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

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OCT 4

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

September 20, 1991

MEMORANDUM

SUBJECT: Mancozeb---A. Review Tox. Data submitted under MRID No. 419036-01
---B. Identify remaining data gaps

Chemical: 913A
RD Record: S397410
HED Project: 1-1472

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

09-20-91

TO: Walt Waldrop/Terri Stowe, PM 71
Reregistration Branch
Special Review and Registration Division (H7508W)

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

9/20/91

Registrant: E. I. du Pont de Nemours, Newark DE.

Request: (A) Review and evaluate the following chronic study performed at du Pont's Haskell Lab. (Newark, DE):

"Combined Chronic Toxicity/Oncogenicity Study with Mancozeb: Two-year Feeding Study in Rats, Project #7859-001/Report No. 259-89, dated September 13, 1990 (EPA MRID 41903601)

TB Conclusions:

Doses Tested: 0, 20, 60, 125, 750 ppm in the diet, equivalent to (actual) compound intakes for males of, respectively: 0, 0.769, 2.33, 4.38 and 30.9 mg/kg/d; and for females: 0, 1.06, 3.06, 6.72 and 40.2 mg/kg/d.

For Chronic Toxicity:

NOEL = 60 ppm
.OEL - 125 ppm; renal pigment

Additionally, at the HDT (750 ppm): Reduced body weight gains, more severe in males; enlarged thyroids (both sexes); increased TSH and decreased T4; increased thyroid follicular cell hyperplasia/hypertrophy; increased thyroid weight (both absolute and relative to body weight); increased incidence and severity of bilateral retinopathy.

For Oncogenicity:

Increased thyroid follicular cell adenomas and carcinomas at the HDT (750 ppm) in both males and females, but statistically significant ($p<0.05$) only for high-dose males

NB: The Agency does not subscribe to the following conclusion of the Dynamac reviewer (found on p22, in the DER section: "Reviewer's Discussion and Interpretation of Results"), which was attributed by that reviewer to Paynter et al. ("Goitrogens and thyroid follicular cell neoplasia: Evidence for a threshold process." Reg. Toxicol. Pharmacol. 8:102-119, 1988):

"Although this mechanism* has been elucidated in rats, a species which is apparently most sensitive to thyroid tumor production, there is no evidence that this mechanism is of any biological significance in humans."

In fact, that was not exactly what Paynter and colleagues intended. The relevance of such a mechanism as demonstrated in rats to humans was expressed in this publication by the full quotation, cited as follows:

"In contrast, humans appear to be less sensitive, since release of stored thyroid hormones causes serum hormone levels to stay normal for weeks in euthyroid humans (Martindale, 1972) and for weeks to several months in hyperthyroid individuals (Odell et al., 1967) despite daily

*The sequence of increased TSH levels, decreased T4 levels, and thyroid hypertrophy/hyperplasia [is] associated with the formation of tumors in rats.

doses of antithyroid drugs sufficient to completely block hormone synthesis. Furthermore, thyroid cancer is statistically a minor health problem accounting for 0.4% of all cancer deaths (DeGroot, 1979)."

(Paynter et al. (1988, p. 103)

In other words, while the risk to humans may be low, the potential hazard remains.

(B) In response to the request to identify remaining data gaps: Adequate data are not yet available from a mouse oncogenicity study.

ATTACHMENT (DER for chronic rat study)

EPA No.: 68D80056
DYNAMAC No.: 392-A
TASK No.: 3-92A
August 2, 1991

00[illegible]

DATA EVALUATION RECORD

MANCOZEB

Chronic Toxicity/Oncogenicity Feeding Study in Rats

APPROVED BY:

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

Signature: William L McLellan for

Date: Aug 2, 1991

EPA No.: 68D80056
DYNAMAC No.: 392-A
TASK No.: 3-92A
August 1, 1991

008639

DATA EVALUATION RECORD

Mancozeb

Chronic Toxicity/Oncogenicity Feeding Study in Rats

REVIEWED BY:

John J. Licciore, Ph.D.
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Date: 8-2-91

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Date: Aug 2, 1991

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EPA Chief
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Signature: Karl F. Baetcke
Date: 9/20/91

DATA EVALUATION RECORD

GUIDELINE § 83-5

STUDY TYPE: Chronic toxicity/oncogenicity study in rats.

MRID NUMBER: 419036-01.

TEST MATERIAL: Mancozeb.

SYNONYM(S): Dithane m-45; manganese, [1,2-ethanediylbis-[carbamodithioatal]](2-)]-, mixture with [[1,2-ethanediylbis-[carbamodithioato]](2-)zinc.

STUDY NUMBER(S): 7859-001 (Project Number); 259-89 (Report Number).

SPONSOR: E. I. du Pont de Nemours and Company.

TESTING FACILITY: E. I. du Pont de Nemours and Company; Haskell Laboratory for Toxicology and Industrial Medicine; Newark, DE 19714.

TITLE OF REPORT: Combined Chronic Toxicity/Oncogenicity Study with Mancozeb Two-Year Feeding Study in Rats.

AUTHOR: Stadler, J.C.

REPORT ISSUED: September 13, 1990.

