for male mice, the authors reported that both the Wilcoxon test ($p=0.0423$) and the log rank test ($p=0.0470$) were statistically significant, indicating that there was differential survival among the four dosed groups. A further examination of the data was then made by comparing each of the non-zero dose groups to control using a Dunnett's approximation for the significance level of each of the three possible tests. These subsequent tests indicated that the statistically significant finding was dominated by the high survival rate in the 100 ppm dose group. There were no statistically significant differences in survival among the four dose groups in females (see Report page 2967 and 2968, Appendix #A-4.10.4 Statistical analysis of survival). Therefore, survival did not appear to be affected by administration of mancozeb in the diet to mice. The increased survival in the 100 ppm group was not explained.

No dose-related increases in the incidence of any clinical sign were observed in male or female mice following exposure to mancozeb. No significant changes in body weight, body weight gain, or food consumption were reported by the authors to be related to mancozeb administration. However, at terminal sacrifice, body weight gain was decreased approximately 13% in male mice and approximately 9% in female mice.

Although effects on thyroid function were observed in this study, the effects were conflicting with an increase in T3 levels in male mice and decreases in T3 and T4 levels in female mice. No statistically significant effects on TSH were observed. Following exposure to ETU, which may be the carcinogenic metabolite of mancozeb, male and female mice exhibited decreased levels of T3 and T4 and increased levels of TSH (NTP, 1989). Therefore, the effects observed on thyroid function concerning T3 levels in male mice and TSH levels in males and females appear to be different from those observed following exposure to ETU; however, this may be a dose-related effect. Since the T3 levels in males were decreased at 12 months, the significant increase by 18 months is unexpected.

The only hematological parameter that may have been altered by mancozeb administration was increases in mean corpuscular hemoglobin concentration (MCHC) in female mice at 18 months (see Section F). There was a dose-related increase in MCHC. Significant decreases in RBC were observed in both male and female mice in the high-dose group at 12 months. Significant increases in mean corpuscular hemoglobin (100 and 1000 ppm) and mean corpuscular volume (1000 ppm) were also observed in female mice at 12 months. However, these significant differences were not observed in animals at terminal sacrifice (18 months).

There were no macroscopic observations that were attributed to mancozeb administration. Increases in the incidences of two endpoints in males (unilateral anatomic variation of the eyes and diffuse discoloration of the lymph nodes) were observed, but these lesions did not appear to be treatment-related. No dose-response patterns were observed for these endpoints.

The microscopic evaluation indicated that the only non-neoplastic responses that were significantly different from controls were increases in calculus of the urinary bladder (100 ppm) in male mice and cystic follicles of the thyroid (100 ppm) and lymphocytosis of the urinary bladder (1000 ppm) in female mice. However, no dose-related patterns were observed in these responses and the increased incidences in the 100 ppm groups may have been a function of the increased survival in these groups; therefore, the incidence of these lesions did not appear to be related to mancozeb treatment.

The Office of Pesticide Programs believes that an MTD can be based on a body weight decrement, and the body weight decrement should reach 10-15%. At terminal sacrifice, a decrease in body weight gain of approximately 13% was observed in males and a decrease of approximately 9% in females. Therefore, the decrease in body weight in males would indicate that an MTD had been achieved. Alterations in thyroid function in male and female mice, and increases in relative liver-to-body weight in females were also noted at the 1000 ppm treatment level.

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REFERENCE

National Toxicology Program (NTP). 1989. Technical Report on the Perinatal Toxicity and Carcinogenicity Studies of Ethylene Thiourea (CAS NO. 96-45-7) in F/344 Rats and B6C3F1 Mice (Feed Studies). NTP TR 388. NIH Publication 90-2843. Peer Review Date November 20, 1989.

III. CONCLUSIONS

Mancozeb administered in the diet to CD-1 mice for eighteen months at concentrations of 0, 30, 100, and 1000 ppm appeared to have the following effects:

1000 ppm - Effects on thyroid function, decreases in T3 and T4 levels in female mice, increases in T3 levels in male mice. 13% decrease in body weight in male mice, increase in relative liver-to-body weight ratio in female mice

100 ppm - No effects

30 ppm - No effects

The Maximum Tolerated Dose (MTD) = 1000 ppm, based on a 13% decrease in body weight gain in high-dose male mice (a 9% decrease was observed in female mice).

Mancozeb did not appear to definitively cause a statistically significant dose-related increase in the incidence of any tumors over control values. Incidences of various non-neoplastic endpoints were found to be significantly increased, including calculus of the urinary bladder (100 ppm) in male mice and cystic follicles of the thyroid (100 ppm) and lymphocytosis of the urinary bladder (1000 ppm) in female mice. However, no dose-related patterns or trends were observed in these responses. Changes in thyroid function were observed. In male mice, triiodothyronine (T3) levels were significantly increased in the high-dose group. In female mice, levels of thyroxine (T4) and T3 were significantly decreased. No significant changes in thyroid stimulating hormone (TSH) levels was reported.

This study is classified Core Minimum.