539
CASWELL FILE

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

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MAY 29, 1992

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Maneb - Chronic Toxicity Study in Dogs

TO: Terri Stowe
PM Team Reviewer (71)
SRRD/RB (H7508W)

FROM: Linda L. Taylor, Ph.D. 5/13/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel 5/19/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. 5/26/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: ATOCHEM North America (Pennwalt Corporation)
Chemical: manganese ethylene-1,2-bisdithiocarbamate; ethylene thiourea
Synonym: Maneb
Caswell No.: 539
Case No.: 818618
Submission No.: S415654
Identifying No.: 014505
DP Barcode: D176784
MRID No.: 422516-01

Action Requested: For Immediate Review - MANEB. Please review Maneb data for the chronic tox. - dog study (GLN 83-1B) and give an updated status report on the tox. requirements for Maneb. A DCI will be going out for all EBDC's and I need the status of the tox. requirements including data gaps and new requirements.

In response to the Agency's April 1, 1987 Data Call-In Notice for registration of Maneb, a chronic oral toxicity study in dogs was submitted: "52-Week Oral Toxicity (Feeding) Study with MANEB TECHNICAL in the Dog". This study has been reviewed, and the DER is appended.

Under the conditions of the study, exposure to Maneb via the diet at dose levels of 50, 200, 1000, and 2200 ppm for one year resulted

in decreased body weight in the females at the highest dose level throughout most of the study, with the males displaying comparable body weights among the groups throughout the study. Body-weight gain and food consumption were decreased at the highest dose level in both sexes, with statistical significance being attained throughout most of the study in the females and for the first 13 weeks in males. Several hematology parameters, suggestive of a hemolytic anemia, were affected by treatment at the two highest dose levels, and several clinical chemistry parameters (consistent with hypothyroidism) were altered. Thyroid (absolute and relative to body/brain) weight was increased in both sexes at the 1000 and 2200 ppm dose levels, but statistical significance was attained only at the highest dose level. Follicular hyperplasia was displayed in all dogs (both sexes) at the two highest dose levels, with the severity increasing with dose.

The NOEL for effects other than neuropathological effects is 50 ppm (1.53 ♂♂/1.71 ♀♀ mg/kg) and the LEL is 200 ppm (6.36 ♂♂/7.18 ♀♀ mg/kg), based on decreased body-weight gain/food consumption, changes in hematology/clinical chemistry parameters indicative of thyroid toxicity/anemia, increased thyroid weight, and follicular (thyroid) hyperplasia. Although none of the neurological parameters examined was affected by treatment, microscopic lesions can occur in the absence of clinical signs. Therefore, neuropathological examination of the sciatic nerve is required before a final determination regarding the NOEL can be made.

Classification: core-Supplementary, pending submission of the neurological examination results of the sciatic nerve, including its extensions, sural, tibial, and peroneal nerves, as well as the interosseus muscle. This study does not satisfy the guideline requirement (83-1B) for a chronic toxicity study in a non-rodent, but it may be upgraded.

With regard to the current status for the toxicology data requirements for Maneb, the toxicological data available on Maneb and the outstanding data requirements were outlined in the Revised Toxicology Chapter of the Registration Standard on Maneb (dated 4/13/88) and the PD 2/3, published in the Federal Register of December 20, 1989 (54 FR 52158). To date, the 2-generation-reproduction study (DER dated 2/27/92; classified Core Minimum; cover memo dated 3/2/92 with update of data requirements provided), the rat developmental toxicity study (DER dated 1/15/92; classified Core Supplementary), and the dog chronic toxicity study (subject of current action) have been submitted and have been reviewed by TB II. The latter study is classified Core Supplementary. As indicated in the 3/2/92 TB II cover memo, a 21-day dermal study was submitted, reviewed (DER dated 3/8/89), and classified core minimum; additional data have been submitted on the mutagenicity studies and these have been upgraded (TB II memo dated 9/27/88 and 10/19/88) to acceptable. Although a 4-week inhalation study was submitted recently (TB II memo dated 11/26/91) to upgrade the 90-

day inhalation study, additional data are required, and the study remains core supplementary. Other data requirements include: (1) a mouse carcinogenicity study; (2) adequate additional data to upgrade the 31-month rat chronic toxicity/carcinogenicity study; and (3) adequate data to upgrade the rabbit developmental toxicity study.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 5/13/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 5/15/92
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic - dog TOX. CHEM NO: 539

MRID NO.: 422516-01

TEST MATERIAL: Technical Maneb

SYNONYMS: Manzate; Dithane M-22

CHEMICAL NAME: manganese ethylene-1,2-bisdithiocarbamate

STUDY NUMBER: RCC Project 206616

SPONSOR: AFOCHEM NORTH AMERICA, INC.