CONCLUSIONS:

Mancozeb was fed to male and female Crl:CD(BR) rats at dietary levels of 0, 20, 60, 125, or 750 ppm (males: 0, 0.769, 2.33, 4.38, and 30.9 mg/kg/day; females: 0, 1.06, 3.06, 6.72, and 40.2 mg/kg/day). Results of the study indicate that the thyroid is a target organ. Mancozeb increased thyroid-stimulating hormone (TSH) levels; decreased thyroxine (T_4) levels; increased the incidences of thyroid follicular cell hypertrophy/hyperplasia, thyroid follicular cell carcinomas and/or adenomas; and enlarged thyroids in the high-dose males and females. Thyroid masses were increased in incidence in the high-dose males. In both sexes receiving the high dose, an increase in absolute and relative thyroid weights was seen at 24 months. An increase in the incidence and severity of bilateral retinopathy was noted in the high-dose rats at 24 months. Granular yellowish-brown pigment was seen microscopically in the kidneys of males and females receiving 125 or 750 ppm at 12 and 24 months; however, there were no accompanying dose-related increases in histopathological lesions in the kidney. Reductions in body weight gains were noted in the high-dose males during the first year and throughout most of the second year of the study. A loss in body weight gain was noted in high-dose females during the first 91 days of treatment; although these females initially recovered from the loss in body weight gain, body weight gains were still lower than controls at the end of 1 year. The incidence of diarrhea was lower in the high-dose males as compared to controls. No effects on survival, food consumption, hematology, ophthalmology, or urinalysis were observed.

Based on effects in kidneys, the LOEL is 125 ppm, and the NOEL is 60 ppm.

Classification: This study satisfies the Guideline requirements for a chronic toxicity/oncogenicity study (83-5).

A. MATERIALS:

1. Test Compound: Mancozeb (Dithane M-45); description: gray-yellow powder; lot No.: 56831; purity: 83.8% (initial purity).

2. Test Animals: Species: rat; strain: Crl:CD BR; age: 22 days at arrival; weight: males--148.8 ± 11.7 g, females--115.2 ± 10.9 g; source: Charles River Laboratories, Inc., Kingston, New York.

B. STUDY DESIGN:

1. Animal Assignment: Rats were acclimated to laboratory conditions 15 days prior to treatment. They were assigned by sex to the following test groups using a computer-generated randomization procedure that stratified the animals within each sex by body weight:

Test Group	Dietary Level (ppm)[a]			Main study (24 Months)		Interim Sacrifice (12 Months)	
	Week:			Males	Females	Males	Females
	1-2	3-4	5-104				
1 Control	0	0	0	62	62	10	10
2 Low	10	15	20	62	62	10	10
3 Mid-1	30	45	60	62	62	10	10
4 MDT-2	65	95	125	62	62	10	10
5 High	375	565	750	62	62	10	10

[a]Diets were mixed at lower concentrations during the first 4 weeks of the study and adjusted upward to target levels. This was done to produce mean daily intake levels of mancozeb (mg/kg) that would be similar in the young, rapidly growing rats to the mean daily intake predicted for mature rats during the remainder of the study.

Dose levels were selected on the basis of the results of previous toxicity studies in rats. These studies were not available for review. In two 13-week studies, rats were administered mancozeb in the diet at concentrations of 100, 1,000, and 10,000 ppm or 25, 50, 75, and 150 ppm. Reduced body weights, decreased food consumption, increased organ weights, and an increase in mortality, when compared to controls, were observed in rats fed 10,000 ppm. Rats fed 10,000 ppm mancozeb showed an increase in thyroid and spleen weights. Thyroid hyperplasia was noted in rats fed 1,000 or 10,000 ppm mancozeb. In another study, rats were administered 625 or 1,250 ppm mancozeb for 12 weeks with a 10-week recovery period. Changes in T_3, T_4, and TSH levels were observed in rats at the end of the feeding period; all but the T_3 levels returned to normal levels prior to the end of the recovery period. Also, mild thyroid hyperplasia

was noted at 12 weeks of treatment; however, there were no histological changes in the thyroid at the end of the recovery period. In a 90-day study, rats were fed 30, 60, 125, 250, or 1000 ppm mancozeb. Decreased body weights and food consumption, changes in clinical chemistry parameters, increased liver and spleen weights, and histopathological changes in the liver, thyroid, pituitary, and adrenal cortex were noted in the high-dose rats. Alterations in T_4 and TSH levels were observed in rats fed 250 or 1000 ppm.

In a chronic toxicity/oncogenicity study in rats, no effects were seen on survival, clinical signs, body weight gain, food consumption, clinical pathology parameters, metabolic rate, or organ weights in rats fed 25, 50, 200, or 1000 ppm mancozeb for 90 weeks. However, a compound-related hyperplasia of the thyroid acinar epithelium was noted in the high-dose rats.

In the present study, rats were initially housed three/cage for acclimation but were individually housed during treatment in a room with temperature and humidity controls set at 23 ± 2°C and 50 ± 10%, respectively, and with a 12-hour light/dark cycle.

2. Diet Preparation: Test diets were prepared by adding an appropriate amount of mancozeb to rodent chow and were thoroughly mixed to assure homogeneous distribution in the diet. All diets were prepared weekly.

At approximately monthly intervals, dietary samples were obtained from each dose group and immediately frozen to prevent the decomposition of mancozeb to ethylene thiourea (ETU). During the first year, samples were analyzed once each month for content of ethylenebisdithiocarbamate (EBDC), the active ingredient in mancozeb, and also analyzed for ETU. During the second year, randomly selected samples from those collected once each month were submitted.

The homogeneity of the test substance (samples derived from the top, middle, and bottom layers of the mixing vessel and immediately frozen) in the diets at levels of 0, 10, 30, 65, and 375 ppm was determined at day -1, at levels of 0, 15, 45, 95, and 565 ppm at day 13, and at levels of 0, 20, 60, 125, and 750 ppm at day 27. Homogeneity samples were also obtained on day 328 when a low-speed mixer was first used to prepare test diets and on day 650 when the quantity of diet at each dietary concentration was reduced. The stability of the test substance in the diet was determined on test days -1, 27, and 174.

