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

008451

6/28/91

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: MANCOZEB - Tox. Data Submitted Under MRID No. 418105-01 - ID No. 014504

Chemical No.: 913A
RD Record No.: S-393537
HED Project No.: 1-0955

FROM: Irving Mauer, Ph.D., Geneticist 05/24/91
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TO: Terri Stowe/Louis Rossi, PM 74
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THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C) 6/28/91

Registrant: Rohm & Haas (R&H), Philadelphia, PA

Request

Review and evaluate the following chronic study, submitted in response to the Mancozeb DCI.

Mancozeb: 52-Week Oral (Dietary Administration) Toxicity Study in the Beagle, performed by Hazleton UK (Report No. 616/3), dated July 28, 1988 (R&H No. 88RC-027) (EPA MRID No. 414486-01).

TB Conclusion

The study is judged CORE-MINIMUM DATA, providing the following parameters:

[Doses tested: 0, 50, 200, 800, 1600 ppm]

NOEL = 50 ppm (males = 1.75 mg/kg/day; females = 1.34 mg/kg/day).

LOEL = 200 ppm (males = 7.26 mg/kg/day; females = 7.02 mg/kg/day). Decreased body weight gain.

Attachment (DER)

Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

6/28/91

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DATA EVALUATION RECORD

008451

I. SUMMARY

MRID No.: 418105-01
ID No.: 014504
RD Record No.: S-393537
Caswell No.: 913A
Project No.: 1-0955

Study Type: (83-1) Chronic Feeding (52-Week) Oral - Dog

Chemical: Mancozeb

Synonyms: Dithane M-45

Sponsor: Rohm & Haas (R&H)
Philadelphia, PA

Testing Facility: Hazleton UK (HUK), No. Yorkshire (UK)

Title of Report: Mancozeb: 52-Week Oral (Dietary Administration) Toxicity Study in the Beagle

Authors: D.C. Shaw

Study Number: R&H NO. 88RC-027 (HUK #616/3)

Date of Issue: July 28, 1988

TB Conclusions:

Doses tested: 0, 50, 200, 800, 1600 ppm in the feed for 52 weeks.

NOEL = 50 ppm (1.75 mg/kg/day - males; 1.84 mg/kg/day - females)

LOEL = 200 ppm (7.26 mg/kg/day - males; 7.02 mg/kg/day - females): Decreased body weight gain.

In addition, at 800 ppm (27.26 mg/kg/day - males; 29.24 mg/kg/day-females): Transient decreases in Hb, PCV.

Further, at 1600 ppm (53.52 mg/kg/day males; 59.72 mg/kg/day - females): Decreased RBC, RETIC; increased CHOL, PT.

Classification (Core-Grade): MINIMUM.

II. DETAILED REVIEW

A. Test Material - Mancozeb (MNCZB) technical (R&H)

Description: Yellowish powder
Batch (Lot): 74222
Purity (%): 30.6 to 84.5% ai (Zn-Mn ethylene bisdithiocarbamate, EBDC; with [redacted] ethylene thiourea, ETU)
Solvent/carrier/diluent: Incorporated in feed

B. Test Organism - Canine

Species: Dog
Strain: Beagle
Age: 5 to 6 months
Weights - Males: 9.45 to 9.79 kg (on receipt)
Females: 6.80 to 8.18 kg (on receipt)
Source: Hazleton Research Products, Denver, PA.

C. Study Design (Protocol) - This study was designed to assess the chronic toxicity potential of mancozeb when administered in the diet to male/female Beagle dogs, according to established Agency and international test guidelines.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. Procedures/Methods of Analysis - Based upon a prior subchronic (13-week) study in Beagles performed at Hazleton America (HLA Project No. 417-416, R&H Report No. 86RC-7), and pilot study in females only at HUK (Project No. 616/1, included in this Final Report as Appendix 19), groups of acclimated/vaccinated male and female animals (4/sex/group) were fed basic powdered dog diet, or diets containing mancozeb (at nominal concentrations of 50, 200, 800, and 1600 ppm w/w). Diets were prepared weekly and analyzed for MNCZ and ETU prior to initiation and in weeks 1, 14, 26, 41, 47, and 52. Animals were observed daily, palpated and weighed weekly, and food conversion efficiency calculated from consumption and body weight gains for the 2 weeks prior to initiation, as well as in study week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, and 52.

Neurological examinations* were performed on all animals by a Board-certified Consultant during study weeks 13, 28, and 50; and ophthalmoscopy performed pre-dose and during weeks 49/50 by the "site" veterinarian.

