



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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUL 20 1986

MEMORANDUM

SUBJECT: Mancozeb (NRDC) - Evaluation of Data Submitted
Under Accession Nos. 261535, 261536, 261537, 261538,
261539

Caswell No. 913A

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Arvella Farmer, PM 61
Special Review Branch
Registration Division (TS-767C)

THRU: Jane E. Harris, Ph.D., Head *Mancozeb* *For JEH*
Section VI, Toxicology Branch *7/17/86*
Hazard Evaluation Division (TS-769C)

Registrant: Rohm & Haas Company

Action Requested:

Review and evaluate the following studies submitted in
response to Data Call-In (DCI):

1. Mancozeb: Hazard Identification, Evaluation, and
Extrapolation to Humans, P.K. Chan, November 7, 1985
(EPA Accession No. 261535).
2. Mancozeb: Three-Month Dietary Toxicity Study in
Rats, P.R. Goldman, H.J. Bernacki, and D.L. Quinn,
Report Number 35R-167, February 27, 1986 (EPA Accession
No. 261536).

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3. Three-Month Dietary Toxicity Study with Mancozeb in Dogs, R.H. Cox, Report Number 86RC-7, February 26, 1986 (EPA Accession No. 261537).
4. Mancozeb: Two-Week Inhalation Toxicity Study in Rats, R.C. Baldwin, J.V. Hagan, and J.R. Fisher, Report Number 85R-190, February 27, 1986 (EPA Accession No. 261538).
5. Mancozeb: Subchronic Inhalation Toxicity Study in Rats/13-Week Interim Report, R.C. Baldwin, J.V. Hagan, and J.R. Fisher, Report Number 86R-0003, February 27, 1986 and Mancozeb/ETU: Rat Inhalation Study - Exposure Phase: Analysis of Urine, Blood, and Thyroids, Report Number ARM-477-86, February 27, 1986 (EPA Accession No. 261539).

Toxicology Branch (TB) Conclusions

These data have been screened for adequacy to satisfy data requirements for the Mancozeb Registration Standard (Memorandum: Mauer to Farmer, March 31, 1986). That preliminary screening concluded that these studies represent those necessary for satisfying data requirements for subchronic testing in rat and dog by the dietary route, and in the rat by the inhalation route.

As detailed in the Data Evaluation Records (DER's) and summarized below, compound-related effects on the thyroid were thoroughly investigated, including residue analysis of both the parent compound (none detected as CS₂ in any EBDC-treated group), and its principal active derivative, ethylene thiourea (dose-related increase of 4 ppm ETU in 30-ppm mancozeb animals to approximately 25 ppm ETU in 1000-ppm animals).

The following summarizes TB's reviews and evaluation of these studies (detailed DER's are attached):

Study (1) is a company-prepared review (with no primary data of Mancozeb and ETU, containing background toxicological information, hazard identification and evaluation, and extrapolations to humans; appendices on other degradation products and contaminants (EBIS, EU); pharmacokinetics, exposure estimates and thyroid dysfunction/tumorigenesis interrelationship.

TB Conclusions: Accepted as submitted for information only (No DER prepared).

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Study (2): Subchronic dietary - rat

Doses fed: 0, 30, 60, 125, 250, and 1000 ppm Mancozeb;
250 ppm ETU.

Reported effects: None for mancozeb at or below 125 ppm; slight change in hormone levels in females fed 250 ppm; depressed body weight and histological changes in thyroid at 1000 ppm.

TB Conclusions: Core-Minimum Data

NOEL (syst) = 60 ppm (equivalent to 3.5/4.4 mg/kg/da, males/females, respectively)

LEL (syst) = 125 ppm (7.4/9.2 mg/kg/da, males/females, respectively), based on renal tubular degeneration in males.

NOEL/LEL for thyroid effects = 125/250 ppm, based on decreases in serum T4 and TSH in females.

Study (3): Subchronic dietary - dog

Doses fed: 0, 10, 100, 1000, and 5000 ppm

Reported effects: None at or below 100 ppm; depressed feed consumption and body weight at 1000 ppm and higher; reduced survival and thyroid effects at 5000 ppm.

TB Conclusions: Core-Minimum Data

NOEL (syst) = 100 ppm (3 mg/kg/da)

LEL (syst) = 1000 ppm (29 mg/kg/da), based on decreased food consumption and body weight gains; cortical lymphoid depletion in thymus; prostatic hypogenesis.

NOEL/LEL for thyroid effects = 1000/5000 ppm (= 102 and 109 mg/kg/day in males and females, respectively), the LEL producing follicular cell hyperplasia, decreased T3 and T4; hypercholesterolemia and hyperbilirubinemia, decreased food consumption and body weight.

Study (4): Subacute inhalation - rat

(To select doses and compare effects of whole body exposure to those of nose-only for the 13-week inhalation study, 5).

Doses tested: 0, 11, 55, and 258 mg/m³ (respirable conc.)

Reported effects: For whole-body exposure, the "maximum" NOEL was 11 mg/m³, and LOEL was 55 mg/m³ (lower body weight gain and T3 levels in males, lower T4 levels in both sexes). For nose-only exposure, NOEL was 55 mg/m³ and LOEL was 258 mg/m³ (lower male body weight gains and T2/T4 levels; histopathological changes in respiratory tract of both sexes).

TB Conclusions: Core-Supplementary Data (range-finding)

NOEL (whole body) = 11 mg/m³ (respirable)
LEL (whole body) = 55 mg/m³ (respirable), based on decreased T4 in males and females; decreased T3 in males.

NOEL (nose only) = 55 mg/m³ (respirable)
LEL (nose only) = 258 mg/m³ (respirable), based on decreased body weight in males; decreased T4 and T3 in males.

(Nose-only exposure selected for Study 5.)

Study (5): Subchronic inhalation - rat

Doses tested (nose-only): 0, 20, 80, and 320 mg/m³
(nominal)
0, 8, 36, and 144 mg/m³
(respirable nose-only aerosol concentration).

Interim sacrifice: One-half of each group at 13 weeks.

Reported effects: None at or below 80 mg/m³ (nominal); body weight changes and thyroid effects at the HDT, 320 mg/m³ (nominal).

Terminal sacrifice scheduled following a 13-week period of nontreatment in order to study reversibility with no mancozeb exposure. Results of this recovery phase not yet submitted.

TB Conclusions (for 13-week interim data only):
Core-Supplementary Data, pending review of the final report including recovery phase data.

NOEL = 8 mg/m³ (respirable)
LEL = 36 mg/m³ (respirable), based on yellow-brown granular pigment in renal tubules of males and females.

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NOEL/LEL for thyroid effects = 36/144 mg/m³
(respirable), based on decreased T4 in females,
accompanied by follicular epithelial hyperplasia.

cc: Judy Hauswirth
Susan Lewis
Joan Warshawsky
Henry Jacoby

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION [EO 12065]

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EPA: 68-02-4225
DYNAMAC No. 1-0098
May 23, 1986

DATA EVALUATION RECORD

MANCOZEB/ETHYLENETHIOUREA

Three-Month Subchronic Toxicity Study in Rats

STUDY IDENTIFICATION: Goldman, P. R., Bernacki, H. J., and Quinn, D. L.
Mancozeb: three-month dietary toxicity study in rats. (Unpublished report
No. 85R-167, protocol No. 85P-134, prepared and submitted by the
Toxicology Department, Rohm and Haas Company, Springhouse, PA; dated
February 27, 1986.) Accession No. 261536.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
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Signature: I. Cecil Felkner

Date: 5-27-86

1. CHEMICAL: Mancozeb; Dithane M 45; ethylenethiourea.
2. TEST MATERIALS: Mancozeb technical, lot No. 43339, TD No. 85-15, was described as a yellow powder containing 84 percent of the active ingredients, a coordination product of zinc ion and manganese ethylenebisdiisocarbamate. Ethylenethiourea, lot Matheson Coleman and Bell: product No. IX0010, TD No. 85-55, was described as a white crystal containing 99.8 percent active ingredient.
3. EXPOSURE ACTION TYPE: Three-month subchronic feeding study in rats.
4. STUDY IDENTIFICATION: Goldman, P. R., Bernacki, H. J., and Quinn, D. L. Mancozeb: three-month dietary toxicity study in rats. (Unpublished report No. 85R-167, protocol No. 85P-134 prepared and submitted by the Toxicology Department, Rohm and Haas Company, Springhouse, PA; dated February 27, 1986.) Accession No. 261536.

5. REVIEWED BY:

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6. APPROVED BY:

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7. CONCLUSIONS:

- A. Under the conditions of this study, mancozeb and its metabolite, ethylenethiourea (ETU), were toxic when fed to rats at the highest dose levels of 1000 and 250 ppm, respectively, for 13 weeks. Males and females in both test groups showed decreased body weights and food consumption, decreased MFO activity, decreased serum T4 (thyroxine) and increased TSH (thyroid stimulating hormone) levels, increased absolute and relative liver and thyroid weights, and follicular epithelial hyperplasia of the thyroid. High-dose mancozeb females showed increased absolute and relative spleen weights.

Diffused hypertrophy and follicular epithelial hyperplasia of the thyroid were observed in males and females dosed with 1000 ppm mancozeb or 250 ppm ETU. In addition, one ETU treated male had a follicular adenoma and multicentric lymphosarcoma of the thyroid. Other major histopathological changes were mostly restricted to the males in the high-dose groups of both test compounds and included centrilobular hepatocellular hypertrophy, and increased amounts of hypertrophied cells in the anterior lobe of the pituitary and in the zona glomerulosa of the adrenal cortex.

The LOEL for systemic subchronic toxicity of mancozeb was 125 ppm, based on histopathologic changes in the kidneys of males and females. No compound-related effects were observed at doses up to and including 60 ppm mancozeb; therefore, the systemic NOEL for mancozeb was 60 ppm (3.5 mg/kg/day in males and 4.4 mg/kg/day in females).

- B. Core Classification: Core Minimum.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):A. Materials and Methods:

- i. The test compound, mancozeb, lot No. 43339, was described as a yellow powder nominally containing 84 percent of the active ingredient, a coordination product of zinc cation and manganese ethylenebisthiocarbamate (Dithane M-45). The second test compound, ethylenethiourea (ETU), (lot Matheson Coleman and Bell, IX0010), was described as a white crystalline solid containing 99.8 percent active ingredient. Individual test diets were prepared each week by homogenizing the test compounds with the basal diet, Purina Certified Rodent Chow #5002-meal (Ralston Purina Co.). The percent of

¹ Only items appropriate to this DER have been included.

active ingredient and homogeneity of mancozeb and ETU were determined in the first batch of diets, and each time the concentrations of these compounds were changed in the test diets. Samples from each test diet were collected at the end of weeks 1, 3, 5, 8, and 12 to determine the stability of the test compounds during the feeding week. Mancozeb and ETU were stored at room temperature.

2. Approximately 4-week-old Crl-CD(SD) rats (Charles River Breeding Laboratories) were acclimated to the animal facility for 2 weeks and randomized according to body weight into a control, five mancozeb, and one ETU dose groups of 14 rats/sex/dietary group.

Animals were caged individually in an environmentally controlled room. In order to maintain approximately the same level of compound intake (mg/kg/day) throughout the feeding period, dose levels in the diets were gradually increased. During the first 2 weeks of study, rats were fed their group diets containing only 50 percent of the final dose levels of the test compounds (15, 30, 62.5, 125, or 500 ppm of mancozeb, and 125 ppm of ETU). The respective dose levels were increased to 70 percent of the final concentrations (21, 42, 87.5, 175, or 700 ppm of mancozeb, and 175 ppm of ETU) during weeks 3-4 of feeding. Between weeks 5-13, rats were fed at the final dose levels of 30, 60, 125, 250, or 1000 ppm for mancozeb and 250 ppm for ETU.

3. Body weights and food consumption were determined 1 week prior to the initiation of treatment, and weekly thereafter. Compound intakes were calculated weekly.
4. Animals were examined daily for signs of toxicity, external lesions, behavior, posture, gait, irregularities in respiration, body temperature, and color and consistency of excreta. Individual ophthalmoscopic examinations were conducted prior to initiation of study and during the last week of treatment.
5. Hematologic (9 tests) and clinical chemistry (16 tests) determinations were made after 13 weeks (day 92) on blood collected from 10 animals/sex/group that had been fasted overnight. Serum samples prepared from the same blood lots were used for assessment of thyroid function by determining thyroxine (tetraiodothyronine, T4), triiodothyronine (T3), and thyroid stimulating hormone (TSH) levels.
6. Representative liver sections were randomly selected from six rats/sex/group (animals bled for laboratory determinations) and processed into microsomal suspensions to determine mixed function oxidase (MFO) activity using the aniline hydroxylation (AH) and aminopyrine (AP)N-demethylation methods.

7. Prior to necropsy, 24-hour urine samples were collected from four rats/sex/group; blood, thyroids, and liver samples were collected at necropsy. Only blood, urine, and thyroid samples were analyzed for ethylenebisdithiocarbamate (EBDC) and ETU residues. Due to the small sample size, thyroids from two 1000-ppm male and female rats were used for EBDC residue analysis. Thyroids from the remaining 1000-ppm males and females were analyzed for ETU residues. Thyroids from other mancozeb groups were analyzed only for ETU residues. All thyroids from ETU rats were analyzed for ETU residue only.
 8. All survivors were necropsied after 13 weeks of treatment. All organs, tissues, and body cavities were examined for gross abnormalities. The adrenals, brain, gonads, heart, kidneys, liver, spleen, and thyroid/parathyroid were removed and weighed. Approximately 40 organ/tissues were examined microscopically.
 9. Analysis of variance was used to assess the significance of intergroup differences for clinical chemistry and hematologic parameters, and organ weights. Analysis of covariance was used to assess body weight and food consumption data. The parameters for thyroid function were assessed using analysis of variance, followed by Dunnett's t-test.
- B. Protocol: A protocol was provided in the study report and is presented in Appendix A of this review.