Results: Mean concentrations of mancozeb in the diets at dose levels of 20, 60, 125, and 750 ppm were 99.3 ± 3.7%, 100.6 ± 2.9%, 99.2 ± 3.0%, and 97.9 ± 2.1% of target dose, respectively. Mean concentrations of ethylene thiourea in the diets at these same dose levels were 0.83 ± 0.28%, 0.79 ± 0.22%, 1.12 ± 0.34%, and 1.08 ± 0.24% of the nominal mancozeb concentrations, respectively. Results of samples analyzed to determine homogeneity of the test substance in the diet at levels of 20, 60, 125, and 750 ppm indicated a homogeneous mix. Results of stability analysis indicated that the test substance, at concentrations of 20, 60, 125, and 750 ppm, was stable over a period of 8 days after mixing.

3. Food and Water Consumption: Animals received food (Irradiated Purina Certified Rodent Chow No. 5002) and water ad libitum.

4. Statistics: Body weight, organ weight and clinical laboratory parameters were analyzed by one-way analysis of variance using the F-test to assess significance. For body weight and clinical laboratory parameters, Dunnett's test was used to determine which means were significantly different from the control. Organ weight comparisons were made with the least-square differences and Dunnett's tests.

   Incidences of clinical observations were assessed by the Fisher's Exact test with a Bonferroni correction and the Cochran-Armitage test for trend. For the evaluation of histopathology data, the Fisher's Exact test was applied.

5. Quality Assurance: A quality assurance statement was signed and dated September 10, 1990.

C. METHODS AND RESULTS:

1. Observations: All animals were observed at least once daily for abnormal behavior and appearance, moribundity, and mortality. Animals were also examined for abnormal behavior and appearance at every weighing.

   Results: Table 1 summarizes data on cumulative mortality and percent survival. The mortality of the animals was not affected by the administration of mancozeb. The only clinical sign attributable to treatment was a significant (0.05) low incidence of diarrhea in the high-dose males (14 of 72 males) versus control males (36 of 72 males). A nonsignificant decrease in the incidence of diarrhea was noted in the high-dose females (4 of 72 high-dose females versus 12 of 72 control females). The significance of this finding is not clear.

TABLE 1. Cumulative Mortality and Percent Survival in Rats Fed Mancozeb for 104 Weeks[a]

Dietary level (ppm)	Mortality (percent survival) at days:					
	378	448	518	588	658	728
			Males			
0	3(95)	5(92)	12(81)	19(69)	33(47)	46(26)
20	0(100)	1(98)	9(85)	20(68)	30(52)	44(29)
60	1(98)	5(92)	11(82)	19(69)	29(53)	38(39)
125	5(92)	7(89)	12(81)	22(65)	36(42)	52(16)
750	1(98)	5(92)	11(82)	20(68)	32(48)	49(21)
			Females			
0	0(100)	5(92)	12(81)	16(74)	29(53)	39(37)
20	2(97)	5(92)	9(85)	16(74)	21(66)	29(53)
60	1(98)	2(97)	6(90)	17(73)	29(53)	38(39)
125	1(98)	3(95)	8(87)	17(73)	32(48)	38(39)
750	2(97)	4(94)	8(87)	14(77)	29(53)	36(42)

[a]Mortality and percent survival were based on 62 rats/sex/dose of the main group. An additional 10 rats/sex/dose survived until their respective scheduled sacrifice dates and are not included in this table.

2. Body Weight: Body weights were recorded weekly during the first 6 months and once every other week thereafter.

Results: Tables 2A and 2B summarize data on mean body weights and men body weight gains, respectively, at selected intervals. Mean body weight gain in the high-dose males was lower than controls during the first year of treatment; the decreases reached statistical significance ($p < 0.05$) at some of the weekly intervals during the first year. At the end of one year, mean body gain in the high-dose males was significantly ($p < 0.05$) lower (8.8%) than controls. Mean body weight gain in the high-dose males was also lower than controls during most of the second year, but the decreases in body weight gain did not reach statistical significance. Between days 0 and 728, mean body weight gain in the high-dose males was 7.8% lower than controls. Between days 0 and 91, mean body weight gain in the high-dose females was significantly ($p < 0.05$) lower (16.3%) than controls. The decrease in body weight gain in the high-dose females was regarded by the study authors to be treatment-related. Following day 91, the high-dose females recovered from the loss in body weight gain, but by the end of the first year of treatment, body weight gain in these females was significantly ($p < 0.05$) lower (11.8%) than controls. Also, at the end of one year of treatment, mean body weight gains in females receiving 20, 60, and 125 ppm were 10.3% ($p < 0.05$), 1.0%, and 10.4% ($p < 0.05$), respectively, than controls. The decrease in body weight gains in low-dose, mid-dose, and high-dose females at 12 months was not considered treatment-related since there was no dose-response relationship. There were no remarkable changes in body weight gains by females of all dose groups between 1 and 2 years of treatment.

3. Food Consumption and Compound Intake: Food consumption was determined weekly. Compound intake (mg/kg/day) was calculated based on food consumption and body weight data.

Results: There was no effect of dosing on food consumption.

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to study initiation and prior to study termination.

Results: There were no abnormalities of the eyes that were induced by treatment with mancozeb.