TESTING FACILITY: a) RCC, Research & Consulting Co. Ltd/Switzerland
b) RCC Umweltchemie AG/Switzerland
c) Experimental Pathology Services (UK) Ltd. & RCC (UK) Ltd/England
d) The Department of Oral Pathology, Dental Institute/England

TITLE OF REPORT: 52-Week Oral Toxicity (Feeding) Study with MANEB TECHNICAL in the Dog

AUTHOR(S): SJ Corney, TR Allen, T. Janiak, Th. Frei, H Luetkemkier, K. Biedermann, Dr. O Vogel, Dr. C Springall

REPORT ISSUED: January 16, 1992

CONCLUSION: Under the conditions of the study, exposure to Maneb via the diet at dose levels of 50, 200, 1000, and 2200 ppm for one year resulted in decreased body weight in the 2200 ppm dose level females throughout most of the study. Male body weight was comparable among the groups throughout the study. Body weight gain and food consumption were decreased at the 2200 ppm dose level in both sexes, with statistical significance being attained throughout most of the study in the females and for the first 13 weeks in males. Several hematology parameters, suggestive of a hemolytic anemia, were affected by treatment at the two highest dose levels. Additionally, several clinical chemistry parameters were altered, which are consistent with hypothyroidism. Thyroid (absolute and relative to body/brain) weight was increased in both sexes at the 1000 and 2200 ppm dose levels, but statistical significance was attained only at the highest dose level. Adrenal gland weight was slightly increased in both sexes at the 2200 ppm dose level, although statistical significance was attained only in males

(relative to body weight). Follicular hyperplasia was displayed in all dogs (both sexes) at the two highest dose levels, with the severity increasing with dose.

The NOEL for effects other than neuropathological effects is 50 ppm (1.53 ♂♂/1.71 ♀♀ mg/kg) and the LEL is 200 ppm (6.36 ♂♂/7.18 ♀♀ mg/kg), based on decreased body-weight gain/food consumption, changes in hematology/clinical chemistry parameters indicative of thyroid toxicity/anemia, increased thyroid weight, and follicular (thyroid) hyperplasia. Although none of the neurological parameters examined was affected by treatment, microscopic lesions can occur in the absence of clinical signs. Therefore, neuropathological examination of the sciatic nerve is required before a final determination regarding the NOEL can be made.

Classification: core-Supplementary, pending submission of the neurological examination results of the sciatic nerve, including its extensions, sural, tibial, and peroneal nerves, as well as the interosseus muscle. This study does not satisfy the guideline requirement (83-1) for a chronic toxicity study in a non-rodent, but it may be upgraded.

A. MATERIALS

1. Test Compound: Technical Maneb; Description:yellow powder; Batch #: B1 280289; Purity: 89.2%.

2. Test Animals: Species: dog, Strain: pure-bred beagle; Age: 5-6 months, pre-test; Weight: ♂ 5.0-8.9 kg, ♀ 4.4-7.5 kg; Source: KFM Kleintierfarm Madörin AG/Switzerland; Breeder: Hazleton-LRE/Kalamazoo, MI, USA.

3. Statistics: Body weights, clinical laboratory data, and organ weights-Univariate one-way analysis of variance to assess the significance of intergroup differences; if the variables assumed to follow a normal distribution, the Dunnett-Test (many to one t-test)based on a pooled variance estimate was applied for comparison between treated and control groups; if a normal distribution was not assumed, the Steel-test (many-one rank test) was applied. Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables.

B. STUDY DESIGN

1. Animal Assignment: Dogs were acclimated for at least 10 weeks (47 dogs)/7 weeks (3 dogs) prior to study initiation. Each was dewormed, vaccinated (distemper, leptospirosis, contagious hepatitis, parvovirus, parainfluenza, bordetella, and rabies), and treated with Telmin KH shortly after arrival and during Week 15 of the study to remove gastrointestinal parasites. During the acclimatization period, 5 dogs per sex were randomly selected for parasitological and bacteriological investigations as an additional health control. Dogs were assigned to groups shortly after arrival by a computer-generated algorithm and, again just prior to the start of dosing, the allocation was adjusted to ensure the use of healthy dogs and mean body weights that were ≈ equal among the groups. The animals in each treatment group were housed in adjacent kennels (minimum of 2.0 square meters floor space each) until the middle of week 8 of treatment. Thereafter, four animals from each group were housed, in adjacent kennels, in Room 1U07; the remaining dog from each group was housed in Room 1U02 (procedure adopted to ensure an even distribution of dogs, which due to study, size could not be housed in same room).