The conventional array of laboratory investigation** were performed from blood (jugular) and urine samples collected twice pre-dose and during study weeks 13, 26, and 52; additional samples were obtained for hematology and clinical chemistry from control and high-dose animals during weeks 10, and for thyroid hormone levels in week

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*Including, but not limited to: Unusual responses of body position, activity level, and coordination of movement and/or gait; unusual behavior; convulsion, tremors, increased levels of lachrymation/salivation, piloerection, pupillary dilation/constriction, vocalization, diarrhea, excessive/diminished urination; altered sensory function, and/or reflexes.

**Hematology (whole blood):

hemoglobin
mean cell volume
red blood cell count and indices:
  mean cell hemoglobin
  packed cell volume
  mean cell hemoglobin concentration
total and differential white blood cell count
platelet count
reticulocyte count
prothrombin time
activated partial thromboplastin time
red blood cell morphology
- plus -
myelograms on bone marrow smears

Clinical Chemistry (serum):

glutamate oxaloacetate transaminase
glutamate pyruvate transaminase
gamma glutamyl transpeptidase (transferase
alkaline phosphatase
creatinine phosphokinase
sodium
potassium
chloride
calcium
inorganic phosphorus
glucose
total bilirubin
blood urea nitrogen
total protein
A/G ratio
albumin
total cholesterol
globulin

Urine analysis (direct catheterization):

color
turbidity
specific gravity
pH
protein
glucose
ketones
total bilirubin
blood
urobilinogen
reducing substances
microscopy of spun deposits

11. In addition, samples of serum were collected twice pre-dose and in study weeks 13, 26, and 52 for despatch to Hazleton Biotechnologies to determine thyroid hormone levels (T_3, T_4) [NB: Thyroid stimulating hormone (TSH) was not measured because specific canine antisera were not available.]

Complete necropsies (pathologist present) were conducted on animals DOS and in survivors at study termination. Organ weights were recorded before fixation from adrenals, heart, brain, kidneys, livers, gonads, spleen, and thyroid (including parathyroids); samples of these tissues plus other organs* as well as all palpable gross lesions and masses (including contiguous apparently normal tissues) were fixed for histological examination.

Data were analyzed by the following statistical methods:

- o ANOVA, followed by pair-wise comparisons using the t-test for: Body weight gains, hematology, absolute organ weights, organ/body weight ratios, and organ/brain weight ratios.
- o Kruskal-Wallis, followed by pair-wise comparisons using the Wilcoxon rank sum test for: Food consumption, clinical chemistry, and thyroid function.

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*bone (costochondral junction)
bone marrow smear (from sternum)
caecum
colon
epididymides
esophagus
femur (with bone marrow)
jejunum
lachrymal gland
lungs with mainstem bronchi
esophagus
pancreas
prostate
rib and bone marrow
sciatic nerve
skin and mammary gland
stomach (fundus, pylorus)
thymus
tongue
urinary bladder
vagina
aorta
brain (including section of medulla/pons, cerebral and cerebellar cortex)
duodenum
eyes (with optic nerves)
gallbladder
ileum
lymph nodes (mandibular and mesenteric)
pituitary
rectum
salivary gland (submaxillary)
skeletal muscle (quadriceps)
spinal cord (cervical, midthoracic, lumbar)
trachea
uterus

- o Nonparametric tests for: Lymphocytes in females at pre-dose week 1, APTT in females at study week 10, absolute male adrenal weights, absolute female thyroid weights, and heart/body weight ratio in females.

All significance tests were performed two-sided, and reported at levels of 5, 1 and 0.1 percent.

E. Results - [NB: data from this study were collected in four figures (illustrating the progression of mean body weights and food consumption), 15 group summary tables, and 18 Appendices from individual animals. Selected (positive) observations were extracted from these tabulations on the following ~R pages.]

The actual concentrations of test article in the four test diets were as follows:

Nominal Group Concentration (ppm)	Actual Concentration ppm (w/w)	
	Weeks 1 - 40	Weeks 41 - 52
0	0	0
50	59	62
200	237	248
800	947	993
1600	1893	1985

Actual intakes of the active ingredient (mg/kg/day) were reported in Report Table 6* (summarized from individual compound consumptions recorded in Appendix 5).

There were no neurological deficits attributed to treatment at any dose level, nor any treatment-related ophthalmological abnormalities, nor any effect on rectal temperatures.

*This is a correction of the text (stated as "Report Table 5") the latter being a tabulation of food consumption, not compound intake.