5. Hematology and Clinical Chemistry: Blood was collected from the orbital sinus for clinical laboratory evaluations at approximately 3, 6, 12, 18, and 24 months after study initiation. With the exception of the 24-month sampling

TABLE 2A. Mean Body Weights at Selected Intervals for Rats Fed Mancozeb for 104 Weeks[a]

Dietary Level (ppm)	Mean body weight (g ± S.D.) at study days: 0	91	182	364	546	728
			Males			
0	148.8 ± 11.7	559.8 ± 52.9	674.2 ± 78.5	798.0 ± 97.3	826.8 ± 136	680.4 ± 183
20	146.7 ± 11.2	550.2 ± 50.4	661.1 ± 72.8	781.9 ± 94.8	829.7 ± 127	717.5 ± 138
60	147.2 ± 11.3	559.1 ± 48.1	671.4 ± 71.1	800.5 ± 102	829.1 ± 138	726.7 ± 112
125	149.3 ± 12.7	568.1 ± 49.7	681.7 ± 73.1	796.6 ± 87.5	833.0 ± 106	742.1 ± 111
750	147.3 ± 11.6	525.1 ± 50.7	630.7 ± 72.6	739.1 ± 92.4	731.5 ± 94.7	633.6 ± 128
			Females			
0	115.2 ± 10.9	282.6 ± 34.8	346.7 ± 48.6	457.3 ± 44.8	520.8 ± 114	522.5 ± 140
20	112.1 ± 10.2	273.3 ± 29.9	328.2 ± 42.7	419.4 ± 66.7	501.4 ± 91.6	531.1 ± 124
60	112.8 ± 10.1	281.7 ± 25.3	340.5 ± 41.8	451.4 ± 69.7	527.1 ± 124	479.6 ± 118
125	113.0 ± 10.7	271.3 ± 29.1	327.5 ± 45.4	419.4 ± 73.7	496.4 ± 940	502.0 ± 107
750	112.7 ± 9.9	252.8 ± 27.9	312.2 ± 47.1	414.3 ± 69.0	490.2 ± 87.3	476.3 ± 146

[a]Data were extracted from study No. 7859-001, Tables 2 and 3.

TABLE 28. Representative Results of Mean Body Weight Gains of Rats Fed Mancozeb for 104 Weeks

Dietary level (ppm)	Mean Body Weight Gain (g ± S.D.) at Days: 0-91	0-364	364-728	0-728
		Males		
0	411.3 ± 50.2	649.7 ± 94.9	-50.2 ± 136	533.7 ± 185
20	403.5 ± 47.9	635.2 ± 92.7	-42.5 ± 155	573.1 ± 133
60	411.9 ± 47.2	653.2 ± 102	-37.3 ± 123	578.7 ± 114
125	419.0 ± 46.9	647.7 ± 84.9	-3.7 ± 142	600.3 ± 110
750	377.7 ± 48.6	591.8 ± 91.0*	-76.0 ± 156	492.0 ± 130
		Females		
0	167.4 ± 31.4	342.1 ± 71.7	69.6 ± 139	410.2 ± 138
20	161.2 ± 28.2	306.9 ± 64.6*	109.9 ± 89.6	419.1 ± 123
60	168.8 ± 23.9	338.6 ± 68.1	39.4 ± 108	367.3 ± 116
125	158.4 ± 27.6	306.6 ± 72.4*	108.1 ± 85.8	389.8 ± 107
750	140.1 ± 28.5*	301.7 ± 69.0*	74.5 ± 144	361.1 ± 143

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

period, twenty male and twenty female rats/group were randomly selected each time for examination). Blood samples from the first 10 rats/group were used for hematology and clinical chemistry evaluations and serum samples from the remaining 10 rats/group were submitted for thyroid function analysis. Rats utilized for hematology and clinical chemistry evaluations were fasted for approximately 16 hours prior to blood collection. Rats designated for thyroid function assays were not fasted. At the 24-month sampling period, only ten rats/group were selected for hematology and clinical chemistry evaluations; no thyroid function assays were performed at this period. The CHECKED (X) parameters were examined:

a. Hematology:

X Hematocrit (HCT)†
X Hemoglobin†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Leukocyte differential count
X Mean corpuscular HCB (MCH)
X Mean corpuscular volume (MCV)

Results: No effects of dosing on hematology parameters were seen.

b. Clinical Chemistry:

Electrolytes

X Calcium†
X Potassium†
X Sodium†

Other

X Albumin†
X Blood creatinine†
X Blood urea nitrogen†
X Cholesterol†
X Globulins
X Glucose†
X Total protein†

Enzymes

X Alkaline phosphatase (ALP)
Cholinesterase
X Creatine kinase†
Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)†
X Serum aspartate aminotransferase (SGOT)†

†Recommended by Subdivision F (November 1984) Guidelines.

Results: Alterations in thyroid function were noted in the high-dose rats (Table 3). Decreased levels of thyroxine (T_4) were seen in high-dose males at months 3, 6, 12, and 18; the decreases were significant ($p < 0.05$) at months 6 and 18. Levels of T_4 were decreased in high-dose females at months 3, 6, 12, 18, and 24; the decreases were significant ($p < 0.05$) at months 3, 18, and 24. Levels of thyroid-stimulating hormone (TSH) were increased in the high-dose males at 3, 6, 12, 18, and 24 months of treatment; the increases were significant ($p < 0.05$) at months 12 and 18. Levels of TSH were also increased in the high-dose females at 3, 6, 12, 18, and 24 months of treatment; the increases were significant ($p < 0.05$) at months 6 and 18. These findings were considered by the study author's to be consistent with an effect on hormones associated with thyroid function. There were no consistent effects on triiodothyronine (T_3) levels except for decreased levels in males receiving 125 ppm and 750 ppm at month 3 and in females receiving 60 ppm, 125 ppm, and 750 ppm at month 3.