12. REPORTED RESULTS:

- A. Test Compound and Dietary Analysis: Technical grade mancozeb (Dithane) and ETU used in this study actually contained 88.6 and 97.8 percent of the active ingredients, respectively.

The test compounds in the diets stored at room temperature remained stable over a 7-day feeding period, averaging 95 and 91 percent of the nominal values for mancozeb and ETU, respectively. Homogeneity values for the two compounds averaged 100 and 92-98 percent of the nominal values. Percent conversion of mancozeb to ETU in the fresh and stored feed samples averaged 1.9 ± 0.8 and 6.3 ± 0.9 , respectively.

The mean intake of mancozeb and ETU over 13 weeks of dosing was calculated as follows (table on p 11 of the CBi report):

Group	Mancozeb Dose (ppm)	Compound Intake (mg/kg/day)	
		Males	Females
2	30	1.78±0.19	2.20± 0.29
3	60	3.49±0.39	4.38± 0.56
4	125	7.42±0.79	9.21± 1.22
5	250	14.98±1.78	17.82± 2.18
6	1000	57.34±6.04	74.64±11.27
7 (ETU)	250	14.28±1.37	17.81± 2.24

- B. Clinical Observations and Mortality: Clinical signs frequently observed in all treatment groups included black crusty material around the eyes, and alopecia. Reportedly, these conditions were of common occurrence in the laboratory rat. Individual ophthalmoscopic examinations revealed no compound-related ocular pathology.

One 125-ppm mancozeb male died during the first week of study. The cause of death was not apparent; however, it was not considered to be compound related.

- C. Body Weights: No compound-related changes in body weights of animals fed up to and including 250 ppm mancozeb were observed. The mean body weight of males receiving 1000 ppm mancozeb was significantly lower ($p \leq 0.05$) than controls (3-8 percent) between weeks 3-13 (Table 1). ETU males showed a significant decrease in body weight (3-7 percent, $p \leq 0.05$) at study weeks 2-13. Females in the 1000-ppm mancozeb group showed a decrease (3-14 percent) in mean body weight between weeks 2-13; however, differences were significant ($p \leq 0.05$) only between weeks 7-10. The decrease in mean body weight of ETU females ranged between 6-8 percent during the 13-week period; however, the change was statistically significant only at week 2.
- D. Food Consumption: No compound-related changes in food consumption were found in groups fed up to and including 250 ppm mancozeb. In general, food consumption in 1000-ppm mancozeb males was significantly depressed (8-15 percent, $p \leq 0.05$) between study weeks 3-13 (Table 2). Food consumption in 1000-ppm mancozeb females was only slightly decreased (1-6 percent) during this period. A significant ($p \leq 0.05$) decrease (8-12 percent) in food consumption in ETU males was observed at study weeks 3-13. ETU females showed a moderate decrease in food consumption (6-10 percent); the change was significant ($p \leq 0.05$) at weeks 1, 3, and 6. At four instances during the study, significant increases (7-9 percent, $p \leq 0.05$) in food consumption were observed at Mancozeb levels of 30-250 ppm. Reportedly, such sporadic increases were not compound related.

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TABLE 1. Summary of Mean Body Weight (g $\pm$ SD) in Rats fed Mancozeb^{a,b} or ETU^b

Test Compound (ppm)	Week								
	0	2	3	4	5	8	10	12	13
<u>MALES</u>									
Control	140.9 $\pm$ 10.1	245.5 $\pm$ 19.2	297.5 $\pm$ 24.0	338.8 $\pm$ 29.0	394.5 $\pm$ 36.4	429.1 $\pm$ 41.5	460.8 $\pm$ 47.9	481.8 $\pm$ 51.5	487.1 $\pm$ 58.2
Mancozeb (1000)	141.7 $\pm$ 9.4	242.5 $\pm$ 18.6	288.7* $\pm$ 22.3	318.3* $\pm$ 32.9	364.8* $\pm$ 49.3	396.8* $\pm$ 47.5	429.2* $\pm$ 49.5	446.2* $\pm$ 49.6	446.7* $\pm$ 55.0
ETU (250)	140.9 $\pm$ 9.7	237.6* $\pm$ 20.7	287.1* $\pm$ 26.5	321.6* $\pm$ 30.5	372.8* $\pm$ 40.5	405.0* $\pm$ 42.9	433.7* $\pm$ 50.5	455.5* $\pm$ 54.6	464.8 $\pm$ 57.8
<u>FEMALES</u>									
Control	113.6 $\pm$ 5.9	164.0 $\pm$ 14.2	185.4 $\pm$ 18.0	203.2 $\pm$ 21.8	231.6 $\pm$ 26.0	247.8 $\pm$ 29.5	262.2 $\pm$ 29.8	272.8 $\pm$ 32.5	278.4 $\pm$ 34.6
Mancozeb (1000)	114.5 $\pm$ 5.5	159.9 $\pm$ 12.3	181.1 $\pm$ 16.1	196.8 $\pm$ 20.3	213.6 $\pm$ 23.0	213.4 $\pm$ 25.2	239.3* $\pm$ 25.0	251.6 $\pm$ 24.1	258.4 $\pm$ 24.9
ETU (250)	112.1 $\pm$ 5.7	153.7* $\pm$ 9.9	172.4 $\pm$ 13.1	190.6 $\pm$ 13.0	215.4 $\pm$ 18.1	228.6 $\pm$ 19.5	241.3 $\pm$ 20.2	249.8 $\pm$ 19.9	257.1 $\pm$ 20.3

Significantly different from control value ($p \leq 0.05$).

Only values from the highest dose groups shown; mean body weights from all lower dose groups were comparable to controls.

Mean values based on 14 rats/sex/group.

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TABLE 2. Summary of Mean Food Consumption (g±SD) in Rats Fed Mancozeb^{a,b} or ETU^b

Test Compound (ppm)	Week								
	0	2	3	4	6	8	10	12	14
<u>MALES</u>									
Control	17.51 ±1.30	23.40 ±2.21	26.62 ±2.59	27.14 ±2.44	27.04 ±2.50	26.36 ±2.28	25.87 ±2.85	26.25 ±3.22	26.33 ±3.92
Mancozeb (1000)	17.58 ±1.54	23.32 ±2.24	24.61* ±2.16	24.23* ±3.77	24.02* ±3.79	22.50* ±5.80	24.23 ±3.06	23.51* ±2.98	23.36 ±4.21
ETU (250)	18.76 ±1.96	23.58 ±2.09	24.62* ±2.65	25.08* ±2.46	23.85* ±2.78	23.27* ±2.39	23.45* ±3.26	24.27* ±1.94	24.34 ±3.50
<u>FEMALES</u>									
Control	15.59 ±1.30	17.54 ±1.63	18.39 ±1.89	18.98 ±2.13	19.50 ±1.95	19.03 ±1.95	19.13 ±1.80	19.25 ±1.73	19.50 ±2.38
Mancozeb (1000)	16.18 ±1.36	17.21 ±1.89	17.57 ±2.01	18.49 ±2.27	18.39 ±1.88	19.51 ±3.76	18.30 ±2.26	18.43 ±1.57	19.23 ±1.89
ETU (250)	15.30 ±1.10	16.51 ±1.26	16.62* ±1.53	17.81 ±1.51	17.47* ±1.54	17.55 ±1.51	17.31 ±1.27	18.23 ±1.35	18.11 ±1.36

*Significantly different from control value ($p \leq 0.05$).^aOnly values from the highest dose groups shown; mean food consumption from all dose groups were comparable to controls.^bMean values based on 14 rats/sex/group.

Intake of mancozeb and ETU (mg/kg/day) was calculated from the nominal dose levels, mean body weights, and food consumption. In general, compound intake in females, especially at the highest dose level, was higher than in males. Compound intake at all dose levels in both sexes was increased at week 5 and leveled off to a fairly consistent value by week 13.

- E. Hematology: Hematological parameters exhibiting significant ($p \leq 0.05$) differences included increased (3 percent) mean corpuscular hemoglobin concentration (MCHC) in 125-ppm mancozeb males, decreased (19 percent) platelets in ETU males, increased white blood cells (WBC) in 60- and 125-ppm mancozeb males (42-43 percent), and decreased monocytes in 1000-ppm mancozeb (82 percent) and ETU (95 percent) males. Reportedly, all of these differences were considered random and not compound related. No significant changes were observed in females.
- F. Clinical Chemistry: ^{There were} ~~Significant compound-related~~ changes in clinical chemistry parameters ~~were restricted~~ to the high-dose groups. Males receiving 1000 ppm mancozeb showed significant ($p \leq 0.05$) increases in blood urea nitrogen (84 percent), creatinine (28 percent), and cholesterol (52 percent). Females in the same dose group showed increased serum alkaline phosphatase (32 percent) and triglyceride (90 percent) levels compared to controls. However, each of these increases resulted primarily from exceptionally high values obtained for male No. 3527 and female No. 3858; therefore, group increases were not considered compound related. Serum cholesterol levels were significantly increased in ETU males (69 percent) and females (30 percent). All statistically significant changes in clinical chemistry parameters in low- and mid-dose mancozeb females were sporadic, and not considered compound related.
- G. Thyroid Function: Serum T4 levels were significantly decreased ($p \leq 0.05$) in 1000-ppm mancozeb males (34 percent) and 250- (28 percent) and 1000-ppm (43 percent) mancozeb females (Table 3). Serum TSH levels were significantly increased in males (26 percent, $p \leq 0.05$) and females (169 percent) receiving 1000 ppm mancozeb. In ETU rats, serum T4 levels were significantly decreased ($p \leq 0.05$) in males (50 percent) and females (65 percent). T3 levels were significantly increased ($p \leq 0.05$) in males (28 percent) and females (16 percent), and TSH levels were significantly increased (408 percent in males, 263 percent in females).
- H. Hepatic Mixed Function Oxidase: Mancozeb at the highest dose level nonsignificantly decreased the MFO activity in males (31 percent) and females (40 percent) when measured by aniline hydroxylation. MFO activity was significantly reduced by 32 percent ($p \leq 0.05$) in ETU males when measured by aminopyrine N-demethylation. A decrease in MFO activity due to mancozeb or ETU treatment was evident irrespective of the basis (per mg microsomal protein, per g liver, or per total liver) for activity determination.

TABLE 3. Mean Serum Levels ($\pm$ SD) of Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH) in Rats Fed Mancozeb or ETU for 13 Weeks^a

Test Compound (ppm)	Males			Females		
	T3 (ng/mL)	T4 (μ g/dL)	TSH (ng/mL)	T3 (ng/mL)	T4 (μ g/dL)	TSH (ng/L)
Control	1.22 $\pm$ 0.17	5.27 $\pm$ 0.98	1.20 $\pm$ 0.58	1.40 $\pm$ 0.17	3.78 $\pm$ 1.08	0.49 $\pm$ 0.23
Mancozeb (30)	1.19 $\pm$ 0.16	5.32 $\pm$ 0.65	1.13 $\pm$ 0.48	1.44 $\pm$ 0.16	3.39 $\pm$ 0.49	0.68 $\pm$ 0.34
Mancozeb (60)	1.16 $\pm$ 0.21	5.35 $\pm$ 1.21	1.75 $\pm$ 1.50	1.42 $\pm$ 0.16	3.55 $\pm$ 0.62	0.66 $\pm$ 0.37
Mancozeb (125)	1.30 $\pm$ 0.15	5.65 $\pm$ 0.85	1.58 $\pm$ 0.94	1.35 $\pm$ 0.13	3.20 $\pm$ 0.41	0.39 $\pm$ 0.34
Mancozeb (250)	1.28 $\pm$ 0.14	5.28 $\pm$ 0.80	1.88 $\pm$ 1.21	1.37 $\pm$ 0.15	2.71* $\pm$ 0.40	0.95 $\pm$ 0.70
Mancozeb (1000)	1.31 $\pm$ 0.19	3.49* $\pm$ 1.05	4.33* $\pm$ 2.53	1.35 $\pm$ 0.19	2.16* $\pm$ 0.62	1.32* $\pm$ 1.02
ETU (250)	1.56* $\pm$ 0.26	2.62* $\pm$ 0.72	6.10* $\pm$ 3.18	1.63* $\pm$ 0.30	1.34* $\pm$ 0.47	1.78* $\pm$ 1.14

*Significantly different from control value ($p \leq 0.05$).

^a Means based on serum samples prepared from 10 animals/sex/group, except for TSH in female control group, where only nine determinations were made.

- I. Residue Analysis: Urine, blood, and thyroid samples were analyzed for presence of EBDC and ETU. No EBDC or ETU residues were detected in the blood samples drawn from the mancozeb-treated animals. The amount of ETU in urine samples from mancozeb-treated animals increased in a dose-related manner from approximately 0.3 ppm at 30-ppm mancozeb to 10 ppm at the 1000-ppm mancozeb dietary concentration. The urine samples from rats fed 125 to 1000-ppm mancozeb also contained 0.10 to 1.1 ppm of EBDC residues. The average total amount of ETU excreted in the urine in 24 hours is summarized in Table 4.

No EBDC residues above the detection limit of 25 ppm were detected in thyroids obtained from the 1000-ppm mancozeb rats. Analysis of thyroid samples of mancozeb-treated animals showed a dose-related increase in ETU residue; values ranged from less than the detection limit of 4 ppm in 30-ppm mancozeb animals to approximately 25 ppm in the 1000-ppm animals.

ETU levels in the blood of ETU-treated animals were found to be marginally above the detection limit of 0.1 ppm; levels ranged from 2.9 to 63 ppm in the urine and from 30 to 53 ppm in the thyroids.