DER Table A: Effects of Mancozeb Fed to Beagle Dogs for 52 Weeks (4/sex/group)[1/]

Observation	Dose Group (ppm)									
	0		50		200		800		1600	
	M	F	M	F	M	F	M	F	M	F
Actual Dietary Conc. (ppm)	- 0 -		- 61 -		- 243 -		- 945 -		- 1939 -	
Actual Intake[2/] (mg ai/kg/d)	- 0 -		1.75	1.84	7.26	7.02	27.26	29.24	53.52	59.72
No. Survivors	4	4	4	4	4	4	4	4	2	4
Mean Body Weight Gain (kg)										
To Week 24	1.70	1.04	1.78	1.44	0.46*	1.35	0.42*	1.00	1.80[3/]	1.15
To Week 28	1.48	0.84	1.69	1.40	0.26*	1.30	0.08**	0.69	1.90[3/]	1.00
To Week 52	2.23	1.47	2.28	1.93	0.65*	1.56	0.43*	0.95	2.23[3/]	1.02
Hematology										
Week 10										
Hb	14.0	16.1							12.1	14.1**
RBC	6.17	6.78							4.98	5.92**
PCV	41.8	47.9							36.1	42.2**
MCV	67.7	70.7							72.9*	71.5
PT	6.6	7.0							6.2	6.4*
RETIC.	0.6	0.6							1.5	1.3**
Tot. WBC	13.0	13.7							14.1	13.2
Neutr.	8.60	8.65							9.60	8.58
Week 13										
Hb	14.4	16.3	14.0	15.7	14.1	15.5	15.0	14.5**	15.0	13.4***
RBC	6.17	6.60	5.77	6.51	5.99	6.63	6.12	5.89	5.84	5.50
PCV	41.7	46.3	40.3	45.6	40.9	44.4	43.2	41.8*	43.2	39.2***
MCV	67.5	70.2	69.9	70.2	68.3	67.3	70.5*	70.9	73.8***	71.5
PT	6.9	6.7	6.9	7.0	6.7	6.9	6.8	7.2	6.8	6.3
RETIC	0.8	1.0	1.1	0.6	1.2	1.0	0.6	0.9	1.0	1.4
Tot. WBC	11.5	13.0	13.1	13.8	10.7	12.8	12.5	10.9	10.3	9.9*
Neutr.	8.08	8.25	9.23	9.45	7.33	8.50	8.45	7.65	7.10	6.48
Week 26										
Hb	15.3	15.6	13.9	15.3	13.7	15.6	15.0	14.2	15.4	14.2
RBC	6.52	6.73	5.80	6.28	5.83	6.76	6.17	5.80	6.03	5.79
PCV	44.2	44.6	40.7	44.1	39.8	45.6	42.9	41.3	44.4	41.1
MCV	67.9	71.5	70.0	70.3	68.4	67.8	69.5	71.3	73.6***	71.0
PT	5.8	6.1	5.7	6.0	6.3	6.2	5.9	5.7	5.9	5.7
Tot. WBC	8.9	10.7	11.5**	11.9	10.0	10.9	8.5	8.8	8.6	9.5
Neutr.	5.30	6.63	7.55	7.30	6.80	7.73	5.43	5.23	5.40	6.05