6. Urinalysis: Urinalysis was performed on 10 rats/group that were designated for hematology and clinical chemistry evaluations. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones
X	pH	X	Bilirubin†
X	Sediment (microscopic)†	X	Blood†
X	Protein†	X	Urobilinogen
X	Osmolarity†		

Results: No effects of treatment with mancozeb on urinary parameters were seen.

7. Sacrifice and Pathology: All animals that died and that were sacrificed (scheduled or unscheduled) were subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examination from control rats, high-dose rats, and rats found dead or sacrificed in extremis. The liver, kidneys, lungs, thyroids, parathyroids, and organs with gross lesions from rats fed 20-, 60-, and 125-ppm diets were also microscopically examined. In addition, the (XX) organs were weighed:

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Thyroid Function Parameters (Mean ± S.D.) in Rats Fed Mancozeb for 104 weeks[a]

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Parameter / Dietary Level (ppm)	Sampling time (months): 3	6	12	18	24
Males					
T_3 (ng/dL)					
0	110 ± 18	90 ± 18	67 ± 14	66 ± 15	47 ± 18
20	128 ± 27	104 ± 14	75 ± 10	52 ± 15	66 ± 62
60	114 ± 26	89 ± 11	66 ± 11	78 ± 13	65 ± 30
125	82 ± 22*	110 ± 26	80 ± 14	85 ± 24	57 ± 8
750	77 ± 12*	111 ± 15*	81 ± 15	80 ± 24	67 ± 10*
T_4 (µg/dL)					
0	3.6 ± 0.6	3.8 ± 0.8	4.0 ± 1.1	3.1 ± 1.2	1.8 ± 0.8
20	4.2 ± 0.8	4.1 ± 0.9	5.2 ± 0.9*	2.8 ± 0.5	2.6 ± 3.0
60	4.5 ± 0.9*	4.1 ± 0.2	3.9 ± 1.1	3.4 ± 1.2	1.5 ± 0.5
125	4.3 ± 0.6	3.8 ± 0.7	4.4 ± 0.8	3.1 ± 1.0	1.4 ± 0.4
750	3.0 ± 0.5	3.1 ± 0.4*	3.4 ± 0.8	2.0 ± 0.7*	1.8 ± 0.5
TSH (ng/mL)					
0	5.6 ± 1.8	4.8 ± 7.5	3.0 ± 0.7	3.6 ± 1.3	2.9 ± 1.1
20	4.9 ± 2.5	5.1 ± 2.2	2.7 ± 1.0	2.5 ± 0.7	3.5 ± 1.2
60	4.1 ± 2.5	3.1 ± 1.7	1.9 ± 0.5*	2.5 ± 1.3	3.3 ± 2.1
125	3.9 ± 1.5	3.8 ± 1.8	2.2 ± 0.3	2.8 ± 1.3	4.3 ± 1.5*
750	6.2 ± 3.0	6.2 ± 2.9	4.0 ± 1.1*	5.5 ± 1.3*	7.0 ± 7.9
Females					
T_3 (ng/dL)					
0	116 ± 25	114 ± 25	78 ± 21	76 ± 13	69 ± 21
20	105 ± 10	126 ± 32	79 ± 15	64 ± 21	68 ± 17
60	91 ± 14*	114 ± 20	74 ± 16	71 ± 8	78 ± 27
125	82 ± 15*	124 ± 15	72 ± 16	78 ± 18	79 ± 20
750	80 ± 14*	120 ± 14	70 ± 16	78 ± 14	86 ± 22
T_4 (µg/dL)					
0	2.2 ± 0.7	2.3 ± 0.5	2.7 ± 1.0	2.7 ± 0.5	1.4 ± 0.2
20	2.3 ± 0.8	2.5 ± 0.6	2.8 ± 0.8	2.1 ± 0.7*	67 ± 0.6
60	2.0 ± 0.7	2.8 ± 0.9	3.5 ± 0.9	2.5 ± 0.3	1.5 ± 0.4
125	2.2 ± 0.5	2.6 ± 0.7	3.3 ± 0.7	2.7 ± 0.7	1.9 ± 0.9
750	1.1 ± 0.5*	1.9 ± 0.5	2.5 ± 1.1	2.1 ± 0.4*	1.1 ± 0.1*
TSH (ng/mL)					
0	3.7 ± 1.6	3.4 ± 0.9	3.0 ± 0.7	2.2 ± 0.8	4.2 ± 1.7
20	3.1 ± 1.2	3.6 ± 1.1	2.8 ± 1.0	2.2 ± 0.9	3.4 ± 1.6
60	2.9 ± 1.4	3.3 ± 1.7	2.6 ± 1.2	3.1 ± 1.8	3.0 ± 1.5
125	3.0 ± 1.99	3.3 ± 1.3	2.6 ± 1.1	2.9 ± 0.8	2.4 ± 1.2
750	4.4 ± 2.1	6.0 ± 2.4*	3.5 ± 1.7	4.7 ± 2.9	5.2 ± 3.5

[a]Data were extracted from study No. 7859-001, Tables 20 and 21.

*Significantly different from control values $p \leq 0.05$.