Five dogs per sex were assigned to each group (control, 30, 200, 1000, and 2200 ppm) and the test material was administered via the diet for 52 weeks. The dogs were provided with 300 grams of granular standard Kliba 335 dog maintenance diet (Kliba Klingentalmühle AG/Switzerland), which was presented at ≈ 10 a.m. daily and removed at 1 p.m., and water ad libitum.

2. Diet Preparation: Feed admixture was prepared every 2 weeks and stored at room temperature for a maximum of 21 hours following storage at -20°C. Technical Maneb was mixed with microgranulated feed (not further described). Samples of treated feed were analyzed for homogeneity and concentration at three-month intervals. Prior to study initiation, a trial mix was performed to ensure that the proposed formulation procedure was acceptable.

RESULTS

The test material was determined to be stable in the diet for at least 20 days frozen at -20°C, and for 1 day at room temperature. The overall mean concentrations found were 100.3%, 106.4%, 103.3%, and 106.8% of the nominal concentration for the 50, 200, 1000, and 2200 ppm dose groups, respectively, with the range from 93.3% to 115.6%. Homogeneity varied in the range from -9% to +7% of the mean concentration.

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily for viability and changes in behavior and appearance.

Toxicity/Mortality (survival)

There were no deaths during the study. Diarrhea was observed more often in dogs at the 2 highest dose levels, compared to the control values, and the mean severity increased with dose. Examination of the estrus cycle records showed that 5/5, 4/5, 4/5, 5/5, and 2/5 dogs at 0, 50, 200, 1000, and 2200 ppm dose levels displayed estrus changes during the study, with 2 of the control, 1 of the 50 ppm group, 3 of the 200 and 1000 ppm dose groups showing two cycles by study termination. Additionally, the high-dose females both cycled latter than 14 of the 18 other females that showed estral changes. No other clinical signs were reported.

Estral Changes

Group (ppm)/dog #	Birth Day	Week during which signs of estrus observed
Group 1 (0)		
26	2/5/89	19-22, 44-48
27	2/1/89	23-26
28	2/2/89	22-25
29	2/4/89	39-42
30	2/3/89	15-19, 43-47

Group (ppm)/dog #	Birth Day	Week during which signs of estrus observed
Group 2 (50)		
31	2/1/89	45-48
32	2/3/89	46-48
33	2/4/89	43-46
34	2/3/89	21-24, 45-48
Group 3 (200)		
36	2/3/89	21-24, 51-53
38	2/5/89	17-20, 45-48
39	2/1/89	17-20, 46-48
40	2/2/89	24-26
Group 4 (1000)		
41	2/3/89	21-25, 50-53
42	2/4/89	27-30, 52-53
43	2/3/89	14-16, 52-53
44	2/5/89	31-34
45	2/3/89	33-36
Group 5 (2200)		
46	2/4/89	40-43
49	2/3/89	37-40

2. Bodyweight

Animals were weighed weekly (from Week 2 pre-test), including the first and last complete day of dosing and before necropsy.

RESULTS

In general, body weight was comparable among the male groups throughout the study. The high-dose females displayed lower body weights compared to the control value from week 3 on.

Body Weight (% of Control)

Week/Dose	50 ppm	200 ppm	1000 ppm	2200 ppm
MALES				
Pre-test	101	104	99	109
1	101	101	97	105
2	101	104	96	101
3	103	104	98	98
4	102	104	98	99
5	104	105	95	100
6	104	106	96	102
7	103	105	95	99
8	103	105	94	98
9	106	106	97	95
10	106	104	94	96
11	106	104	96	98
12	107	103	96	96
13	107	104	96	97
26	106	103	95	96
40	106	103	94	95
52/52	106/107	101/102	92/93	94/94
FEMALES				
Pre-test	102	108	98	102
1	102	106	95	97
2	101	109	96	96
3	101	110	96	90
4	104	106	96	90
5	103	106	96	90
6	103	106	97	92
7	100	104	96	89
8	100	103	96	89
9	100	103	94	88
10	101	104	97	87
11	101	101	96	87
12	101	103	95	87
13	101	103	95	87
26	100	100	99	88
40	102	100	101	90
52/52	99/100	101/101	102/101	89/88

The authors presented the body-weight gain data on a % basis; the 2200 ppm males showed a statistically significant decrease from weeks 2 through 4, 7-14, 17, and 23 of the study. The 2200 ppm females also showed a decrease in body-weight gain on a % basis, with statistical significance attained during weeks 3, 5, 7-13, 16, 23, 24, 26, and 27. NOTE: TB II was unable to verify the data presented as a %, apparently due to the rounding of the numbers in the computer-generated tables.