- J. Organ Weights: Compound-related changes in organ weights were restricted to the highest dose groups. Relative liver weights were significantly increased ($p \leq 0.05$) in 1000-ppm mancozeb males (11 percent) and females (24 percent) and ETU males (12 percent) and females (15 percent) (Table 5). Mean absolute and relative weights of thyroids were significantly ($p \leq 0.05$) increased in mancozeb (32 and 49 percent) and ETU males (80 and 84 percent); in mancozeb and ETU females relative thyroid weights (33 and 30 percent, respectively) were significantly ($p \leq 0.05$) increased. Relative spleen weights were also significantly ($p \leq 0.05$) increased in 1000-ppm mancozeb females (22 percent) and ETU females (16 percent).

Because of increased body weights, absolute liver, heart, and kidney weights in 60-ppm mancozeb males were significantly ($p \leq 0.05$) increased. In addition, a few random but statistically significant changes in organ weights in 30-, 250- and 1000-ppm mancozeb animals were observed.

- K. Gross Pathology: Three ETU-treated males showed enlarged livers. Prominent lobular architecture and pale or discolored livers, observed in many of the treatment groups, were not considered compound related.
- L. Histopathology: The major compound-related histopathological changes were restricted to the liver, kidneys, thyroid, adrenal, and pituitary glands (Table 6). Thyroids in 1000-ppm mancozeb and ETU rats showed a diffused hyperplasia of the follicular epithelium. In the thyroid of one 1000-ppm mancozeb and three

TABLE 4. Mean Levels of ETU ($\mu\text{g} \pm \text{SD}$) Excreted in 24-Hour Urine of Rats Fed Mancozeb or ETU for 13 Weeks^a

	Mancozeb (ppm)						ETU (ppm)
	0	30	60	125	250	1000	250
Males	<0.030	1.35 ± 1.28	3.99 ± 2.46	14.91 ± 6.17	18.45 ± 12.88	97.29 ± 31.47	52.58 ± 28.64
Females	<0.010	6.93 ± 0.51	2.43 ± 0.81	5.3 ± 1.14	18.09 ± 10.03	83.07 ± 29.80	98.90 ± 92.46

^aMeans based on 4 animals/sex/group.

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TABLE 5. Mean Absolute (g) and Relative Organ Weights (Organ Wt. x 10,000/Body Wt.) of Rats Fed Mancozeb^{a,b} or ETU for 13 Weeks^b

Test Compound (ppm)	Males						Females					
	Liver		Spleen		Thyroid		Liver		Spleen		Thyroid	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Control	12.29	271	0.667	14.9	0.025	0.551	6.52	256	0.390	15.5	0.019	0.755
	2.38 ^c	21	0.135	2.4	0.004	0.070	0.70	24	0.049	2.9	0.004	0.105
Mancozeb (1000)	12.51	300*	0.694	17.1	0.033*	0.822*	7.56	317*	0.447	18.9*	0.024	1.005*
	2.11	35	0.100	5.1	0.010	0.425	1.76	44	0.071	2.8	0.002	0.091
ETU (250)	13.55	303*	0.589	13.0	0.045*	1.013*	6.97	295*	0.426	18.0*	0.032	1.358*
	2.05	32	0.101	1.9	0.014	0.245	0.60	22	0.079	2.6	0.004	0.189

*Significantly different from control value ($p \leq 0.05$).

^a Only values for the highest dose groups shown.

^b Means based on 10 animals/sex/group, except for thyroid and spleen in male ETU group, where organs from 9 animals were weighed.

^c Standard deviation.

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TABLE 6. Summary of Histopathological Observations in Rats Fed Mancozeb or ETU for 13 Weeks

Organ/Lesion ^a	Males							Females						
	Dose Group ^c							Dose Group ^c						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
<u>Adrenal Glands</u>	<u>10^b</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>
Cortical vacuolation	3	3	2	5	6	1	4	0	0	0	0	0	0	0
Hyperrophy, zona glomerulosa	1	0	1	2	2	6	6	1	1	1	3	1	3	2
<u>Kidneys</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>
Yellow-brown pigment, cortical tubules														
—minimal	0	0	0	2	4	4	0	0	0	0	8	4	1	0
—slight	0	0	0	7	4	5	0	0	0	0	1	4	4	0
—moderate	0	0	0	0	2	1	0	0	0	0	0	2	5	0
Multifocal cortical tubular degeneration	2	2	5	8	4	4	2	0	1	0	0	1	2	0
Hyaline material, cortical tubules	6	5	6	7	6	4	2	0	0	0	0	0	0	0
<u>Liver</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>
Hypertrophy, centri-lobular hepatocytes														
—minimal	0	0	0	0	0	2	4	0	0	0	0	0	0	
—slight	0	0	0	0	0	0	2	0	0	0	0	0	0	0
—moderate	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Multifocal mononuclear cellular infiltration	8	9	7	7	9	9	5	5	5	4	2	5	2	0
<u>Pituitary</u>	<u>10</u>	<u>10</u>	<u>9</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>9</u>
Hypertrophied/vacuolated cells														
—minimal amount	5	8	5	5	6	3	0	6	2	3	3	4	4	
—small amount	4	2	4	3	3	1	2	0	1	1	2	0	1	4
—moderate amount	1	0	0	1	1	4	4	0	0	0	0	0	0	
—marked amount	0	0	0	0	0	2	3	0	0	0	0	0	0	0
<u>Spleen</u>	<u>10</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>10</u>	<u>10</u>
Hemosiderosis														
—minimal	1	0	0	0	0	1	1	0	0	0	0	0	0	0
—slight	3	0	0	0	0	1	1	0	0	0	0	0	0	0
—moderate	5	0	0	0	0	3	2	0	0	0	0	0	2	0
—marked	1	0	0	0	0	0	0	7	0	0	0	0	8	0
Lymphosarcoma	0	0	0	0	0	0	1	0	0	0	0	0	0	0

^aSelected from Appendix 14 of the CBI report as recording compound-related changes/lesion.^bNumbers of organs examined are underlined.^cDose groups: 1, control; 2-6, 30, 60, 125, 250, and 1000 ppm mancozeb; 7, 250 ppm ETU.

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Continued TABLE 6. Summary of Histopathological Observations in Rats Fed Mancozeb or ETU for 13 Weeks

Organ/Lesion	Males							Females						
	Dose Group							Dose Group						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
<u>Thyroid</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>
Hyperplasia, follicular epithelium	0	0	0	0	0	9	10	0	0	0	0	0	9	10
Basophilic focus/foci	0	0	0	0	0	1	3	0	0	0	0	0	0	0
Cystic follicle(s)	0	0	1	0	1	1	2	0	0	0	0	1	0	0
Utimobronchial bodies	1	3	3	3	1	1	1	1	2	2	2	1	1	4
Utimobronchial bodies, cystic	2	2	1	3	1	3	4	3	1	0	0	3	3	2

ETU males, there was a small, well-defined basophilic focus of hyperplastic follicular epithelial cells. One ETU male had follicular adenoma and a multicentric lymphosarcoma in the thyroid.

All treatment groups including controls contained large hypertrophied vacuolated cells of the pituitary; however, the number of these cells was increased in the 1000-ppm mancozeb males. There was an apparent increase in severity (moderate to marked) in males receiving the highest dose levels of mancozeb and ETU.

The kidneys from males and females fed 125 to 1000-ppm mancozeb had minimal to moderate amounts of a yellow-brown pigment in the lumen of the cortical tubules. Pigmentation was reportedly due to deposits of ethylenebis(isothiocyanate) (EBIS), a yellow-colored excretory metabolite of mancozeb; no histopathological changes were associated with this pigmentation.

An increased incidence of hypertrophy of cells of the zona glomerulosa of the adrenal cortex occurred in the 1000-ppm mancozeb and ETU males; incidence was low in the other treatment groups. Cortical vacuolization of the adrenal gland was found in all male dose groups.

Two 1000-ppm mancozeb males, four ETU males, and one ETU female showed hypertrophy of the centrilobular hepatocytes. Livers from all males and females in groups, including controls, showed a high incidence of multifocal mononuclear infiltration.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that:

1. No compound-related clinical signs or mortalities due to mancozeb or ETU feeding were observed.
2. Food consumption and body weights for both sexes at the highest dose levels of mancozeb and ETU were reduced.
3. None of the mancozeb groups showed any significant compound-related changes in hematologic or clinical chemistry parameters. ETU animals showed increased levels of serum cholesterol; ETU males showed significant decreases in platelet counts.
4. Serum T4 levels were decreased in 1000-ppm mancozeb males and 250 to 1000-ppm mancozeb females. TSH levels were increased in the 250-ppm mancozeb females and 1000-ppm mancozeb males and females.

ETU males and females showed decreased serum T4 and increased T3 and TSH levels.

5. Hepatic mixed function oxidase activity was reduced in the 1000-ppm mancozeb animals and ETU males.
6. Mean absolute and relative liver and thyroid weights were increased in the high-dose animals for both the test compounds; absolute and relative weights for spleen were increased in 1000-ppm mancozeb females.
7. Deposits of yellow-brown granular pigments were seen in the lumen of the cortical tubules of the kidney in rats at 125 to 1000 ppm mancozeb; however, this condition was not considered a manifestation of compound-related toxicity.
8. Follicular epithelial hyperplasia of the thyroid was observed in high-dose mancozeb and ETU animals; males in both dose groups showed increased amounts of hypertrophied cells in the anterior lobe of the pituitary, and hypertrophy of the cells of zona glomerulosa of the adrenal cortex. Centrilobular hepatocellular hypertrophy was observed in all ETU rats and 1000-ppm mancozeb males.
9. The NOEL for mancozeb was established at 125 ppm and the LOEL was 250 ppm based on decreases in serum T4 and TSH levels in females.
10. No EBDC or ETU residues were detected in the blood samples of mancozeb animals; a dose-related increase was found in the amount of ETU in the urine of mancozeb-treated animals. EBDC residues of 0.1 to 1.1 ppm were also detected in rats fed 125 to 1000 ppm mancozeb. No EBDC residues above the detection limit of 25 ppm were detected in thyroids from high-dose mancozeb rats. ETU residues in thyroids of mancozeb-treated animals increased in a dose-related manner. ETU levels in the blood of ETU-fed animals were found to be slightly above the detection limit of 0.1 ppm; levels in urine ranged from 2.9 to 63 ppm and levels in thyroids ranged from 30 to 53 ppm.
8. A quality assurance statement was signed and dated February 12, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The experimental design was complete and adequate for assessment of the systemic subchronic toxicity of mancozeb and its metabolite (ETU). The summary data tables were supported by individual animal data. The report is well organized and technically sound. Under the conditions of the study, compound-related effects were restricted to the 1000-ppm mancozeb and 250-ppm ETU (highest doses tested) animals, to a major extent in the males. These effects in males and females of both groups included decreased body weights and food consumption, decreased MFO activity, decreased serum T4 and increased TSH levels.

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and increased absolute and relative liver and thyroid weights. Follicular epithelial hyperplasia of the thyroid was observed in both sexes of the 1000-ppm mancozeb and ETU animals. High-dose mancozeb females showed increased absolute and relative spleen weights.

Histopathological changes that specifically occurred in the males of 1000-ppm mancozeb and 250-ppm ETU groups were centrilobular hepatocellular hypertrophy, increased amount of hypertrophied cells in the anterior lobe of the pituitary and in the zona glomerulosa of the adrenal cortex, and basophilic foci of hyperplastic follicular epithelium. In addition, follicular adenoma and multicentric lymphosarcoma of the thyroid were found in one ETU male.

The compound-related lesions in the mancozeb-fed rats were histologically similar to those that occurred in the same tissues of the ETU-fed rats.

Many nonspecific clinical conditions, including black crusty material around the eyes and alopecia, were frequently observed in all the treatment groups.

There were no toxicologically important effects on mortality or ocular pathology. One death (125-ppm mancozeb male), though it remained unexplained, was not considered compound related.

Although discounted by the authors as being compound-related, the deposits of yellow-brown pigment present in the lumen of renal cortical tubules (at all doses above 60 ppm) were accompanied by an increased incidence of multifocal cortical tubular degeneration in 125 ppm mancozeb males. These inclusions may represent a form of kidney urolithiasis as seen with gout, cystinuria, or hyperoxaluria. Therefore, these renal inclusions cannot be ignored.

Based on histopathological changes in the kidneys at levels of 125 ppm and above, we assess that a systemic NOEL for mancozeb is 60 ppm (3.5 mg/kg/day in males and 4.4 mg/kg/day in females).

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 48-60.

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APPENDIX A

Protocol

MANCOZEB

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DYNAMAC No. 1-009-C
May 23, 1986

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DATA EVALUATION RECORD

MANCOZEB

Subchronic Oral Toxicity Study in Dogs

STUDY IDENTIFICATION: Cox, R. H. Mancozeb: Three-month dietary toxicity study in dogs. (Unpublished study No. 86RC-7 prepared by Hazleton Laboratories America, Vienna, VA, for Rohm and Haas Co., Spring House, PA, and E.I. du Pont de Nemours and Co., Wilmington, DE; dated February 26, 1986.) Accession No. 261537.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 10-23-86

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1. CHEMICAL: Mancozeb; Dithane M-45; Manzate 200; coordination product of zinc ion and manganese ethylenebisdithiocarbamate; $C_4H_6N_2S_4MnZn$.
2. TEST MATERIAL: Mancozeb (lot No. 43339; TU No. 85-15) was described as a yellow powder containing 83.35 percent active ingredient.
3. STUDY/ACTION TYPE: Subchronic oral toxicity study in dogs.
4. STUDY IDENTIFICATION: Cox, R. H. Mancozeb: Three-month dietary toxicity study in dogs. (Unpublished study No. 86RC-7 prepared by Hazleton Laboratories America, Vienna, VA, for Rohm and Haas Co., Spring House, PA, and E.I. du Pont de Nemours and Co., Wilmington, DE; dated February 26, 1986.) Accession No. 261537.