Observation	Dose Group (ppm)									
	0		50		200		800		1600	
	M	F	M	F	M	F	M	F	M	F
Week 52										
Hb	16.3	16.5	15.7	16.6	14.7	16.1	15.8	14.5*	16.8	14.6*
RBC	6.99	6.73	6.53	6.96	6.41	6.97	6.61	5.59	6.78	6.03
PCV	47.7	47.7	65.6	48.2	43.4	47.2	46.0	42.4	49.5	43.1
MCV	68.1	70.9	69.9	69.4	67.8	68.2	69.7	70.8	73.1**	71.4
PT	6.4	6.6	6.2	6.2	6.7	6.2	6.0	6.2	5.6*	5.8
Tot. WBC	10.9	10.3	10.9	11.9	9.9	10.3	10.4	9.9	11.1	9.9
Neutr.	6.73	5.78	6.20	7.08	6.35	5.10	6.18	5.95	6.65	6.08
CLINICAL CHEMISTRY										
Total Cholesterol										
Week 10	133	129	124	118	118	142	150	158	181	182*
26	126	136	123	128	113	158	160	205	188	210*
52	114	128	117	129	102	137	145	195	171	184
Necropsy (n)										
Adrenals, red	0	0	0	0	0	1	0	0	0	0
Lung, abnormal	0	0	0	0	1	0	0	1	1	2
Spleen, hyperplasia	1	1	1	3	1	0	4	0	1	2
Prostatitis	1	-	1	-	0	-	3	-	1	-
Thymus										
- Involuted	2	0	4	1	4	0	3	3	1	2
- Cystic	2	3	4	2	4	0	3	3	1	3
- Hyperplastic	0	0	0	0	0	1	0	0	0	1
Thyroid										
- Hyperplastic	1	2	0	0	0	1	0	0	0	0
- Distended follicles	0	0	0	0	0	0	0	0	2	4
- Cystic	1	1	0	0	0	0	0	0	1	0
Lungs										
- Leucocytosis	1	1	2	2	0	3	4	1	2	2
Pituitary										
- Cystic	1	1	1	0	0	2	0	3	0	2
Histopathology										
Prostatitis	1	-	0	-	0	-	0	-	1 [4/]	-
Ulcer, dermat.	0	1	1	1	0	0	2	0	0 [5/]	0 [3,3,10/]
Kupffer pigment	0	0	0	1	1	1	1	0	2 [5/]	3
Arteritis	0	0 [6/]	0	0	1	0	0	0	0 [4,5/]	0 [3,3,1]
Thyroid (folliculitis)	0	1	0	0	0	0	0	0	3&2	2&3

Observation	Dose Group (ppm)									
	0		50		200		800		1600	
	M	F	M	F	M	F	M	F	M	F
Histopathology (cont'd)										
Pneumonitis	0	0	0	0	0	0	0	0 7/	1 4/	0
Salivary (glandulitis)	0	1	0	0	0	0	0	1	0	0
Gastritis	0	1 5/	0	0	0	0	0	0	0	0
Pituitary cysts	0	0	0	0	0	1	0	0 7/	0	0
Mesentery lymphodenitis	0	0	0	0	0	1	0	1	0	0
Gallbladder hyperplasia	0	0	0	0	0	0	0	1	0	0

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
**Significant at $p < 0.001$.
1/Representative (mainly significant and/or positive) findings extracted from Tables 1-15 and Appendices 1 through 18 of the FINAL REPORT.
2/Mean intakes over the 52 weeks of the study, calculated by the reviewer.
3/These weights are from the two survivors of the HDT male group, and exclude moribund sacrifices (M808, M810) at weeks 10 and 11.
4/Hi-dose M807 (terminal sacrifice)
5/Hi-dose M809 (terminal sacrifice)
6/Control F812 (terminal sacrifice)
7/800 mg/kg F825 (terminal sacrifice)
8/Hi-dose F827 (terminal sacrifice)
9/Hi dose F829 (terminal sacrifice)
0/Hi-dose F830 (terminal sacrifice)

Two high-dose animals had to be sacrificed in extremis early in the study (Tables 1,2; APPENDIX 1). M810 ate only about one-half its food during week-9, and was judged anemic based upon results of hematological determinations, in addition to showing an increased reticulocyte count. Despite meat supplements provided for the next week, this animal's condition did not improve and--- subsequent to severe body weight loss during week 10 compounded by lethargy, labored breathing---M810 was killed and necropsied. Hematological parameters and pathological findings in this animal were consistent with chronic regenerative anemia, additionally with manifestations of diffuse centrilobular necrosis and extramedullary hematopoiesis, erythroid hematopoiesis with pigment in spleen and bone marrow, and evident reticulocytosis. The other premature high-dose loss was animal M808 which also reduced its food consumption (by two-thirds) and lost over 700 g in body weight during weeks 9 to 10. White blood cell count in that animal was elevated in both weeks 10 and 11; additional meat supplements were also unavailing and this animal also had to be necropsied (study week 11) following profuse hematuria and distended bladder palpated the night before sacrifice. Urethral calculi were observed on gross examination, as well as microscopic evidence of hydronephrosis with tubular dilatation, coincident with renal necrosis and congestion and urinary tract lesions (urethritis, prostatitis, cystitis, and ureteritis), with associated acute peritonitis. All other animals in this and other dose groups survived in apparent good health, with no adverse clinical signs or treatment-related palpable masses.

In addition to severe weight loss among high-dose males (due mainly to the two moribund sacrifices), both mid-dose groups of males (200 and 800 ppm) gained significantly less weight ($p < 0.05$) than controls* (Tables 3,4; Figures 1,2; APPENDIX 2). There were no significant changes in food consumption (other than in the two high-dose sacrifices) nor in feed conversion efficiency (Table 5; Figures 3,4; APPENDICES 3,4).