Body-Weight Change (%)

Week/Group	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
MALES					
2	2.4	3.0	4.0	1.5	-1.7*
3	4.2	6.0	5.9	3.3	-4.3**
4	6.6	8.8	9.0	6.0	0.0**
7	11.4	15.0	14.3	9.2	4.2*
8	12.7	16.2	15.8	9.7	3.7*
13	16.7	24.1	19.4	14.2	7.1*
16	19.9	25.4	21.9	17.7	10.5
18	20.6	27.7	24.5	20.2	10.9
26	22.1	30.7	23.9	18.2	10.1
52	25.8/25.1	34.4/33.9	25.0/24.2	18.7/17.8	11.9/11.0
FEMALES					
2	1.4	2.1	2.9	1.5	0.7
3	3.4	3.1	5.8	2.4	-4.2*
4	7.0	7.4	6.1	8.0	0.6
5	8.8	10.6	7.6	8.8	1.5*
7	13.3	12.6	10.6	14.4	5.1**
8	15.1	13.4	11.1	15.8	7.3*
13	19.7	19.7	15.0	18.8	7.9*
16	20.9	19.5	15.1	23.8	10.2*
18	25.5	21.9	16.9	26.8	15.8
23	26.4	25.8	16.8	26.8	14.1*
26	27.0	24.3	16.8	30.0	14.4*
52	28.2/28.2	23.9/25.7	20.3/20.1	36.1/34.4	17.7/15.8

TB II calculated the body-weight gain/week for each group for the first 13 weeks, as well as the overall body-weight gain for this time period (see table below). but no statistical analysis was performed.

Mean Body-Weight Change (kg)

Interval/Group	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
MALES					
Pre-test	0.24	0.24	0.16	0.06	0.34
1-8	0.20	0.22	0.34	0.14	-0.14
8-15	0.14	0.24	0.12	0.12	-0.20
15-22	0.18	0.18	0.26	0.18	0.34
22-29	0.10	0.14	0.14	-0.02	0.20
29-36	0.06	0.06	0.06	0.04	0.14
36-43	0.24	0.28	0.18	0.24	0.02
43-50	0.12	0.08	0.12	0	-0.02
50-57	0.06	0.28	0.12	0.18	-0.14
57-64	0.18	0.14	0.14	0.02	0.16
64-71	-0.04	0	0.02	0.04	0.14
71-78	0	0.14	-0.22	0.02	-0.12
78-85	0.1	0.06	0.22	0.10	0.16
85-92	0.16	0.02	0.28	0.22	0.06
1-92	1.48	1.84	1.78	1.28	0.62

Interval/Group	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
FEMALES					
Pre-test	0.04	0	0.18	0.14	0.06
1-8	0.12	0.10	0.26	0.12	0.02
8-15	0.12	0.08	0.18	0.06	-0.30
15-22	0.22	0.28	0.02	0.34	0.28
22-29	0.12	0.18	0.10	0.02	0.08
29-36	0.04	0.04	0.04	0.12	0.12
36-43	0.24	0.10	0.16	0.26	0.10
43-50	0.14	0.06	0.06	0.08	0.14
50-57	0.04	0.14	0.04	-0.12	0.04
57-64	0.12	0.12	0.16	0.30	-0.08
64-71	0.10	0.14	-0.08	0.04	0.18
71-78	-0.01	-0.04	0.06	-0.1	0.08
78-85	0.10	0.06	0.08	0.08	0.12
85-92	0.02	0.04	0.1	0.2	0.14
1-92	1.28	1.3	1.14	1.40	0.62

3. Food Consumption and Compound Intake

Food consumption was recorded daily for each dog.

RESULTS

Group mean food intake was decreased in males at the 2200 ppm dose level mainly during the first 13 weeks of the study and in females at 2200 ppm for the first 26 weeks of the study. The other groups showed comparable intake values to those of the controls.