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Date: _____

7. CONCLUSIONS:

Under the conditions of this study, dose levels of 1000 and 5000 ppm of mancozeb in the diet of dogs induced anorexia, loss of body weight, and pale mucous membranes. The dogs in the high-dose group (5000 ppm) had a reduction in red cell mass with associated changes in hematological parameters (decreased hematocrit and hemoglobin levels and decreased erythrocyte counts), as well as increased total bilirubin and cholesterol values and decreased T3 and T4 (thyroid functions) values. Histopathologic examination revealed hypothyroidism, thymic hypoplasia, and hypoplasia of the gonads and sex organs in the mid- (1000 ppm) and high-dose (5000 ppm) animals. Three dogs (two males and one female) in the high-dose group were sacrificed in extremis because of a deterioration in physical condition due to malnutrition. Based on the results of this study, the NOEL for subchronic toxicity is 100 ppm and the LOEL is 1000 ppm for both male and female dogs.

Core Classification: The study is Core Minimum.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, mancozeb (Dithane M-45) from lot No. 4339, was received in the laboratory in two shipments. It was described as a yellow powder containing 83.35 percent active ingredient. All dose calculations in this study were adjusted to 100 percent active ingredient. Mancozeb is insoluble in water and decomposes at 100°C in the presence of moisture; however, it is stable at room temperature under normal light. The details of the chemical profile of the compound are on file with the registrant. Mancozeb was stored refrigerated in a desiccator during the study.
2. The test animals were beagle dogs obtained from Hazleton Research Products, Inc. (Denver, PA). The dogs were quarantined and acclimatized in the laboratory for at least 20 days. Following quarantine, 60 healthy dogs (30 males and 30 females) were selected and randomly assigned to five dose groups, each containing 6 animals/sex. Groups 1-5 received 0, 10, 100, 1000, or 5000 ppm of mancozeb in the diet, respectively. The dogs were 25 to 30 weeks old and weighed between 6.5 and 9.6 kg (males) and 5.2 and 8.2 kg (females) at study initiation. The dogs were housed individually (in cages) in an environmentally controlled room with a 12-hour light/dark cycle. Purina Certified Canine Diet No. 5007,

¹ Only items appropriate to this DER have been included.

containing the specific dose levels of the test material, was provided to each dog for 2 hours a day through the early part of week 5 and 6 hours each day thereafter except on the days prior to blood collection for clinical laboratory evaluations. Approximately 400 g of feed was offered to each dog through week 3; thereafter, it was reduced to 300 g/day. Water was available ad libitum.

3. The diets were prepared by premixing a weighed amount of the test material with approximately 1 kg of dog feed. The premix was then added to the appropriate amount of feed for each group and mixed in a Patterson-Kelly blender. Fresh diets were prepared once each week. Samples from each group were collected once prior to study initiation and from the 7- and 14-week mixings and were analyzed for homogeneity. Stability of the test material in the diet was determined for samples collected at each mixing during the study including pretest and following 7-day storage at room temperature.
4. The dogs were observed twice daily for mortality and overt toxic effects. A detailed physical examination was performed on all dogs once a week for the first 4 weeks and biweekly thereafter. Ophthalmoscopic examinations were performed on all dogs, once prior to study initiation and again during week 13. Individual food consumption was recorded daily, and body weights were recorded weekly throughout the study. Blood samples were collected from all dogs during weeks -1, -2, 5, and 13 for hematology (10 parameters) and clinical chemistry (16 parameters). Blood collected during weeks 5 and 13 was also used to determine serum concentrations of T3 and T4 (RIA) for evaluation of thyroid function. Levels of TSH were not determined because of the stated unavailability of reliable RIA assay kits.
5. After week 13 or the study, all surviving dogs were sacrificed by exsanguination following sodium thiobarbiturate anesthesia. Necropsies were performed on all dogs, and all gross findings were appropriately recorded.

Terminal body weights and the weights of the following organs of each animal were recorded: adrenals, brain, testes with epididymides, ovaries, thyroid/parathyroid, kidneys, liver/gallbladder, spleen, and heart. The organ/body and organ/brain weight ratios were calculated from these values.

Representative portions of 36 organs and tissues of all dogs, in addition to any gross lesions, were fixed in 10% neutral buffered formalin, processed, and examined microscopically after staining with hematoxylin and eosin.

6. Prior to necropsy, 24-hour urine samples were collected from four males and four females randomly selected from each

group. Blood samples were also taken from the same animals. These samples, along with one lobe of the thyroid of all surviving animals, were frozen immediately and sent to Enviro-Bio-Tech (Bernville, PA) for analysis of ethylenebisdi-thiocarbamate (EBDC) and ethylenethiourea (ETU) residues. Two liver samples were also taken from the same animals from which urine and blood were collected; these samples were frozen and saved for possible future analysis.

7. Body weights, body weight gains, food consumption, hematology, clinical chemistry, and organ weight data were analyzed using appropriate statistical methods. A difference in mean values between dose groups and controls was considered significant at the $p \leq 0.05$ level.

B. Protocol: The study protocol is presented in Appendix A.

12. REPORTED RESULTS:

- A. Diet Analysis and Compound Intake: The results of the diet analyses confirmed the homogeneity of the diet preparation. The test material was found to be stable in the diet during the assay period. The average concentration of mancozeb was 106 ± 6.7 percent of the target values for the various diet preparations.

The mean intake of mancozeb over the 13 weeks of dosing was calculated for each group as shown in Table 1.

TABLE 1. Mean Mancozeb Intake^a

Group	Dose (ppm)	Compound Intake (mg/kg/day $\pm$ SD)	
		Males	Females
1	0	0	0
2	10	0.29 ± 0.025	0.32 ± 0.024
3	100	2.98 ± 0.021	3.35 ± 0.189
4	1000	28.62 ± 2.315	28.91 ± 3.016
5	5000	101.53 ± 13.342	108.67 ± 19.152

^a Calculated from 13 weekly values of mean body weight and mean food consumption.

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- B. Mortality: Two males and one female in the 5000-ppm dose group were sacrificed in extremis due to a deterioration in physical condition which was caused by anorexia and malnutrition; these findings were reported to be compound related.
- C. Clinical Observations: Dose-related clinical signs of dehydration, thinness, and pale mucous membranes were noted in animals of both sexes in the 5000-ppm dose groups, and occasional instances of dehydration were seen in animals in the 1000-ppm dose group (Table 2). These signs were considered the result of malnutrition due to anorexia caused by dosing with the test material.

Physical examination of the animals revealed thinness, dehydration, and pale mucous membrane in high-dose groups as noted in the clinical observations. These were considered to be compound related. Ophthalmoscopic examination of the dogs did not reveal any compound-related effects.

TABLE 2. Incidence of Selected Clinical Findings in Dogs Fed Mancozeb for 3 Months

	Male Dose Group (ppm)					Female Dose Group (ppm)				
	0	10	100	1000	5000	0	10	100	1000	5000
Dehydration	1	0	0	4	6	0	0	1	2	6
Few or no feces	2	1	0	1	6	0	2	1	2	5
Languid	0	0	0	0	1	0	0	0	0	1
Thin	1	0	0	0	2	0	0	0	0	6
Pale mucous membranes	0	0	0	0	2	0	0	0	0	2
Sacrificed in extremis	0	0	0	0	2	0	0	0	0	1

- E. Food Consumption and Body Weight: Anorexia occurred as a compound-related effect in animals of both sexes in 1000- and 5000-ppm dose groups beginning at week 1 and continuing throughout the study. The decrease in food intake was approximately 10-20 percent in the 1000-ppm dose group and amounted to a decrease of approximately 40 percent in the 5000-ppm dose group (Table 3).

The decrease in food intake by animals in the 5000- and 1000-ppm groups affected body weight. Males and females in the 5000-ppm dose group lost an average body weight of 0.8 and 1.5 kg, respectively, whereas the control males and females gained an average of 1.1 and 1.0 kg of respectively, over the 13-week study period (Table 4). Body weight gains of both males and females fed 1000 ppm were less than one-half of the control values.

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TABLE 3. Selected Mean Food Consumption in Dogs Fed Mancozeb for 3 Months^a

Dose Level (ppm)	Group Mean Food Consumption (g/week) $\pm$ S.D. at Week				Total Food Consumption (Weeks 1-13)
	0	4	8	13	
Males^a					
0	1595.7 $\pm$ 421.45	1869.3 $\pm$ 126.92	1914.8 $\pm$ 175.37	1886.5 $\pm$ 162.74	23946.2 $\pm$ 1586.8
10	2047.7 $\pm$ 265.03	1898.3 $\pm$ 110.31	1930.0 $\pm$ 169.04	1911.3 $\pm$ 202.50	25641.3 $\pm$ 1947.8
100	1775.0 $\pm$ 232.26	1953.7 $\pm$ 84.61	1940.2 $\pm$ 97.76	1896.3 $\pm$ 172.83	25036.2 $\pm$ 1179.4
1000	1243.5 $\pm$ 202.14	1650.8 $\pm$ 261.73	1830.7 $\pm$ 118.33	1651.3 $\pm$ 99.70	21504.0 $\pm$ 1634.1*
5000	688.8 $\pm$ 133.51*	1099.8 $\pm$ 141.38*	1020.5 $\pm$ 364.78*	1051.3 $\pm$ 192.73*	14419.5 $\pm$ 1541.1*
Females^b					
0	1525.3 $\pm$ 194.20	1628.7 $\pm$ 216.60	1516.8 $\pm$ 137.83	1450.0 $\pm$ 314.66	20268.2 $\pm$ 2259.3
10	1653.5 $\pm$ 219.74	1772.3 $\pm$ 115.05	1573.7 $\pm$ 130.43	1478.5 $\pm$ 132.49	21372.3 $\pm$ 1213.1
100	1649.3 $\pm$ 232.68	1640.8 $\pm$ 313.12	1784.5 $\pm$ 216.18*	1597.2 $\pm$ 240.41	21967.0 $\pm$ 2375.9
1000	952.7 $\pm$ 158.30*	1358.5 $\pm$ 113.74	1343.0 $\pm$ 86.39	1230.3 $\pm$ 202.57	16480.3 $\pm$ 954.5*
5000	601.0 $\pm$ 309.38*	924.3 $\pm$ 196.42*	1031.6 $\pm$ 141.28*	977.8 $\pm$ 177.86*	11662.2 $\pm$ 1035.7*

^a Mean values based on 6 animals/group except for the 5000-ppm males at week 13, which included 4 animals.

^b Mean values based on 6 animals/group except for the 5000-ppm females at weeks 8 and 13, which included 5 animals.

* Significantly different from control value ($p \leq 0.05$).

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TABLE 4. Selected Mean Body Weights in Dogs Fed Mancozeb for 3 Months

Dose Level (ppm)	<u>Group Mean Body Weight (kg) $\pm$S.D. at Week</u>				Mean Body Weight Change (from wk 0-13)
	0	4	8	13	
<u>Males^a</u>					
0	8.3 $\pm$ 1.03	8.5 $\pm$ 0.92	9.1	9.4 $\pm$ 0.93	1.1 $\pm$ 0.42
10	8.8 $\pm$ 0.40	9.4 $\pm$ 0.51	9.1	10.0 $\pm$ 0.81	1.2 $\pm$ 0.56
100	8.2 $\pm$ 0.91	8.9 $\pm$ 0.99	9.4 $\pm$ 1.19	9.8 $\pm$ 1.17	1.7 $\pm$ 0.71
1000	8.0 $\pm$ 0.65	8.0 $\pm$ 0.74	8.4 $\pm$ 0.82	8.5 $\pm$ 0.87	0.5 $\pm$ 0.55
5000	8.0 $\pm$ 1.03	7.1 $\pm$ 0.86*	7.0 $\pm$ 1.25*	7.4 $\pm$ 0.91*	-0.8 $\pm$ 0.30*
<u>Females^b</u>					
0	6.3 $\pm$ 0.44	6.8 $\pm$ 0.34	7.1 $\pm$ 0.44	7.3 $\pm$ 0.64	1.0 $\pm$ 0.50
10	6.8 $\pm$ 0.48	7.2 $\pm$ 0.34	7.5 $\pm$ 0.25	7.5 $\pm$ 0.28	0.7 $\pm$ 0.25
100	6.4 $\pm$ 0.54	6.7 $\pm$ 0.49	7.5 $\pm$ 0.86	7.7 $\pm$ 1.04	1.3 $\pm$ 0.72
1000	6.3 $\pm$ 0.90	6.1 $\pm$ 0.78	6.3 $\pm$ 0.77	6.2 $\pm$ 0.66	-0.1 $\pm$ 0.50*
5000	6.8 $\pm$ 1.37	5.6 $\pm$ 1.28	6.0 $\pm$ 0.79*	5.6 $\pm$ 0.67*	-1.5 $\pm$ 0.65*

^a Mean values based on 6 animals/group except for the 5000-ppm males at week 13, which included 4 animals.

^b Mean values based on 6 animals/group except for the 5000-ppm females at weeks 8 and 13, which included 5 animals.

* Significantly different from control value ($p \leq 0.05$).