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

Results:

a. Organ Weights: Table 4 presents selected organ weight data for rats fed mancozeb for 1 year or 2 years. After 24 months of treatment, absolute and relative (to body weight) thyroid/parathyroid weights of the high-dose males were significantly ($p < 0.05$) increased by 60% and 62 %, respectively, when compared with controls. Also, absolute and relative (to body weight, thyroid/parathyroid weights of the high-dose females were significantly ($p < 0.05$) increased by 43% and 47%, respectively, when compared to controls at 24 months of treatment. The increases in absolute and relative thyroid/parathyroid weights in high-dose males, and females were considered by the study authors to be treatment-related.

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 4. Selected Organ Weights (Mean ± S.D.) and Organ-to-Body Weight in Rats Fed Mancozeb for 1 Year or 2 Years[a]

Organ/Dietary Level (ppm)	12 month		24 month	
	Absolute (g)	Relative-to-body (%)	Absolute (g)	Relative-to-body (%)
		Males		
Liver				
0	26.53 ± 3.74	3.24 ± 0.32	22.24 ± 6.20	3.25 ± 0.48
20	25.87 ± 3.78	3.29 ± 0.28	24.37 ± 4.96	3.38 ± 0.51
60	26.00 ± 3.15	3.28 ± 0.35	24.51 ± 3.58	3.48 ± 0.60
125	27.34 ± 3.37	3.32 ± 0.28	2.07 ± 2.61	3.25 ± 0.65
750	26.70 ± 3.75	3.69 ± 0.31*	24.63 ± 4.98	4.20 ± 1.18*
Testes				
0	3.76 ± 0.17	0.46 ± 0.04	3.11 ± 0.80	3.45 ± 0.07
20	3.57 ± 0.81	0.46 ± 0.06	3.23 ± 0.63	0.46 ± 0.13
60	3.64 ± 0.26	0.47 ± 0.08	3.35 ± 0.81	0.47 ± 0.10
125	3.82 ± 0.34	0.47 ± 0.03	3.22 ± 0.75	0.44 ± 0.13
750	3.86 ± 0.39	0.54 ± 0.06*	3.41 ± 0.59	0.57 ± 0.13
Thyroids/parathyroids				
0	0.046 ± 0.014	0.0056 ± 0.0019	0.047 ± 0.015	0.0073 ± 0.030
20	0.049 ± 0.024	0.0062 ± 0.0028	0.045 ± 0.013	0.0064 ± 0.0024
60	0.041 ± 0.008	0.0052 ± 0.0014	0.049 ± 0.012	0.0070 ± 0.0023
125	0.039 ± 0.013	0.0047 ± 0.0015	0.0052 ± 0.007	0.0071 ± 0.0015
750	0.048 ± 0.008	0.0068 ± 0.0012	0.075 ± 0.017*	0.0118 ± 0.0034*
		Females		
Liver				
0	14.76 ± 4.80	3.26 ± 0.42	17.67 ± 4.36	3.50 ± 0.76
20	13.79 ± 2.51	3.27 ± 0.26	18.30 ± 5.37	3.44 ± 1.06
60	14.54 ± 2.33	3.17 ± 0.47	15.99 ± 4.37	5.43 ± 0.73
125	13.42 ± 1.18	3.04 ± 0.38	16.65 ± 3.43	3.44 ± 0.54
750	15.74 ± 3.23	3.64 ± 0.50	18.70 ± 4.21	3.88 ± 0.68
Thyroids/parathyroids				
0	0.037 ± 0.011	0.0084 ± 0.0028	0.037 ± 0.007	0.0078 ± 0.0024
20	0.027 ± 0.008*	0.0065 ± 0.0020	0.040 ± 0.012	0.007, ± 0.0025
60	0.037 ± 0.005	0.0080 ± 0.0013	0.036 ± 0.009	0.0078 ± 0.0021
125	0.032 ± 0.004	0.0076 ± 0.0011	0.041 ± 0.010	0.0085 ± 0.0018
750	0.039 ± 0.006	0.0090 ± 0.0014	0.053 ± 0.011*	0.0115 ± 0.0036*

[a]Data were extracted from study No. 7859-001, Tables 24-27.

*Significantly different from control value, $p < 0.05$.

At the interim sacrifice (12 months), the liver and testes weights relative to body weights of the high-dose males were significantly ($p < 0.05$) increased; however, absolute liver and testes weights were similar to controls. Also, the liver weights relative to body weights of the high-dose males at 24 months of treatment was significantly ($p < 0.05$) increased, but absolute liver weights were similar to control. The study author attributed the increases in the relative weights of the liver and testes to the lower body weights of the high-dose males.

b. Gross Pathology: The incidence of enlarged thyroids was higher in males receiving 750 ppm (14 of 61 males) when compared to controls (2 of 60 males). Also, the incidence of thyroid masses was higher in males receiving 750 ppm (9 of 61 males) when compared to controls 90 of 60 males). The incidence of enlarged thyroids was also high in females receiving 750 ppm (5 of 61 females) when compared to controls (0 of 62 females). Other gross pathological findings were considered by the study author to be incidental.

c. Microscopic Pathology:

1) Nonneoplastic: Follicular cell hypertrophy/hyperplasia of the thyroid gland was noted in the high-dose males (10 of 11 males) and females (10 of 11 females) that died or were sacrificed during the first year of treatment. The incidence of this thyroid lesion in the high-dose rats was significantly ($p < 0.05$) increased over control incidence. The severity of this lesion was greater in the males than females. Granular, yellow-brown pigment was present in the kidneys of rats fed 125 ppm (observed in 2 of 14 males, and in 10 of 11 females) or 750 ppm (observed in 10 of 11 males, and in 11 of 11 females) for 1 year. However, there were no corresponding pathological changes in the kidney. Treatment-related findings were noted in males and females treated with 750 ppm mancozeb for 2 years (results summarized in Table 5). The incidences of thyroid C-cell nodular hyperplasia and thyroid follicular cell hypertrophy/hyperplasia were significantly ($p < 0.05$) increased is the high-dose males and females. High-dose males and females also exhibited significantly ($p < 0.05$) increased incidences and severity of bilateral retinopathy. Although the incidence of bilateral retinopathy was significantly ($p < 0.05$) increased in females receiving 125 ppm as assessed by Fisher's Exact

TABLE 5. Selected Nonneoplastic Findings in Main Study Rats Fed Mancozeb for 104 Weeks[a]

Organ/Finding	Dietary level (ppm)									
	Males					Females				
	0	20	60	125	750	0	20	60	125	750
Adrenal cortex	(60)[b]	(47)	(40)	(51)	(61)	(62)	(34)	(42)	(42)	(61)
Necrosis	0	1	3	2	7*	0	1	2	1	3
Cecum	(55)	(40)	(29)	(39)	(55)	(61)	(29)	(36)	(38)	(59)
Inflammation	0	1	2	1	7*	2	1	1	2	5
Eyes	(60)	(46)	(37)	(58)	(61)	(62)	(60)	(62)	(61)	(60)
Retinopathy, bilateral	4	2	1	3	19*	21	28	24	31*	49*
Retinopathy, unilateral	8	7	0	8	9	14	10	10	6*	3*
Kidneys	(60)	(62)	(61)	(58)	(61)	(62)	(60)	(62)	(61)	(61)
Pigment, yellow-brown, glassy, globular	0	0	0	15*	25*	0	0	0	27*	53*
Lungs	(60)	(62)	(61)	(58)	(61)	(62)	(60)	(62)	(61)	(61)
Histiocytosis, focal	8	11	8	8	10	13	7	7	5*	3*
Lymph node-mandibular	(60)	(44)	(37)	(48)	(61)	(61)	(30)	(40)	(38)	(61)
Lymphangiectasis	10	5	2	2	1*	8	2	1	1	0*
Pituitary	(60)	(55)	(45)	(51)	(61)	(62)	(49)	(57)	(56)	(61)
Hyperplasia, pars intermedia	1	0	1	3	6	0	0	0	0	0
Thyroid	(60)	(62)	(60)	(58)	(61)	(62)	(60)	(62)	(61)	(61)
Hyperplasia, nodular C-cell	2	7	2	5	3	2	6	5	3	7
Hyperplasia, nodular, follicle	0	1	3	2	15*	0	2	2	0	11*
Hypertrophy/hyperplasia follicle cell	1	1	2	1	34*	1	0	1	0	24*

[a]Data were extracted from study No. 7859-001, Tables 34 and 35.

[b]Numbers in parentheses indicate number of animals with tissues examined.

*Significantly different from control group by Fisher's Exact test ($p \leq 0.05$).

test, results of the Cochran-Armitage test for trend indicated that only the high-dose rats had a significant increase in this lesion.

2) Neoplastic: A summary of the incidence of neoplasms in the thyroid of rats treated with mancozeb for 2 years is presented in Table 6. The incidence of thyroid follicular cell carcinomas or adenomas was increased in the high-dose males and females, as compared to controls. The incidences of rats with carcinomas or adenomas were higher for males (14/61 and 20/61, respectively) than for females (4/61 and 6/61). Six high-dose males exhibited both carcinomas and adenomas. Those males and females with thyroid carcinomas or adenomas also demonstrated decreased levels of thyroxine and elevated levels of thyroid-stimulating hormone. The study author attributed these thyroid changes to ethylene thiourea (ETU), a known thyroid carcinogen and a metabolite of mancozeb.

D. STUDY AUTHOR CONCLUSIONS:

Mancozeb was administered via the diet to five main groups of Crl:CD(BR) rats (62/sex/group) at dose levels of 0, 20, 60, 125, or 750 ppm for 2 years. In addition, five groups of satellite rats (10/sex/group) received 0, 20, 60, 125, or 750 ppm for 1 years. A statistically significant loss in body weight was seen in the high-dose males when compared to controls during the first year of the study. The lower mean body weight in the high-dose males persisted throughout the second year of the study, although the loss in body weight did not reach statistical significance. The loss in body weight is attributed to the intake of the test substance. A compound-related and statistically significant low body weight gain was seen in high-dose females during days 0-91; the high-dose females recovered from the loss in body weight gain after the first 3 months. Over the course of the first year, body weight gains for females receiving 20, 125, and 750 ppm were lower than controls; the loss in body weight in these females was not attributed to intake of mancozeb.

There was no treatment-related effect on food consumption. The mean daily intake of males receiving 0, 20, 60, 125, or 750 ppm was 0, 0.769, 2.33, 4.83, and 30.9 mg mancozeb/kg bw/day, respectively. The mean daily intake in females receiving the same dietary concentrations was 0, 1.06, 3.06, 6.72, and 40.2 mg mancozeb/kg bw/day.