Mean Food Consumption (g/dog/day; % of control)

Week/Group	50 ppm	200 ppm	1000 ppm	2200 ppm
MALES				
Pre-test	95	94	92	99
1	104	100	90	76
2	101	102	87	65
3	99	99	91	71
4	99	100	91	80
5	99	100	94	81
6	99	100	92	81
7	101	101	88	78
8	100	99	96	82
9	101	100	92	78
10	101	100	93	88
11	100	101	93	84
12	101	95	95	82
13	100	100	97	85
16	100	101	98	90
23	99	100	97	95
35	98	100	98	91
42	99	100	98	92
52	98	100	98	92
1-52	98	100	96	90

Week/Group	50 ppm	200 ppm	1000 ppm	2200 ppm
FEMALES				
Pre-test	101	105	109	102
1	101	103	91	65
2	104	104	91	64
3	101	103	93	72
4	103	105	101	79
5	103	105	101	79
6	103	108	103	84
7	103	109	103	82
8	101	103	101	83
9	102	103	97	83
10	99	103	100	77
11	104	114	108	88
12	100	107	95	78
13	95	102	96	80
16	99	106	102	89
22	103	106	104	87
26	100	104	101	84
38	106	108	106	88
39	105	110	104	97
40	108	109	105	96
44	104	104	95	87
52	106	111	103	93
1-52	102	106	104	89

Test material intake is listed below for each sex and dose level.

Dose Level (ppm)	Test Material Intake (mg/kg/day)	
	MALES	FEMALES
50	1.53	1.71
200	6.36	7.18
1000	33.84	35.25
2200	66.47	72.93

4. Ophthalmological examination

Each dog was examined for abnormalities of the eyes ≈ 20 minutes after the instillation of 0.5% tropicamide solution (Mydriaticum, Dispersa AG) using a binocular indirect ophthalmoscope (all pupil model, Keeler Instruments, Inc., USA). The observation area included the cornea, lens, conjunctiva, sclera, iris, and fundus. Examinations were performed pre-test, and at weeks 13, 26, and 52.

RESULTS

There were no adverse effects noted at any dose level in either sex throughout the study.

5. Clinical Laboratory Investigations

Blood and urine samples were collected from all dogs (fasted overnight, but allowed access to water ad libitum. In order to

reduce biological variation caused by circadian rhythms, the samples were collected between 7 and 9:45 a.m.. Blood samples were drawn from the jugular vein, and urine was collected using a catheter. Blood and urine were collected twice pre-test and at weeks 13, 26, and 52.

a. Hematology: The CHECKED (X) parameters were examined. The anticoagulants EDTA-K3 (hematology) and sodium citrate (coagulation) were used during blood collection.

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Red cell morphology
X	(Thromboplastin time)		
X	(Activated partial thromboplastin time)		
X	(partial thromboplastin time)		
X	Nucleated erythrocytes normoblasts		

RESULTS

Slight decreases in mean erythrocyte count, hemoglobin concentration, and hematocrit were reported in both sexes at the 200, 1000, and 2200 ppm dose levels at each investigation, although these were not always dose-related and/or statistically significant. Additionally, increased mean cell volumes were displayed at 13 weeks in the 2200 ppm males and the 1000 ppm females, and/or decreased mean cell hemoglobin concentration were displayed at 13 and 26 weeks in both sexes at the 2200 ppm dose level, although the females did not attain statistical significance at week 52. Slight reticulocytosis and polychromasia was observed in females at the 2200 ppm dose level at each investigation, although statistical significance was attained only at weeks 13 and 26. Slight reticulocytosis and polychromasia were evident also in one 2200 ppm male at weeks 52 and 13, respectively. Increased platelet counts were observed in both sexes at the 2200 ppm dose level and in males at 1000 ppm at each investigation (statistical significance was not attained at week 13 in the 2200 ppm males and at week 52 in the 1000 ppm males).

Hematology Findings (males)♥

Parameter/Dose	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
Erythrocytes					
pre-test	5.95	6.19	5.83	6.39	6.43
pre-test	5.75	5.87	5.81	6.36*	6.21
13	6.43	6.30	6.08	5.78	5.92
26	6.80	6.84	6.38	6.15	6.21
52	6.98	6.93	6.41	6.09	6.23
Hemoglobin					
pre-test	7.9	8.2	7.9	8.6	8.6
pre-test	7.7	7.8	7.9	8.4	8.3
13	9.0	8.8	8.6	8.3	8.6
26	9.4	9.5	9.0	8.7	9.0
52	9.9	9.9	9.1	8.6*	8.8
Hematocrit					
pre-test	0.40	0.41	0.40	0.42	0.43
pre-test	0.38	0.39	0.39	0.41	0.41
13	0.43	0.42	0.41	0.40	0.42
26	0.45	0.44	0.42	0.41	0.43
52	0.47	0.47	0.43	0.41	0.43
MCV					
pre-test	66.8	66.9	67.7	66.2	67.1
pre-test	66.3	66.5	67.4	65.2	66.2
13	66.3	66.7	67.6	68.6	70.6**
26	65.7	65.0	66.0	66.6	68.7
52	67.3	67.6	67.0	67.9	69.0
MCHC					
pre-test	19.9	19.9	20.0	20.2	20.0
pre-test	20.2	20.1	20.1	20.3	20.3
13	21.1	21.1	20.9	20.9	20.4**
26	20.8	21.4	21.3	21.4	21.1
52	21.2	21.1	21.2	20.8	20.3**
Reticulocytes					
pre-test	0.006	0.009	0.009	0.006	0.008
pre-test	0.008	0.006	0.010	0.005	0.009
13	0.006	0.005	0.004	0.006	0.010
26	0.007	0.005	0.007	0.010	0.010
52	0.014	0.006	0.007	0.008	0.015
Platelets					
pre-test	302	334	286	362	363
pre-test	295	350	316	370	332
13	282	298	282	408*	398
26	246	286	268	392**	424**
52	286	321	288	427	480*