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- F. Clinical Pathology (hematology, clinical chemistry, and thyroid function tests): Decreased erythrocyte counts, and hematocrit and hemoglobin levels (Table 5) as well as decreased T3 and T4 values and increased total bilirubin and cholesterol values (Table 6) were observed in animals of both sexes in 500- and 1000-ppm dose groups. These findings were considered to be due to compound-related hypothyroidism. Decreased alanine aminotransferase (ALT) and calcium values noted in these animals were also considered to have the same etiology. However, the authors also considered the possibility that anorexia and body weight loss may have caused the abnormal clinicopathological findings in the 1000- and 5000-ppm dose groups. No compound-related ophthalmologic changes were reported.
- G. Organ Weights: Significantly increased mean absolute and relative thyroid/parathyroid weights were seen in the 5000-ppm males and females and were considered to be the direct result of dosing with mancozeb (Table 7). Other changes in organ weights noted in this study were considered to be due to body weight losses resulting from anorexia.
- H. Gross Observations: Enlarged and/or dark thyroid/parathyroids and decreased thymus size were seen in the 1000- and 5000-ppm males and females and were considered compound related. A pale appearance of the visceral organs was noted in two males and one female in the 5000-ppm group which were sacrificed in extremis. No other findings were considered treatment related.
- I. Histopathology: Compound-related histomorphological tissue alterations include thyroid follicular cell hyperplasia in both males and females in the 5000-ppm dose group, thymic cortical lymphoid depletion in the 1000- and 5000-ppm animals (both sexes), hypoplastic changes in the reproductive systems of males and females in the high-dose group as well as prostatic hypogenesis in males receiving 1000 ppm, pallor of the zona fasciculata of the adrenal gland in high-dose males and females, and hematopoietic alterations in the spleen and liver of high-dose (5000 ppm) males and females. Normal staining variability in the adrenal cortex was also noted in animals from all groups, including controls. No other compound-related histopathologic alterations were noted. Table 8 summarizes selected microscopic observations.
- J. Residue Analysis: The analyses were performed by the gas chromatographic method. ETU and EBDC (as CS₂) were detected in the urine in a dose-dependent manner. Blood levels of ETU were slightly above the detection limit, which is 0.040 ppm, in animals in the 5000-ppm dose group. EBDC was not detected in thyroid; however, ETU was detected in the thyroids of animals of both sexes. The average ETU concentration in the thyroids was 7.78 and 12.02 ppm for males and 5.35 and 10.66 ppm for females in 1000 and 5000 ppm dose groups, respectively (Table 9).

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TABLE 5. Selected Mean ($\pm$ S.D.) Hematology Values of Dogs Fed Mancozeb for 3 Months

Dose Level (ppm)	HGB (g/dl)		HCT (%)		MCV (fl)		RBC ($10^6/\mu l$)		MCH (pg)	
	Week: 5	13	5	13	5	13	5	13	5	13
<u>Males</u>										
0	14.5 ± 1.11	15.3 ± 1.12	40.2 ± 3.97	43.1 ± 3.02	65.3 ± 2.18	65.5 ± 2.57	6.15 $\pm .662$	6.59 $\pm .554$	23.5 ± 0.85	23.2 ± 0.33
10	13.9 ± 0.59	14.7 ± 0.84	38.8 ± 1.76	42.2 ± 2.20	66.4 ± 1.54	66.3 ± 1.65	5.84 $\pm .248$	6.35 $\pm .262$	23.8 ± 0.73	23.2 ± 0.33
100	14.4 ± 0.78	15.2 ± 1.62	40.2 ± 2.67	43.1 ± 4.74	64.8 ± 2.24	65.5 ± 1.77	6.22 $\pm .577$	6.59 $\pm .799$	23.3 ± 0.98	23.2 ± 0.33
1000	14.0 ± 0.73	14.2 ± 1.06	39.3 ± 2.46	40.1 ± 3.28	67.0 ± 2.40	67.6 ± 1.87	5.86 $\pm .413$	5.94 $\pm .547$	24.0 ± 0.92	23.8 ± 0.33
5000	12.2* ± 1.82	11.5 ± 5.72	34.1* ± 4.89	32.9 ± 15.58	67.5 ± 3.58	72.6* ± 5.63	5.06* $\pm .752$	4.66 ± 2.411	24.1 ± 1.54	25.2* ± 0.15
<u>Females</u>										
0	15.3 ± 1.89	15.5 ± 1.40	42.9 ± 5.52	43.9 ± 3.89	65.5 ± 0.51	65.9 ± 1.01	6.55 $\pm .807$	6.66 $\pm .539$	23.3 ± 0.33	23.3 ± 0.16
10	14.3 ± 0.85	14.4 ± 0.88	40.2 ± 2.85	40.9 ± 2.40	65.5 ± 0.97	65.7 ± 1.61	6.13 $\pm .424$	6.21 $\pm .358$	23.4 ± 0.45	23.2 ± 0.51
100	14.3 ± 0.94	14.1 ± 1.26	41.9 ± 2.98	40.3 ± 3.49	66.4 ± 0.98	67.1 ± 1.21	6.31 $\pm .504$	6.01 $\pm .520$	23.5 ± 0.62	23.3 ± 0.31
1000	13.0* ± 1.02	13.9 ± 1.59	36.1* ± 3.31	39.4 ± 4.10	67.3 ± 2.22	68.0 ± 1.87	5.36* $\pm .540$	5.81 $\pm .724$	24.3 ± 0.36	24.2 ± 0.33
5000	12.0* ± 1.59	11.9* ± 2.55	33.3* ± 4.83	33.8* ± 6.65	67.9* ± 1.07	70.8* ± 4.03	4.91* $\pm .769$	4.81* ± 1.128	24.4* ± 0.88	24.2* ± 0.33

*Significantly different from control value ($p \leq 0.05$).

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TABLE 6. Selected Mean ($\pm$ S.D.) Clinical Chemistry and Thyroid Function Values of Dogs Fed Mancozeb for 3 Months

Dose Level (ppm)	TBILI (mg/dl)		Calcium (mg/dl)		TCHOL (mg/dl)		ALT (μ /L)		T ₃ (ng/dl)		T ₄ (ng/dl)	
	Week: 5	13	5	13	5	13	5	13	5	13	5	13
Males												
0	0.1 ± 0.00	0.1 ± 0.08	10.6 ± 0.08	10.7 ± 0.31	187 ± 19.5	179 ± 25.9	25 ± 2.6	23 ± 5.0	1.2 ± 0.27	1.0 ± 0.14	2.3 ± 1.06	1.8 ± 0.54
10	0.1 ± 0.04	0.1 ± 0.04	10.6 ± 0.31	10.6 ± 0.35	186 ± 25.8	169 ± 18.5	32 ± 7.0	100 ± 21.7	1.0 ± 0.08	1.0 ± 0.12	1.5 ± 0.49	1.2 ± 0.38
100	0.1 ± 0.04	0.1 ± 0.00	10.7 ± 0.18	10.4 ± 0.44	174 ± 24.5	152 ± 20.5	27 ± 5.2	75 ± 15.5	1.0 ± 0.15	0.9 ± 0.14	1.9 ± 0.65	1.4 ± 0.64
1000	0.3* ± 0.17	0.1 ± 0.05	10.7 ± 0.12	10.6 ± 0.19	208 ± 37.1	208 ± 43.7	21 ± 8.9	20 ± 6.5	0.9 ± 0.21	1.0 ± 0.15	1.4 ± 0.50	1.4 ± 0.3
5000	0.2 ± 0.12	0.2 ± 0.10	10.1 ± 0.68	10.0 ± 0.78	335* ± 72.8	383* ± 53.1	15* ± 5.1	15* ± 3.6	0.5* ± 0.22	0.9 ± 0.45	0.3* ± 0.25	0.5* ± 0.4
Females												
0	0.1 ± 0.08	0.1 ± 0.05	10.8 ± 0.29	10.8 ± 0.53	159 ± 23.9	174 ± 26.6	24 ± 3.2	21 ± 1.4	1.0 ± 0.13	1.0 ± 0.19	2.1 ± 0.51	2.1 ± 0.5
10	0.1 ± 0.05	0.1 ± 0.05	10.5 ± 0.43	10.4 ± 0.40	160 ± 31.6	139 ± 25.8	20 ± 3.1	19 ± 2.1	1.1 ± 0.15	1.0 ± 0.10	2.3 ± 0.90	1.8 ± 0.4
100	0.2 ± 0.08	0.1 ± 0.05	10.7 ± 0.37	10.3 ± 0.35	178 ± 24.7	174 ± 27.1	21 ± 4.6	19 ± 3.4	1.1 ± 0.26	1.0 ± 0.10	1.8 ± 0.76	1.6 ± 0.48
1000	0.2 ± 0.05	0.1 ± 0.05	10.4 ± 0.50	10.5 ± 0.36	229* ± 52.2	245* ± 55.7	21 ± 1.3	21 ± 2.4	1.1 ± 0.14	1.2 ± 0.10	2.0 ± 0.46	2.3 ± 0.8
5000	0.5* ± 0.05	0.2* ± 0.08	10.1* ± 0.33	10.1 ± 0.33	254* ± 87.6	289* ± 62.8	14* ± 2.2	13* ± 3.4	2.6* ± 0.19	0.9 ± 0.22	0.4* ± 0.24	0.3* ± 0.18

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

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TABLE 7. Mean Absolute and Relative^a Thyroid/Parathyroid Weights ($\pm$ S.D.) of Dogs Fed Mancozeb for 3 Months

Sex/Parameter	Dose Level (ppm)				
	0	10	100	1000	5000
<u>Males</u>					
-Absolute organ weight (g)	0.98 $\pm 0.24^b$	0.85 0.06	0.98 ± 0.22	1.02 ± 0.19	2.23* ± 0.87
-Organ weight relative to final body weight (%)	0.010 ± 0.003	0.008 ± 0.001	0.010 ± 0.002	0.012 ± 0.003	0.031* ± 0.014
-Organ weight relative to brain weight (%)	0.013 ± 0.004	0.010 ± 0.001	0.013 ± 0.003	0.014 ± 0.003	0.031* ± 0.011
<u>Females</u>					
-Absolute organ weight (g)	0.80 ± 0.19	0.80 ± 0.23	0.77 ± 0.22	0.77 ± 0.12	1.73* ± 0.94
-Organ weight relative to final body weight (%)	0.011 ± 0.003	0.011 ± 0.003	0.010 ± 0.002	0.012 ± 0.001	0.031* ± 0.017
-Organ weight relative to brain weight (%)	0.011 ± 0.003	0.011 ± 0.003	0.010 ± 0.003	0.011 ± 0.002	0.025* ± 0.013

^a Organ weight relative to final body weight and organ weight relative to brain weight.

^b Standard deviation.

* Significantly different from control value ($p \leq 0.05$).

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TABLE 8. Incidence of Selected Microscopic Observations in Dogs Fed Mancozeb for Three Months

Organ/Observation	Sex/Dose Level (ppm)									
	Males					Females				
	0	10	100	1000	5000	0	10	100	1000	5000
Thyroid	(6) ^a	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
-follicular cell hyperplasia	0	0	0	0	6	0	0	0	0	6
Thymus	(6)	(6)	(6)	(6)	(5)	(6)	(6)	(6)	(6)	(5)
-lymphoid depletion, cortex	0	0	0	6	5	0	0	0	6	5
Testes	(6)	(6)	(6)	(6)	(6)	-	-	-	-	-
-aspermato-genesis	0	0	0	0	3	-	-	-	-	-
-hypospermato-genesis	0	0	0	0	2	-	-	-	-	-
-hypogenesis	0	0	0	0	1	-	-	-	-	-
Epididymides	(6)	(6)	(6)	(6)	(6)	-	-	-	-	-
-hypogenesis	0	0	0	0	2	-	-	-	-	-
Prostate	(6)	(6)	(6)	(6)	(6)	-	-	-	-	-
-hypogenesis	0	0	0	2	6	-	-	-	-	-
Ovaries	-	-	-	-	-	(6)	(6)	(6)	(6)	(5)
-hypogenesis	-	-	-	-	-	0	0	0	0	1
Uterus	-	-	-	-	-	(6)	(6)	(6)	(6)	(6)
-hypogenesis	-	-	-	-	-	0	0	0	0	1
Liver	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(5)
-extramedullary hemato-poiesis, increased	0	0	0	0	2	0	0	0	0	2
-sinusoidal cell pigmentation	0	0	0	0	4	0	0	0	0	2
Adrenals	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)	(6)
-pallor, zona fasciculata	0	0	0	0	4	0	0	0	0	3

^aNumber in parenthesis is the number of tissues examined.

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TABLE 9. Selected Mean and Range of Ethylenethiourea (ETU) Values Observed in the Blood and Thyroid Glands of the Dogs Fed Mancozeb for 3 Months

Specimen	Sex	Dose Group (ppm)	ETU (Mean) (ppm)	ETU (Range) (ppm)
Blood	F	100	ND*	—
	M	100	ND	<0.040-0.070
	F	1000	0.053	<0.040-0.070
	M	1000	0.043	<0.040-0.050
	F	5000	0.15	0.14-0.19
	M	5000	0.14	0.10-1.9
Thyroid	F	100	0.716	0.45-0.90
	M	100	1.83	0.57-4.0
	F	1000	5.35	4.1-9.2
	M	1000	7.78	4.1-9.9
	F	5000	10.66	7.0-15.0
	M	5000	13.02	6.1-18.0

*Not detectable; limit of detection = 0.040 ppm.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

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- A. Dietary administration of mancozeb (Dithane M-45) to beagle dogs at dose levels of 1000 and 5000 ppm resulted in significant toxic effects. A compound-related decrease in the triiodothyronine and thyroxine thyroid hormones (T3 and T4) was seen in 5000-ppm dose groups. Hypercholesterolemia and hyperbilirubinemia accompanied hypothyroidism. A moderate to marked decrease in food consumption in dogs in the 5000-ppm dose group resulted in loss of body weight and dehydration. Dogs in the 1000-ppm dose group did not gain weight during the study and occasionally appeared dehydrated. The authors concluded that the toxicological significance of some of the changes in clinical laboratory values and the microscopic alterations that were seen in the gonads, thymus, adrenals, and possibly in some of the hematopoietic tissues may have been confounded by malnutrition.

Based on the results of the study, the NOEL for subchronic toxicity of mancozeb in the dog was considered to be 100 ppm in the diet.