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*Which persisted to study termination (see DER Table A), despite statements in text by the investigators to the contrary that the weight changes among 200 ppm males were considered not related to treatment.

Average group mean test compound intake ranged from 1.4 to 2.3, 6.3 to 9.9, 24.1 to 33.1 and 43.6 to 72.6 mg/kg/day for both sexes in low (50 ppm), both intermediate (200, 800 ppm), and high-dose (1600 ppm) level groups, respectively (Table 6; APPENDIX 5).

Significant hematological changes were recorded in high-dose (1600 ppm) females in study week 10 and among mid-dose (800 ppm) females measured in week 13 (Tables 8; APPENDIX 7). Specifically depressed values ($p < 0.01$) for hemoglobin (Hb), and packed cell-volume (PCV) were evident as well as transiently increased reticulocyte counts (RETIC). In addition, increased mean cell volumes (MCV) were recorded early among high-dose males (in weeks 10 and 13), which persisted to study termination. The hematological changes also persisted in both 800 and 1600 ppm females to study termination (52 weeks). No dose-related treatment changes were found in terminal myelographic determinations (Table 9; APPENDIX 8).

High-dose females registered significantly higher total cholesterol (Tot. Chol.) throughout the study, significantly so ($p \leq 0.05$), at weeks 13 and 26. (Table 10; APPENDIX 9). The same trend was apparent in surviving high-dose males, but did not reach statistically significant values. Additional sporadic but significant clinical chemistry changes were also recorded (e.g., in albumin, phosphorus, inter alia), but were considered biologically not relevant in the absence of other correlated changes.

There were no statistically significant (or biologically relevant) treatment-related changes among test groups for thyroid function (Table 11; APPENDIX 10) or for urinalyses (APPENDIX 11). Major organ weight changes recorded were a significant increase compared to controls in thyroid weight-to-body weight ratio among high-dose males ($p < 0.05$) and females ($p < 0.001$), as well as apparent (but nonsignificant) increases in group mean liver weights and liver-to-body weight and liver-to-brain weight ratios (Table 12 to 14; APPENDICES 12 to 14). Additionally, group mean testes weights for all test groups except 50 ppm males were lower than controls, but again did not achieve the 5 percent level of statistical significance, and as well did not appear to be dose-related.

Other than the severe pathological changes already described in the two high-dose male moribund sacrifices (acute urogenital tract lesion in M808, but unrelated to the chronic regenerative anemia found in M810), the

overall nature and incidence of gross necropsy findings in test and control survivors to study termination were minimal and in keeping with the expected background for lab-bred Beagles (Tables 15.1 and 15.2). Except as noted for high-dose decendants, the incidence of histopathological changes was generally similar in both control and treated animals were: Leukocyte foci in liver and tongue; hyperplasia in mesentery and mandibular lymph nodes; involution with cysts in thymus; and, (pulmonary) pneumonitis (Tables 15.3, 15.4, 15.5). The singular exception was Kuppfer cell pigment deposits, with an increased incidence in high-dose (1600 ppm) animals, and thyroid follicular distention present in all 1600 ppm animals surviving to the 52 weeks sacrifice.

The investigators concluded that the only consistent treatment-related effects of the chronic administration of dietary mancozeb were: Reduced T_4 levels correlated to increased thyroid weight and follicular distention at the high-dose level (1600 ppm); reductions in hemoglobin, red blood cell counts and packed cell volume, together with concurrent increases in total cholesterol in mid-dose (800 ppm) and high-dose (1600 ppm) females. Hence, in their view, the no-observed effect level after 52 weeks exposure of Beagle dogs to mancozeb was 200 ppm.

TE Evaluation: Core-Minimum Data

The study was apparently well organized and performed adequately to reflect most determinations required by Agency Test Guidelines (83-1) for non-rodents. We agree with the investigators with respect to the evaluations of the data generated in this chronic study, except for the lowest effect level (800 ppm) inferred by them from significant hematological values significantly different from concurrent controls (and background lab data apparently on hand). Although not clearly dose-related, there were significant changes in body weight gain and food consumption at all levels (including 200 ppm) except at the LDT (50 ppm).

Therefore, we judge 50 ppm to be the NOEL for chronic dietary mancozeb administration in dogs.

Attachments: Data Tables

A T T A C H M E N T S

Data Tables

Page_____ is not included in this copy.

Pages 15 through 75 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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