Hematology Findings (females)♥

Parameter/Dose	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
Erythrocytes					
pre-test	6.33	5.95	6.08	6.04	6.52
pre-test	6.19	5.96	6.00	5.89	6.39
13	6.38	6.56	6.13*	5.73**	6.05*
26	6.49	6.75	6.20	5.67	6.11
52	6.62	6.64	6.09	6.09	6.32
Hemoglobin					
pre-test	8.7	8.2	8.2	8.1	8.6
pre-test	8.4	8.1	8.2	7.9	8.3
13	9.9	9.4	8.7*	8.5**	8.5**
26	9.3	9.7	8.8	8.4	8.7
52	9.5	9.6	8.7	9.0	8.8

Parameter/Dose	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
Hematocrit					
pre-test	0.43	0.41	0.41	0.41	0.43
pre-test	0.42	0.41	0.40	0.39	0.42
13	0.47	0.45	0.42*	0.41*	0.42
26	0.44	0.45	0.41	0.39	0.41
52	0.46	0.46	0.42	0.43	0.43
MCV					
pre-test	68.6	68.6	67.7	67.7	66.0
pre-test	67.7	68.0	66.9	66.9	65.3
13	67.8	68.2	68.2	71.5*	69.8
26	67.2	67.4	66.6	69.5	67.7
52	69.5	69.3	68.4	71.0	68.8
MCHC					
pre-test	19.9	20.0	20.0	19.8	19.9
pre-test	20.2	19.9	20.5	20.1	20.0
13	21.2	21.0	20.9	20.7	20.2**
26	21.4	21.4	21.2	21.2	21.1
52	20.7	20.9	20.9	20.8	20.3
Reticulocytes					
pre-test	0.011	0.008	0.009	0.010	0.012
pre-test	0.007	0.009	0.006	0.008	0.012
13	0.006	0.007	0.003	0.007	0.015*
26	0.004	0.008	0.004	0.009	0.018**
52	0.009	0.007	0.006	0.011	0.014
Platelets					
pre-test	376	371	387	305	405
pre-test	363	384	383	280	398
13	337	329	367	319	512**
26	343	330	448	318	495*
52	373	402	450	352	557**

♥ units: RBC-T/l; HB-mmol/l; HCT-l/l; MCV-fl; MCHC-mmol/l; ret-l

b. Clinical Chemistry: The CHECKED (X) parameters were examined. The anticoagulant lithium heparin was used during blood collection.

X	Electrolytes:
X	Calcium
X	Chloride
X	Magnesium
X	Phosphorous
X	Potassium
X	Sodium
X	Iron
	Enzymes
X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
X	Creatine kinase (CK)
X	Lactate dehydrogenase (LDH)
X	Serum alanine aminotransferase
X	Serum aspartate aminotransferase
X	Gamma glutamyl transferase (GGT)
X	Glutamate dehydrogenase (GLDH)

X	Other:
X	Albumin
X	Blood creatinine
X	Blood urea nitrogen
X	Total cholesterol
X	Globulins
X	Glucose
X	Phospholipids
X	Total bilirubin
X	Total Protein
X	Triglycerides
X	Lipids, total
X	Triiodothyronine, total T3

|X| Ornithine carbamyltransferase (OCT)
|X| protein electrophoresis*
|X| Thyroxine, total T4

* Electrophoretic fractions: Albumin, α1-globulin, α2-globulin, β1-globulin, β2-globulin, sβ (sum of beta globulins), gamma-globulin