- B. A quality assurance statement was dated January 29, 1986 for the toxicity study; a quality assurance statement was dated November 26, 1985 for the residue analyses report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Three dogs, two male and one female, in the 5000-ppm dose group were sacrificed in moribund conditions caused by anorexia. Decreases in body weights and food consumption were noted both in the 1000- and 5000-ppm groups of both sexes. The animals in the high-dose group were thin, dehydrated, and had pale mucous membranes. Reductions of red cell mass and related values were also noted in the high-dose group. In addition, the histomorphological evidence of hypothyroidism, pallor in zona fasciculata of the adrenals, and hypoplasia of the gonads and sex organs were present in the high-dose group in dogs of both sexes, including the animals which were sacrificed in extremis.

Evidence of the concentration of the metabolic product (ETU) of mancozeb in the thyroid relative to blood was noted in this study. In the absence of a control study using ETU, it appears that mancozeb (or its metabolite) directly affected the thyroid and possibly the other endocrine glands (adrenal and gonads). Hypothyroidism is known to affect the hematologic parameters adversely and to cause increased cholesterol levels, as noted in the high-dose animals in this study. However, anorexia (due to unpalatable food) and reduced food consumption, noted in 1000 and 5000 ppm dose groups, most likely caused the loss of body weight. These effects could have also conceivably caused the reduction in hematologic values.

Thyroid stimulating hormone (TSH) analyses, although important criteria to consider when the thyroid is a primary target organ, were not performed in this study due to the unavailability of suitable radioimmunoassay kits. In the final analysis, it appears that both anorexia and hypothyroidism acted synergistically to precipitate the toxic effects of mancozeb in the dogs of the high dose groups.

In summary, the NOEL in this study is 100 ppm which is equivalent to intakes of 3.0 and 3.4 mg/kg/day in males and females, respectively. The LOEL is 1000 ppm which is equivalent to 29 mg/kg/day in both sexes. At this dose there was decreased food consumption and body weight gains, transient reduction in RBC mass (counts, hematocrit, and hemoglobin value), cortical lymphoid depletion in the thymus, and prostatic hypogenesis. Compound-related thyroid changes only occurred at the HDT, 5000 ppm (102 and 109 mg/kg/day in males and females, respectively).

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol, CBI pp. 98-114.

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APPENDIX A
Study Protocol

MANCOZEB

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Page _____ is not included in this copy.

Pages 55 through 71 are not included.

The material not included contains the following type of information:

- ☐ Identity of product inert ingredients.
- ☐ Identity of product impurities.
- ☐ Description of the product manufacturing process.
- ☐ Description of quality control procedures.
- ☐ Identity of the source of product ingredients.
- ☐ Sales or other commercial/financial information.
- ☐ A draft product label.
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EPA: 68-02-4225
DYNAMAC No. 1-009E
June 4, 1986

DATA EVALUATION RECORD

MANCOZEB

Subchronic Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Hagan, J. I., Fisher, J. R., and Baldwin, R. C.
Mancozeb: Subchronic inhalation study in rats--thirteen-week interim
report. (Unpublished study No. 86R-003 prepared by Rohm and Haas Co.,
Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I.
du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.)
Accession No. 261539.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 6-4-86

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1. CHEMICAL: Mancozeb; dithane M-45; Manzate 200; coordination product of zinc ion and manganese ethylenebisdithiocarbamate; $C_4H_6N_2S_4MnZn$.
2. TEST MATERIAL: Mancozeb (lot No. 4339; TD No. 85-015; product code 6-2804) was described as a yellow powder containing 83.35 percent active ingredient.
3. STUDY/ACTION TYPE: Subchronic inhalation toxicity study in rats.
4. STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Subchronic inhalation study in rats--thirteen-week interim report. (Unpublished study No. 86R-003 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261539.

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7. CONCLUSIONS:

A. Groups of 38 male and 38 female rats were exposed to mancozeb target concentrations of 0, 20, 80, or 320 mg/m³ for 13 weeks. Groups of five males and five females were sacrificed after 4 weeks of exposure, and groups of 16 males and 16 females were necropsied at the end of the 13 week exposure. In addition, 17 males and 17 females were held for an additional 13 weeks following the exposure phase of the study as a recovery study. [The results of the recovery study, were not included in this report.]. The actual mean respirable concentrations to which the rats were exposed were 0, 8, 36, and 144 mg/m³, respectively. The male rats exposed to 144 mg/m³ exhibited significant ($p \leq 0.05$) reduced mean body weight and body weight gain for most of the exposure period, whereas no such effects were noted in female rats. Mean corpuscular volume, mean corpuscular hemoglobin concentration, serum triglyceride levels, and inorganic phosphorus levels were significantly ($p \leq 0.05$) altered; however, they were within normal ranges for rats and were not considered of biological relevance. Thyroid function tests revealed significantly reduced T4 serum levels in female rats exposed to 144 mg/m³ for 13 weeks. In samples collected at the termination of exposures, blood, urine, and thyroid samples exhibited an exposure-response increase in ethylenethiourea (ETU) and ethylenebisdithiocarbamate (EBDC) concentrations. These data support the hypothesis that mancozeb is metabolized to ethylenethiourea. Organ weight changes included reduced kidneys and heart weights in male rats exposed to 144 mg/m³; this may have reflected the reduced body weight of these animals. No remarkable ophthalmologic findings were reported. Among the histologic findings, hyperplasia of the follicular epithelium was noted in 3 of 10 females exposed to 144 mg/m³, yellow-brown granular pigment in kidneys of both males and females exposed to 36 or 144 mg/m³, and several lesions in the respiratory tract. The thyroid changes were related to exposure to mancozeb whereas the respiratory tract lesions are typically observed following exposure to dusts. The respiratory tract lesions may be indicative of a progressive disease. The authors considered renal inclusions to represent the elimination of a urinary metabolite. However, the granular form of the pigmented material may be indicative of progressive disease or lead to chronic lesions following the inhalation of mancozeb. Based on the renal inclusions, the LOAEL is 36 mg/m³ and the NOAEL is 8 mg/m³ for rats exposed to mancozeb dust aerosols via nose-only exposure.

Item 8--see footnote 1.

¹ Only items appropriate to this DER have been included.

9. BACKGROUND: The exposure levels selected for this subchronic study were derived from a 2-week inhalation study designed to determine exposure levels and the mode of exposure. Nose-only was selected over whole body exposure. From the 2-week study, the NOAEL was 55 mg/m³ and the LOAEL was 258 mg/m³ for rats exposed to mancozeb for 10 exposures based on the respirable concentrations of mancozeb in the chambers.

Item 10—see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. The test material, mancozeb, contained 33.35 percent active ingredient, which was a coordination product of zinc ion and manganese ethylenebisdithiocarbamate. The exposure concentrations used in this study were based on the formulated test material, as received. Four groups of animals, designated 1, 2, 3, and 4, were exposed to target aerosol concentrations of mancozeb of 0, 20, 80, or 320 mg/m³. Animals were exposed (nose-only) 6 hours a day, 5 days a week.
2. Thirty-eight male and 38 female Crl:CS(SD)BR rats (Charles River-Lakeview, Newfield, NJ) were randomly assigned to each of the four exposure groups. The rats were 35 days old at the initiation of the study and weighed between 157 and 214 g (males) and 124 and 168 g (females). Due to an error in sexing, group 2 contained 37 males and 39 females. The animals in each of the four groups were further divided into three subgroups, designated A, B, and C. Animals from subgroup A were necropsied after 4 weeks, and subgroup B animals were necropsied after 13 weeks of exposure. Subgroup C animals were to be necropsied after a 13-week post-exposure recovery period; data for the recovery period will appear in the final report.
3. The animals were housed individually in stainless steel wire-mesh cages in an environmentally controlled room while they were not in the exposure chambers. The room was maintained at a temperature ranging between 70 and 80°F (21-27°C), a relative humidity of 30-70 percent, and a 12 hour light, 12 hour dark cycle. During exposure, the exposure chambers were maintained under similar conditions. Animals were provided food and water ad libitum except during exposure and during the pre-necropsy fasting period. During exposure, animals were housed in individual PVC nose-only restraining tubes which were attached to the front of the exposure chambers.

4. The powdered test material was placed in a sample reservoir, and was forced through the funnel-shaped bottom of the reservoir by a plunger, into a conducting duct which contained a blow-jet nozzle. From the conducting duct, the test material was incorporated into an air stream which entered the air-mixing turret of a stainless steel and glass exposure chamber. The configuration and stroke frequency of the plunger were varied in order to achieve the different exposure concentrations. The exposure chambers were supplied with filtered room air, and the chamber air flow rate was monitored. The aerosol concentrations in each exposure chamber were also monitored periodically each day in order to check and adjust the aerosol output of the dust generators. The actual chamber analytical concentrations and the particle size distributions were determined gravimetrically. Samples were taken daily from all four chambers to determine chamber analytical concentrations. Particle size samples were taken weekly from chambers 2, 3, and 4, which housed groups 2, 3, and 4, respectively; particle size samples were not taken from chamber 1 (control chamber). The exposure concentrations cited in the report were the respirable dust concentrations, which were calculated from the total analytical dust concentrations and the respirable fraction. The respirable fraction was calculated from the mass median diameter and the geometric standard deviation. Temperature and humidity were monitored continuously in the chambers and in the animal holding room.
5. All animals were examined and weighed the day before the first exposure (week 0), and then weekly until study termination. Animals were examined before, during, and after each exposure for signs of toxicity and mortality. Each animal was given an ophthalmologic examination prior to the first exposure and again during week 12. At the scheduled intervals, after 4 weeks and after 13 weeks of exposure, animals were sacrificed and necropsied. Blood samples were drawn from all animals scheduled for histopathologic evaluation. These samples were used to evaluate eight hematologic and 15 clinical chemistry parameters. Thyroid functions (T3, T4, and TSH serum levels) were also evaluated.

At necropsy, all animals were examined macroscopically. After 4 weeks of exposure, the following target organs from all animals sacrificed were removed, fixed in formalin, and histologically examined: lungs; lymph node (peribronchial); nasal turbinates; trachea; and thyroid/parathyroid. Absolute organ weights were recorded and relative organ weights (organ-to-body weight ratios) were determined for lungs and thyroid/parathyroid. After 13 weeks of exposure, the absolute and relative weights of 10 organs from all sacrificed animals were recorded. Tissues from the above-mentioned target organs, as well as liver and kidney tissues and all gross lesions and masses were histologically

examined for rats exposed to 20 or 80 mg/m³. A complete histopathological examination was performed on rats exposed to 0 or 320 mg/m³, which included tissues of 40 organs and all gross lesions and masses.

Six male and six female rats from each group had previously been designated for residue analysis, and were not sacrificed at study termination. After 13 weeks of exposure, these rats were placed in metabolism cages for 24 hours, during which time the total urine output was collected and frozen. After the 24-hour period, these animals were sacrificed, and the lungs, trachea, and livers were removed and placed in frozen storage. The blood and thyroids were also removed and frozen; the frozen samples and urine were sent to Enviro-Bio-Tech, Ltd. (Bernville, PA) for determination of residual levels of ETU and EBOC as carbon disulfide.

6. The data for body weights, body weight changes, hematologic parameters, clinical chemistry parameters, thyroid function parameters, organ weights, and organ-to-body weight ratios were evaluated using appropriate statistical methods. A difference between exposure groups and controls was considered statistically significant at $p \leq 0.05$.

8. Protocol: The study protocol is included in Appendix A.

12. REPORTED RESULTS:

- A. Chamber Conditions: It was reported that the air flow rate during exposure in all chambers was 400 L/min., which resulted in a 99 percent aerosol equilibrium time (t_{99}) of 14.4 min. or 4.0 percent of the exposure time. The chamber concentration and aerosol characterization data are shown in Table 1.

1. 4 Weeks of Exposure: The mean analytical aerosol concentrations of mancozeb in the chambers were found to be 0 (group 1), 22 (group 2), 86 (group 3), and 308 (group 4) mg/m³; these values corresponded to target concentrations of 0, 20, 80, and 320 mg/m³. The corresponding respirable concentrations were 0, 8, 40, and 127 mg/m³ for groups 1, 2, 3, and 4, respectively. The mean mass median diameter (MMD) of the aerosol particles ranged from 3.7 to 4.4 micrometers, the mean geometric standard deviation (GSD) ranged from 2.1 to 2.3, and the respirable fraction ranged from 42 to 47 percent. The mean temperature in the chambers ranged from 20.0 to 21.8°C, and the mean relative humidities ranged from 71 to 74 percent.
2. 13 Weeks of Exposure: The mean analytical aerosol concentrations of mancozeb were 0, 18, 79, and 326 mg/m³; these values corresponded to target concentrations of 0, 20, 80, and 320 mg/m³ respectively. The corresponding respirable

TABLE 1. Chamber Concentration and Aerosol Characterization

Target Exposure Concentration ($\mu\text{g}/\text{m}^3$)	Mean Analytical Exposure Concentration (mg/m^3)	Range of Daily Exposure Concentration (mg/m^3)	Respirable Exposure Concentration (mg/m^3)	Mean Mass Median Diameter (micrometers)	Geometric Standard Deviation	Respirable Fraction (percent)
<u>After 4 Weeks</u>						
0	0	0	0	0	0	0
20	22	1- 70	8	4.1	2.1	43
80	86	36-152	40	3.7	2.2	47
320	308	161-572	127	4.4	2.3	42
<u>After 13 Weeks</u>						
0	0	0	0	0	0	0
20	18	7- 30	8	3.9	2.1	45
80	79	47-117	36	3.8	2.1	46
320	326	215-514	144	4.2	2.1	42

concentrations were 0, 8, 36, and 144 mg/m³, respectively. The aerosol particles had mean MMD of 3.8 to 4.2 micrometers, mean GSD of 2.1, and respirable fraction ranged from 42 to 46 percent. The mean chamber temperatures ranged from 19.6 to 21.1°C, and mean relative humidities ranged from 68 to 77 percent.