TABLE 6. Neoplastic Findings in the Thyroid of Main Study Rats Fed Mancozeb for 104 Weeks[a]

Organ/ Finding	Dietary level (ppm)									
	Males					Females				
	0	20	60	125	750	0	20	60	125	750
Thyroid	(60)[b]	(62)	(60)	(58)	(61)	(62)	(60)	(62)	(61)	(61)
Adenoma (benign), C-Cell	2	5	7	6	4	9	6	6	6	3
Adenoma (benign), follicular cell	0	1	1	0	20*	1	1	1	1	6
Carcinoma (malignant, primary) C-Cell	0	0	1	0	1	2	4	0	1	1
Carcinoma (malignant, primary) epidemial	0	1	0	0	0	0	0	0	0	0
Carcinoma (malignant, follicular cell	0	0	2	2	14*	0	0	0	1	4
Malignant (metastatic/ multicenter) leukocyte granulocyte	0	0	0	0	0	0	0	1	0	0

[a]Data were extracted from study No. 7859-001, Tables 34 and 35.

[b]Numbers in parentheses indicate number of animals with tissues examined.

*Significantly different from control group by Fisher's Exact test ($p \leq 0.05$).

The incidence of diarrhea was lower in the high-dose males; this effect was considered compound related. There was no effect of dosing on ophthalmology, mortality, or urinalysis. Mean thyroxine (T_4) levels were decreased, and mean levels of thyroid-stimulating hormone (TSH) were increased in high-dose males and females when compared to controls. At interim sacrifice, significant increases in mean relative liver and testes weights were seen in the high-dose males; however, the increases in the relative organ weights were attributed to the lower body weights in the males. After 24 months of treatment, mean relative liver weights were increased in high-dose males, while mean absolute and relative thyroid/parathyroid weights were increased in both high-dose males and females.

Enlarged thyroids in both sexes receiving 750 ppm and thyroid masses in males receiving 750 ppm were observed during the second year of treatment. Follicular cell hypertrophy/hyperplasia of the thyroid gland was noted in the high-dose males and females at the 12-month necropsy; this finding was more severe in males than in females. Granular pigment was present in males and females receiving 125 ppm or 750 ppm during the first and second years of the study. Male and female rats fed 750-ppm diets exhibited increased incidences of thyroid follicular cell carcinomas, adenomas, nodular hyperplasia, and hypertrophy/hyperplasia. These thyroid changes are considered to be related to ethylene thiourea (ETU), a known thyroid carcinogen and product of mancozeb metabolism in the rat. Bilateral retinopathy was noted in high-dose males and females. The No-Observed-Effect Level (NOEL), based on the presence of renal pigment, was 60 ppm. The No-Observed-Adverse-Effect Level (NOAEL) for rats fed diets containing mancozeb is 125 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was complete and adequate, and the data were well reported. Summary data were supported by individual animal data, and mean values that were validated showed agreement with the author's values. Dose levels were selected on the basis of the results of previous toxicity studies.

The results of thyroid function tests and gross and histologic examinations indicated the thyroid as a target organ. There were increases in thyroid-stimulating hormone (TSH) levels, decreases in thyroxine (T_4) levels, thyroid follicular cell hypertrophy/hyperplasia, and thyroid follicular cell carcinomas and/or adenomas in high-dose males and females. These results are consistent with findings that the sequence of increased TSH levels, decreased T_4 levels, and thyroid hypertrophy/hyperplasia is associated with the formation of thyroid

tumors in rats.[1] Although this mechanism has been elucidated in rats, a species which is apparently most sensitive to thyroid tumor production, there is no evidence that this mechanism is of any biological significance in humans.[2] The histopathologic findings in the thyroids of the high-dose rats correlated with gross pathologic changes, i.e., increased incidences of enlarged thyroids in high-dose males and females and the presence of thyroid masses in high-dose males. Also, absolute and relative (to body weight) thyroid weights were significantly increased in the high-dose rats. Previous studies have established the role of ethylene thiourea (ETU), a metabolite of mancozeb, in the induction of thyroid tumors.[3] In the present study, dietary samples were carefully prepared and handled to minimize the decomposition of mancozeb to ETU.

Note Comment - Cover memo KB 9/20/91

Besides histologic findings in the thyroid, there was a significant increase in the incidence and severity of age-related bilateral retinopathy in the high-dose rats at 24 months of treatment; however, this finding did not correlate with ophthalmologic changes. Granular, yellowish-brown pigment was also noted microscopically on the cortical tubular lumina of kidneys in males and females receiving 125 or 750 ppm at 12 and 24 months of treatment; although this effect was dose-dependent, there were no accompanying dose-related increases in renal lesions. The renal pigment may be due to the excretion of a metabolite of the test substance in the kidney. Renal pigment has also been reported at 120 ppm in parental animals of a reproduction study (HED Document No. 8038), and renal tubular degeneration accompanying such pigment in 125 ppm males of a 3-month feeding study (HED Document No. 05318).

Treatment-related decreases in body weight (when compared to controls) were noted in the high-dose males throughout the study and in the high-dose females during the first 91 days of the study. The high-dose females recovered from the loss in body weight gain; however, body weight gains were lower than controls at the end of 12 months. There was no effect of dosing on mortality, hematology parameters, ophthalmology, food consumption, or urinalysis.

Based on effects in kidneys, the LOEL is 125 ppm, and the NOEL is 60 ppm.

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[1]Hill, R.N. et al. (1989) Review: Thyroid follicular cell carcinogenesis. Fundam. Appl. Toxicol. 12:629-697.

[2]Paynter, O.E. et al. (1988). Goitrogens and thyroid follicular cell neoplasia: Evidence for a threshold process. Reg. Toxicol. Pharmacol. 8:102-119.

[3]Graham, S.L. et al. (1975) Effects of prolonged ethylene thicurea ingestion on the thyroid of the rat. Food Cosmet. Toxicol. 13:493-499.