RESULTS

Increased group mean values for cholesterol and/or total lipids and phospholipid levels were reported in females at the 1000 ppm dose level and in both sexes at the 2200 ppm dose level, although statistical significance was not always attained and a dose response was not always evident. Slightly increased group mean total bilirubin concentrations were observed in males at the 1000 ppm dose level and in both sexes at the 2200 ppm dose level. It is to be noted that the 1000 ppm males displayed a statistically significant increase at the first pre-test measurement and higher values than those of the 2200 ppm males at each time point during the study. Statistical significance was attained in the 2200 ppm females at week 13 only. Thyroxine (T_4) values were decreased in both sexes at the 2200 ppm dose level at each time point, although the males did not attain statistical significance at week 52. Triiodothyronine (T_3) values were decreased (72% ♂/80% ♀ of control value at week 52) in both sexes at the 2200 ppm dose level, although statistical significance was not attained at any time point. Males at the 2200 ppm dose level displayed increased triglyceride values, attaining statistical significance at week 13 only, but the values during the pre-test period were also greater than control. A comparable increase was not observed in the females. Alkaline phosphatase values were increased at the 2200 ppm dose level in both sexes throughout the study (except ♂♂ at week 52) compared to the control values, but statistical significance was not attained at any time point.

MALE CLINICAL BIOCHEMISTRY FINDINGS∇

Parameter/Dose	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
Bilirubin					
pre-test	4.2	4.2	4.2	5.6*	4.2
pre-test	3.8	4.1	3.8	4.5	4.1
13	3.8	4.3	4.3	5.4**	5.3**
26	4.1	3.9	4.2	5.9**	5.3*
52	4.8	4.5	4.3	5.7	5.4
Total Lipids					
pre-test	2.6	3.1	3.2	3.1	3.4
pre-test	2.4	2.9	2.6	3.0	2.9
13	2.9	3.3	3.4	3.7	4.5**
26	3.0	3.4	3.4	3.9	4.1
52	3.5	3.9	3.6	3.8	4.2

Parameter/Dose	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
Cholesterol					
pre-test	3.54	4.02	4.03	4.02	4.25
pre-test	3.45	4.02	3.74	3.83	3.73
13	3.56	4.25	4.19	4.42	5.20*
26	3.31	4.38	4.37	4.78	4.80
52	3.50	4.47	4.13	3.85	4.72
Phospholipids					
pre-test	3.63	3.91	4.03	4.04	4.18
pre-test	3.30	4.05	3.87	3.61	3.55
13	3.97	4.50	4.26	4.45	4.88
26	3.59	4.36	4.10	4.33	4.39
52	3.81	4.53	4.15	3.97	4.37
Triglycerides					
pre-test	0.39	0.48	0.50	0.48	0.53
pre-test	0.37	0.37	0.35	0.45	0.44
13	0.38	0.36	0.41	0.46	0.60**
26	0.46	0.44	0.44	0.53	0.57
52	0.53	0.54	0.46	0.67	0.60
Thyroxine (T_4)					
pre-test	50.2	49.6	52.9	55.6	50.8
pre-test	50.7	52.0	50.2	53.9	42.3
13	35.2	39.7	36.1	40.0	24.6*
26	47.0	56.0	48.7	42.4	35.2**
52	37.8	40.9	44.2	40.4	31.9
Alkaline Ph.					
pre-test	6.60	6.56	5.44	5.52	5.61
pretest	5.91	5.69	5.18	4.91	5.13
13	3.22	3.01	2.91	3.97	3.85
26	2.59	2.53	2.60	3.21	3.21
52	2.56	2.33	2.35	2.64	2.49
Triiodothyronine (T_3)					
pre-test	1.27	1.12	1.21	1.37	1.16
pretest	1.28	1.27	1.31	1.47	1.21
13	2.09	2.45	2.19	2.57	1.72
26	1.55	1.61	1.80	1.33	1.35
52	2.39	2.35	1.81	1.93	1.72