B. Clinical Observations:

1. 4 Weeks of Exposure: Six animals, distributed among the four groups, died during this study period. The authors attributed these deaths to asphyxiation caused by excessive restraint in the nose-only restraining tubes. Alopecia and missing tail tips were noted for several animals; these findings were attributed to injury by the restraining tubes.
2. 13 Weeks of Exposure: During week 5 to week 13, five additional deaths occurred, two of these were attributed to excessive restraint. One female, exposed to 80 mg/m³, died as the result of gastric torsion. One male exposed to 320 mg/m³ died with a prostate abscess. One male exposed to 20 mg/m³ exhibited gasping, rales, bradypnea, dyspnea, a red serosanguinous exudate on the muzzle, and bright red spotting on the dropping sheet during week 5. The animal appeared emaciated through week 11 with misaligned incisors at week 7, and died during exposure in week 12 with a nasal abscess around a tooth. Several animals exhibited alopecia, dark brown staining of the fur, abrasions, missing tail tips, wet abdominal fur, and/or red exudate around the eyes, all of which were attributed to the restraining method.

C. Body Weights and Body Weight Gains: A summary of the mean body weight data is presented in Table 2.

1. 4 Weeks of Exposure: Mean body weights and mean body weight gains of males exposed to 320 mg/m³ were significantly reduced ($p \leq 0.05$) compared to controls during weeks 2 through 4. No other exposure-related body weight effects were noted during the first 4 weeks of exposure.
2. 13 Weeks of Exposure: Males exposed to 320 mg/m³ exhibited significantly reduced ($p \leq 0.05$) mean body weights and mean body weight gains compared to controls during weeks 7 through 13. Females exposed to 20 mg/m³ exhibited a significantly increased ($p \leq 0.05$) body weight gain as compared to controls. No other exposure-related body weight effects were noted during this time period.

D. Hematology:

1. 4 Weeks of Exposure: An insufficient amount of blood was obtained from 11 of 20 females at the 4-week necropsy interval. The data, therefore, were insufficient to make a meaningful statistical evaluation of female hematology.

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TABLE 2. Selected Mean Body Weights ($\pm$ SD) and Total Body Weight Gain ($\pm$ SD) in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m ³)	<u>Group Mean Body Weight (g) at Week</u>				Total Body Weight Gain (Week 0-13) ^a
	0	4	8	13	
<u>Males</u>					
0	182.97 ±12.67	336.4 ±30.0	399.3 ±30.2	360.3 ±40.6	277.1 ±34.0
20	180.27 ±9.83	327.2 ±30.0	383.2 ±40.7	440.1 ±50.6	259.6 ±45.8
80	182.45 ±11.79	329.7 ±22.9	393.3 ±32.1	451.9 ±40.7	271.0 ±37.1
320	181.37 ±10.64	318.0* ±23.3	375.8* ±29.5	429.0* ±34.8	248.3* ±30.5
<u>Females</u>					
0	145.05 ±9.19	200.1 ±17.5	223.0 ±16.3	241.1 ±18.3	95.7 ±16.8
20	142.49 ±9.56	203.3 ±18.3	228.5 ±19.4	251.9 ±25.8	110.1* ±20.1
80	143.92 ±6.19	197.1 ±12.9	225.2 ±14.0	241.6 ±17.4	98.5 ±14.8
320	146.13 ±9.86	205.4 ±14.7	228.4 ±15.7	245.9 ±19.3	99.5 ±14.6

^aTotal weight gain for rats alive at week 13.*Significantly different from control value ($p \leq 0.05$).

parameters. No hematologic effects were seen for males in any exposure group.

2. 13 Weeks of Exposure: Females exposed to 320 mg/m³ exhibited a significant increase ($p \leq 0.05$) in mean corpuscular volume (MCV) and a significant decrease ($p \leq 0.05$) in mean corpuscular hemoglobin concentration (MCHC) compared to controls. However, the parameters from which the MCV and MCHC values were derived (hematocrit, red blood cell count, and hemoglobin) were not affected. Therefore, these differences were not considered toxicologically relevant. No other hematologic effects were seen in males or females. Table 3 summarizes mean values of selected hematologic parameters for females.

E. Clinical Chemistry:

1. 4 Weeks of Exposure: No effects were seen in clinical chemistry parameters for males or females in any group after 4 weeks of exposure.
2. 13 Weeks of Exposure: A significant reduction ($p \leq 0.05$) was seen in triglyceride levels in males exposed to 320 mg/m³ after 13 weeks of exposure. The authors considered this effect to be related to the reduced body weights which were seen these males, and was, therefore, a secondary effect of mancozeb exposure.

Inorganic phosphorus was significantly reduced ($p \leq 0.05$) in females exposed to 80 mg/m³ compared to controls. This effect was not observed in females exposed to 320 mg/m³, and was, therefore, not considered toxicologically relevant by the authors. No other clinical chemistry effects were seen in males or females.

F. Thyroid Function Data:

1. 4 Weeks of Exposure: T3, T4, and TSH serum levels were not affected in males or females of any group during the first 4 weeks of exposure.
2. 13 Weeks Exposure: A significant reduction ($p \leq 0.05$) in T4 serum level was noted in females exposed to 320 mg/m³; this was considered to be exposure related. No other effects on thyroid function were noted. Female T4 serum levels are presented in Table 4.

- G. Residue Analysis (conducted by subcontractor and included in Appendix of report): Urine, blood, and thyroid samples collected after 13 weeks of exposure were analyzed for ETU and EBDC; the data are presented in Table 5. The subcontractor reported that EBDC residues increased in urine at 20, 80, and 320 mg/m³ and in blood at 320 mg/m³. The ETU residues increased in urine and blood at 20, 80, and 320 mg/m³ and in thyroid at 80 and 320 mg/m³.

TABLE 3. Selected Mean Hematology Values ($\pm$ SD) in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m ³)	Parameter/Group Mean Value After 13 Weeks				
	RBC (10E6/mm ³)	HCT (%)	HGB (g/100 mL)	MCV (μ m ³)	MCHC (%)
<u>Males</u>					
0	8.63 ± 0.45	50.4 ± 2.0	14.4 ± 0.6	58 ± 2.0	28.6 ± 0.4
20	8.38 ± 0.41	49.3 ± 0.9	14.1 ± 0.7	59 ± 2.0	28.5 ± 0.8
80	8.47 ± 0.34	49.9 ± 1.4	14.1 ± 0.4	59 ± 2.0	28.4 ± 0.4
320	8.49 ± 0.40	50.0 ± 2.9	14.4 ± 0.7	59 ± 2.0	28.7 ± 0.7
<u>Females</u>					
0	7.95 ± 0.27	47.2 ± 1.7	13.8 ± 0.4	60 ± 1.0	28.9 ± 0.5
20	8.12 ± 0.39	49.5 ± 2.6	14.1 ± 0.7	61 ± 1.0	28.5 ± 0.4
80	8.09 ± 0.40	49.3 ± 1.5	14.1 ± 0.6	62 ± 2.0	28.3 ± 0.3
320	7.81 ± 0.52	48.5 ± 3.5	13.6 ± 0.9	62* ± 1.0	28.3* ± 0.5

*Significantly different from control value ($p \leq 0.05$).

TABLE 4. Selected Thyroid Function Data for Rats Exposed to Mancozeb For 13 Weeks

Target Exposure Concentration (mg/m ³)	Group Mean ($\pm$ SD) T4 Serum Level (μ g/dl)	
	After 4 Weeks	After 13 Weeks
<u>Males</u>		
0	4.61 $\pm$ 1.22	4.39 $\pm$ 0.76
20	5.40 $\pm$ 0.75	4.17 $\pm$ 0.31
80	4.71 $\pm$ 0.59	4.50 $\pm$ 0.67
320	4.13 $\pm$ 1.11	4.02 $\pm$ 0.60
<u>Females</u>		
0	3.59 $\pm$ 0.77	3.17 $\pm$ 0.60
20	32.5 $\pm$ 1.13	3.11 $\pm$ 0.76
30	3.23 $\pm$ 0.53	2.77 $\pm$ 0.75
320	2.77 $\pm$ 0.54	2.18* $\pm$ 0.74

*Significantly different from control value ($p \leq 0.05$).

TABLE 5. Residue Analyses in Rats Exposed to Mancozeb for 13 Weeks

Target Exposure Concentration (mg/m ³)	Residue Levels (ppm); range or mean $\pm$ SD					
	Blood		Urine		Thyroid	
	ETU	EBOC	ETU	EBOC	ETU	EB
<u>Males</u>						
0	<0.07 to 0.14	<0.80 ^b	0.10 $\pm$ 0.07	<0.04 to 0.2	<5.9 to 13	-
20	<0.12 to 0.16	<0.80	0.32 $\pm$ 0.22	0.12 $\pm$ 0.07	<5.6 to 8.8	-
80	0.18 $\pm$ 0.15	<0.80	8.9 $\pm$ 5.9	0.66 $\pm$ 0.5	5.1 $\pm$ 2.5	-
320	0.14 $\pm$ 0.04	0.86 $\pm$ 0.13	13.0 $\pm$ 9.6	0.53 $\pm$ 0.32	7.7 $\pm$ 2.5	-
<u>Females</u>						
0	<0.07 to 0.22	<0.80	0.11 $\pm$ 0.07	<0.01 to 0.6	<10 to 14	-
20	<0.10 to 0.14	<0.80 to 1.6	0.17 $\pm$ 0.68	0.29 $\pm$ 0.13	<5.9 to 13	-
80	0.17 $\pm$ 0.10	<0.80	16.0 $\pm$ 8.3	1.3 $\pm$ 0.83	11.0 $\pm$ 5.0	-
320	0.45 $\pm$ 0.22	0.91 $\pm$ 0.5	69.0 $\pm$ 66	3.1 $\pm$ 2.2	28.0 $\pm$ 21	-

^aNot analyzed due to limited sample size.

^bValues that include symbol for less than (<) indicate the concentration was below limits of detection.

H. Absolute and Relative Organ Weights:

1. 4 Weeks of Exposure: The authors reported no effects on thyroid or lung weights of males or females in any group after 4 weeks of exposure.
2. 13 Weeks of Exposure: Absolute kidneys and heart weights were significantly reduced ($p \leq 0.05$) in males exposed to 320 mg/m^3 after 13 weeks of exposure. The authors concluded that these reductions resulted from the reduced terminal body weights, and were secondary effects of exposure to mancozeb. No other absolute or relative organ weight effects were observed in either sex in any other group. Table 6 presents mean absolute and relative lung, thyroid, kidney, and heart weights for males and females after 13 weeks of exposure.

- I. Ophthalmology: After 12 weeks of exposure to mancozeb, no exposure-related effects were observed in the eyes of any animal in any group. Bilateral retinal degeneration was observed in all of the animals whose cages were in the topmost position on the rack, and was attributed to an excessive amount of room light reaching these cages. One occurrence of urethritis and scattered occurrences of focal retinopathy were noted but were not considered exposure related by the authors.

J. Histopathology:

1. 4 Weeks of Exposure: Several gross and microscopic changes were seen in the tissues examined from the 4-week necropsy. These were scattered among the dosage and control groups, and the authors did not consider any of these changes to be exposure related.
2. 13 Weeks of Exposure: No exposure-related lesions were observed in males or females exposed to 20 mg/m^3 after 13 weeks of exposure to mancozeb. Males and females exposed to 30 or 320 mg/m^3 exhibited yellow-brown granular pigment in the lumen of the cortical tubules of the kidney. The authors considered this to be the result of the elimination of a pigmented metabolite which was considered to be produced as a consequence of exposure, but not toxicologically significant because there were no histopathologic changes seen in the kidney in animals of either group.

The occurrence of mild hyperplasia of the follicular epithelium in the thyroid glands of three females exposed to 320 mg/m^3 was considered to be related to exposure. No exposure-related lesions were seen in the thyroid glands of male rats.

TABLE 6. Selected Organ Weight Data for Rats Exposed to Mancozeb for 13 weeks

Target Exposure Concentration (mg/m ³)	Group Mean Value ($\pm$ SD) at 13 Weeks							
	Lung		Kidneys		Heart		Thyroid	
	Absolute (g)	Rel. to BW (x 1000)	Absolute (g)	Rel. to BW (x 1000)	Absolute (g)	Rel. to BW (x 1000)	Absolute (mg)	Rel. to BW (x 1000)
Males								
0	2.55 $\pm$ 0.29	6.01 $\pm$ 0.61	3.54 $\pm$ 0.29	8.35 $\pm$ 0.69	1.64 $\pm$ 0.15	3.87 $\pm$ 0.31	28.9 $\pm$ 4.1	0.07 $\pm$ 0.01
20	2.48 $\pm$ 0.29	5.89 $\pm$ 0.56	3.25 $\pm$ 0.43	7.73 $\pm$ 0.83	1.53 $\pm$ 0.17	3.64 $\pm$ 0.29	26.5 $\pm$ 2.4	0.06 $\pm$ 0.01
80	2.67 $\pm$ 0.79	6.25 $\pm$ 1.86	3.48 $\pm$ 0.29	8.15 $\pm$ 0.52	1.61 $\pm$ 0.12	3.78 $\pm$ 0.27	26.0 $\pm$ 5.1	0.06 $\pm$ 0.01
320	2.44 $\pm$ 0.31	6.08 $\pm$ 0.64	3.14* $\pm$ 0.32	7.84 $\pm$ 0.71	1.48* $\pm$ 0.13	3.70 $\pm$ 0.20	26.9 $\pm$ 3.3	0.06 $\pm$ 0.01
Females								
0	1.86 $\pm$ 0.16	8.53 $\pm$ 0.89	2.10 $\pm$ 0.13	9.65 $\pm$ 0.84	1.00 $\pm$ 0.07	4.59 $\pm$ 0.51	20.5 $\pm$ 3.5	0.07 $\pm$ 0.01
20	1.99 $\pm$ 0.35	8.38 $\pm$ 1.85	2.20 $\pm$ 0.31	9.17 $\pm$ 0.93	1.07 $\pm$ 0.10	4.45 $\pm$ 0.28	20.6 $\pm$ 4.5	0.08 $\pm$ 0.01
80	1.84 $\pm$ 0.17	8.20 $\pm$ 0.64	2.15 $\pm$ 0.30	9.55 $\pm$ 1.20	1.00 $\pm$ 0.09	4.45 $\pm$ 0.33	22.0 $\pm$ 2.2	0.09 $\pm$ 0.01
320	2.09 $\pm$ 0.38	9.08 $\pm$ 1.07	2.32 $\pm$ 0.70	10.84 $\pm$ 2.48	1.06 $\pm$ 0.14	4.64 $\pm$ 0.62	22.1 $\pm$ 2.4	0.05 $\pm$ 0.01

*Significantly different from control value (p $\leq$ 0.05).

Several histopathologic lesions were noted in the respiratory tract, but these were considered to be spontaneous and not related to exposure to mancozeb. Table 7 presents the incidence of findings in the respiratory tract, thyroid, and kidney.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. Four groups of 38 male and 38 female rats were exposed to target dust aerosol concentrations of 0, 20, 80, and 320 mg/m³ for 13 weeks. The actual respirable concentrations for the 13 weeks were 0, 8, 36, and 144 mg/m³, respectively.

After 4 weeks of exposure, significant ($p < 0.05$) reductions in body weight and body weight gain were noted for male rats exposed to the target concentration of 320 mg/m³. No other effects were considered to be related to exposure by the authors.

After 13 weeks of exposure, significant reductions were found in body weight and body weight gain in males as well as reductions in T4 levels in females exposed to the target concentrations of 320 mg/m³. In addition, microscopic examination of the thyroid glands in these females revealed hyperplasia of the follicular epithelium. No other effects were considered to be related to exposure to mancozeb.

There was a yellow-brown granular pigment in the convoluted tubules of male and female rats exposed to 80 or 320 mg/m³. The authors did not consider this pigment a manifestation of toxicity but likely to be the accumulation of the metabolite, ethylenebisisothiocyanate sulfate.

Based on these results, the NOAEL for mancozeb in rats was considered to be 36 mg/m³ and the LOAEL was considered to be 144 mg/m³ based on respirable concentration data.

- B. Signed quality assurance statements were dated February 27, 1986 for toxicity and January 13, 1985 for the residue report (Note: this is probably an error in year).

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Considerable variation in chamber concentrations was noted, especially early in this study. The ranges in daily mean chamber concentrations were narrower after the first 4 weeks. The characterization of the dust aerosol was adequate, and it is appropriate to base the toxicological findings on the respirable concentrations of 0, 8, 36, and 144 mg/m³ for the target concentrations of 0, 20, 80, and 320 mg/m³, respectively, as reflected in the following assessment.

TABLE 7. Incidence of Selected Histopathologic Lesions Found in Rats Exposed to Mancozeb for 13 Weeks

Organ/Finding	Target Exposure Concentration (mg/m ³)							
	Males				Females			
	0	20	80	320	0	20	80	320
<u>Kidney</u>								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--yellow-brown pigment, cortical tubules	0	0	5	8	0	0	10	9
<u>Thyroid</u>								
No. examined	(10)	(10)	(10)	(11)	(10)	(10)	(10)	(10)
--hyperplasia, follicular epithelium	0	0	0	0	0	0	0	3
<u>Lung</u>								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--multifocal interstitial inflammation	3	3	1	4	9	1	1	8
--foci of alveolar macrophages	5	0	0	3	2	0	1	4
--focal/multifocal hemorrhage	0	0	0	1	0	0	1	0
--diffuse acute congestion	0	1	0	1	0	1	1	1
<u>Nasal Turbinates</u>								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--congestion	0	0	0	0	0	0	1	1
--multifocal mononuclear cellular infiltration	2	2	3	4	0	1	0	2
--hemorrhage	0	0	0	0	0	0	1	1
<u>Trachea</u>								
No. examined	(10)	(11)	(10)	(11)	(10)	(11)	(11)	(11)
--focal tracheitis	1	0	1	3	0	2	2	3

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The body weight data revealed an exposure-related, significant reduction in body weight and weight gain for male rats exposed to 144 mg/m³. A significant increase in body weight gain in females exposed to 8 mg/m³ was also noted; however, these females were slightly smaller at study initiation, and the effect was not seen at higher exposure levels; this weight gain is therefore not considered related to exposure.

The hematology data reflect the general good health of these rats with low white cell counts and no unusual data among the red cell indices. The significant increase in mean corpuscular volume and decrease in mean corpuscular hemoglobin concentration are within the normal range of values for rats and were not considered to be related to exposure to mancozeb.

Triglyceride levels in males exposed to 144 mg/m³ and inorganic phosphorus levels in females exposed to 36 mg/m³ were significantly reduced when compared to controls. These changes were within the normal range of values for rats and were not considered to be related to exposure to mancozeb.

The significant reduction in serum T4 levels in females exposed to 144 mg/m³ noted at week 13 was considered to be related to exposure to mancozeb.

Residue analyses in blood, urine, and thyroids revealed increasing concentrations of ethylenethiourea and ethylenedisithiocarbamate with increasing exposure concentration. These data support the hypothesis that mancozeb is metabolized to ethylenethiourea and that the thyroid is a target organ for mancozeb toxicity.

The absolute weights of kidneys and heart were significantly reduced in males exposed to 144 mg/m³. The authors concluded that these differences were due to reduced body weight in these males. However, we calculated organ-to-brain weight ratios and found that the relative kidneys and heart weights were reduced, although not significantly for males exposed to 144 mg/m³.

No remarkable findings were noted during the ophthalmological examinations.

Microscopic examination of tissues taken at termination (13 weeks) necropsy revealed the presence of yellow-brown, granular pigment in males and females exposed to 36 mg/m³. The pigment was not present at the 4-week interim sacrifice and, thus, may represent possible progressive lesions in the kidney. In addition, mild hyperplasia of the follicular epithelium of the thyroid occurred in three males exposed to 144 mg/m³. These changes may be due at least in part to a compensatory reaction to hypothyroidism. Since hyperplasia was not present after the initial 4 weeks of exposure, it is possible that these changes indicate progressive

lesions in female rats. As expected for dust aerosol exposures, there were minor histologic changes in the nasal turbinates and trachea as well as congestion in the lungs of rats exposed to mancozeb. These changes may indicate progressive lung disease leading to the formation of granulomas in the lung and bronchial lymph nodes. Based on these histologic findings, the thyroid, kidneys, and respiratory tract are target organs in rats exposed to dust aerosols of mancozeb.

- B. The study was conducted in an acceptable manner and appropriate quality assurance inspections were reported.
- C. The effects on body weight in males and on the thyroid in females are similar to effects seen in other studies of mancozeb. The findings of the yellow-brown granular pigment in the kidneys, accompanied by a decrease in kidney weight, has not been reported previously. This may mean that the metabolic fate from inhaled mancozeb is at least slightly different from the fate of ingested mancozeb. The fact that the pigment is granular may indicate a serious kidney problem in rats exposed beyond 13 weeks. These inclusions were not present at the 4-week interim sacrifice while the incidence was high in animals exposed to 36 or 144 mg/m³. These inclusions may represent a form of kidney urolithiasis as seen with gout, cystinuria, or hyperoxaluria. Stones can form from oxalate, cysteine, uric acid, calcium carbonate, or calcium phosphate. Uric acid is particularly sensitive to pH with stones forming below pH 5.5. The exact nature of mancozeb and/or its metabolites in kidney function is not known; however, these renal inclusions cannot be ignored. An ongoing recovery study will reveal whether or not the pigment is cleared from the kidneys over time. The changes in the respiratory tract are not unexpected for dust aerosol exposures. These types of lesions can progress to the formation of granulomas in the lung and microgranulomas in the bronchial lymph nodes. The ongoing recovery study will give some indications of whether or not these are progressive lesions of the respiratory tract.

Based on these results, there are definite effects of exposure to a respirable concentration of 144 mg/m³ mancozeb; for males in this study it was body weight and for females effects were on thyroid function and were verified by histology on the thyroid. The seriousness of the renal inclusions (granular pigment) cannot be assessed. The inclusions were not present at 4 weeks, while the incidence was high in the 36 and 144 mg/m³ groups at study termination. Thus, we cannot ignore inclusions and based on these data, the LOAEL for rats exposed to mancozeb is 36 mg/m³ and the NOAEL is 9 mg/m³.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol.

MANCOZEB

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EPA: 68-02-4225
DYNAMAC No. 7-0090
May 27, 1986

DATA EVALUATION RECORD

MANCOZEB

Two-week Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Hagar, J. V., Fisher, J. R., and Baldwin, R. C.
Mancozeb: Two-week inhalation toxicity study in rats. (Unpublished study
No. 85R-190 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and
Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co.,
Wilmington, DE; dated February 27, 1986.) Accession No. 261538.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature:

I. Cecil Felkner

Date:

5-27-86

1. CHEMICAL: Mancozeb; Dithane M-45; Manzate 200; coordination product of zinc ion and manganese ethylenebisdithiocarbamate; $C_4H_6N_2S_4MnZn$.
2. TEST MATERIAL: Mancozeb (lot No. 43339; TD No. 85-015; product code 6-2804) was described as a yellow powder containing 83.35% active ingredient.
3. STUDY/ACTION TYPE: Two-week inhalation toxicity study in rats.
4. STUDY IDENTIFICATION: Hagan, J. V., Fisher, J. R., and Baldwin, R. C. Mancozeb: Two-week inhalation toxicity study in rats. (Unpublished study No. 85R-190 prepared by Rohm and Haas Co., Philadelphia, PA, for Rohm and Haas Co., Spring House, PA, and E. I. du Pont de Nemours and Co., Wilmington, DE; dated February 27, 1986.) Accession No. 261538.

5. REVIEWED BY:

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Jane Harris, Ph.D.
EPA Section Head

Signature: Jane HarrisDate: 5-27-86

7. CONCLUSIONS:

The purpose of this 2-week inhalation study was to select exposure levels and the mode of exposure for a 90-day inhalation study on mancozeb in rats. Four groups of 12 male (weighing 121-180 g) and 12 female (weighing 98-135 g) Crl:CD(SD)BR rats were exposed to dust aerosols of mancozeb for 6 hours per day for 10 days. Respirable concentrations were 0, 11, 55, or 258 mg/m³ with mass median diameters of 3.5 to 4.9 μ m and geometric standard deviations between 2.2 and 2.5. The four groups were further subdivided into whole-body exposed rats and nose-only exposed rats. Considerable difficulty was experienced in generating the dust aerosols because the daily mean concentrations varied from 5 to 82 mg/m³ in the low-exposure group, 61 to 300 mg/m³ in the mid-exposure group, and 402 to 681 mg/m³ in the high-exposure group. These daily concentrations yielded total mean aerosol concentrations of 0, 23, 138, and 519 mg/m³; however, the respirable fraction ranged from 38 to 49%, so that the mean respirable aerosol concentrations were 0, 11, 55, and 258 mg/m³, respectively.

Whole-Body Exposure: No deaths occurred during this study. Significant reductions were found in body weight and weight gain for rats exposed to 258 mg/m³, as well as significant reductions in female body weight and male and female weight gain in rats exposed to 55 mg/m³. No exposure-related effects were noted in rats exposed to 11 mg/m³. Male and female thyroxine (T₄) levels and male 3,5,3'-triiodo-L-thyronine (T₃) levels were significantly decreased after exposure to 55 or 258 mg/m³ mancozeb for 2 weeks. Thyroid-stimulating hormone (TSH) levels were nonsignificantly increased in rats exposed to 258 mg/m³ mancozeb. In addition, male and female lung weights and lung-to-body weight ratios were significantly increased in rats exposed to this same concentration. Exposure-related multifocal interstitial inflammation, microgranulomas, multifocal mixed inflammatory cell infiltration, focal or multifocal necrosis in the respiratory tract, and reactive lymphoid hyperplasia of the peribronchial lymph nodes were also found at 258 mg/m³.

Nose-Only Exposure: One male exposed to 258 mg/m³ died during this study due to asphyxiation caused by the animal twisting its head away from the nasal opening in the exposure tube. Alopecia was noted around the eyes of one female exposed to 55 mg/m³. No adverse effects were noted in males or females exposed to 11 or 55 mg/m³. Significant reductions in mean body weight (week 2) and mean body weight gain were noted during weeks 1 and 2 in males exposed to 258 mg/m³. There were no body weight effects in females exposed to 258 mg/m³. In addition, the T₃ and T₄ levels were significantly reduced and the lung-to-body weight ratio was increased in males exposed to 258 mg/m³. Microscopic examination of nasal turbinates revealed an increased incidence and degree of multifocal mixed inflammatory cell infiltration, i.e., mononuclear cells and neutrophils and multifocal or focal necrosis of the turbinate mucosa in four males and two females exposed to 258 mg/m³.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

- A. The study was conducted in a reasonable manner, and a signed quality assurance statement dated February 17, 1986, was included.
- B. Based on these results, nose-only was selected as the mode of exposure for the 90-day study, and based on the nose-only exposures, 258 mg/m³ is the LOEL and 55 mg/m³ is the NOEL for rats exposed to mancozeb for 10 exposures.
- C. Whole-body exposed animals were exposed to mancozeb dermally through fur and skin aerosol impaction and orally through preening (during and postexposure) and normal lung clearance mechanisms in addition to exposure via chamber air inhalation. As a result, it was assessed that these animals received a greater concentration of mancozeb than the nose-only exposed rats, as evidenced by the lower LOEL and NOEL, 55 mg/m³ and 11 mg/m³, respectively.
- D. This 2-week study was not designed as a Core study, but provides useful information. There were deficiencies in the generation system.

9. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-4.

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APPENDIX A
Materials and Methods

MANCOZEB

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