♥ units: CHOL/PL/TG-mmol/l; TL-g/l; T_4-nmol/l; AlkP-ukat/l

FEMALE CLINICAL BIOCHEMISTRY VALUES♥

Parameter/Dose	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
Bilirubin					
pre-test	4.6	3.7	5.1	4.3	3.8
pre-test	3.8	4.8	5.2	4.2	4.6
13	4.8	4.3	5.0	5.2	6.2*
26	5.2	5.0	4.9	5.3	5.7
52	5.2	5.6	5.3	5.4	6.1
Total Lipids					
pre-test	2.8	2.9	3.4	3.0	2.9
pre-test	2.8	2.6	2.9	3.0	2.9
13	3.4	3.7	3.7	4.4	4.5
26	3.7	3.8	4.2	4.4	4.1
52	4.5	5.2	5.3	4.9	4.7
Cholesterol					
pre-test	3.52	3.80	4.02	3.95	3.65
pre-test	3.42	3.69	3.89	4.00	3.76
13	3.60	3.85	4.16	5.14	5.21*
26	4.35	4.09	4.96	5.21	5.08
52	4.54	4.90	5.51	5.60	5.20
Phospholipids					
pre-test	3.46	3.65	3.92	3.68	3.54
pre-test	3.60	3.62	3.81	3.76	3.42
13	3.98	4.21	4.20	4.73	4.89
26	4.32	4.20	4.56	4.68	4.56
52	4.55	4.69	4.87	4.80	4.61
Triglycerides					
pre-test	0.49	0.50	0.59	0.49	0.48
pre-test	0.44	0.37	0.44	0.42	0.43
13	0.46	0.49	0.46	0.44	0.47
26	0.51	0.59	0.51	0.60	0.50
52	0.69	0.91	0.69	0.63	0.63
Thyroxine(T_4)					
pre-test	50.7	52.7	62.5	51.0	51.5
pre-test	48.0	49.9	56.5	48.3	50.7
13	40.7	43.6	47.3	34.3	27.6*
26	54.6	49.3	45.0	42.3	34.1**
52	45.7	46.5	45.8	45.4	31.7**
Alkaline Ph.					
pre-test	5.84	6.80	4.88	6.66	5.65
pre-test	5.16	5.67	4.22	5.88	4.68
13	2.99	3.97	3.25	4.41	4.53
26	2.87	3.14	3.14	4.06	4.39
52	3.00	3.83	3.50	4.13	4.23
Triiodothyronine (T_3)					
pre-test	1.12	1.26	1.51	1.23	1.15
pre-test	1.24	1.35	1.48	1.40	1.29
13	2.18	2.08	1.97	2.15	2.06
26	1.50	1.49	1.21	1.52	1.44
52	2.02	1.87	1.68	2.11	1.62

♥ Units: CHOL/PL/TG-mmol/l; TL-g/l; T_3/T_4-nmol/l; AlkP-ukat/l; BIL-umol/l

6. Urinalysis: The CHECKED (X) parameters were examined.

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

RESULTS

None of the parameters was affected by treatment.

7. Neurological Examinations

Neurological examinations, including examination of gait, postural reactions, spinal reflexes, and cranial nerves, were performed on each dog pre-test and at weeks 14, 26, 40, 46, and 51.

(a) **Postural Reactions:**
(1) Wheelbarrowing, wheelbarrowing with extended neck;
(2) Hopping - thoracic and pelvic limbs;
(3) Extensor postural thrust;
(4) Hemistanding and hemiwalking;
(5) Placing thoracic limbs - visual and tactile;
(6) Tonic neck reaction;
(7) Proprioceptive positioning.

(b) **Spinal Reflexes**
(1) Muscle tone;
(2) Patellar reflexes;
(3) Biceps and triceps reflex;
(4) Flexor reflexes - pelvic and thoracic limbs;
(5) Pain perception;
(6) Crossed extensor reflex
(7) Perineal reflex;
(8) Panniculus reflex.

(c) **Cranial Nerves**
The cranial nerves examined are in brackets ( ). The head was observed for evidence of abnormal posture (vestibular VIII), facial muscle weakness or contracture (VII), or atrophy of the muscles of mastication (Motor V). With one eye covered, the other eye was menaced (II-VII). If the response was absent, the eyelids were checked for their ability to close (VII). The symmetry of the pupils and their reaction of light were observed (II-III). The eyes were checked for evidence of abnormal posture, strabismus (III, VI, vestibular VIII), or abnormal nystagmus (vestibular VIII). The corneal and palpebral reflexes (sensory V-VII), ear movement, (VII)< and the position of the philtrum (VII) were tested. The commissure of the lips was checked for hypertonia (VII). The skin sensation was checked over the entire head (sensory V). The

jaws were observed for normal closure (motor V). The resistance to opening of the mouth was tested for normality (motor V). The position of the tongue, its movements, and size (atrophy) and strength were tested (XII). The gag reflex was checked (IX, X).

RESULTS

There were no treatment-related effects reported on any of the neurological parameters examined.

8. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The following organs were weighed: adrenal glands, brain (including brainstem), heart, kidneys, liver, pituitary, testes with epididymides, thyroid gland with parathyroid.

X	Digestive system
X	Tongue
X	Salivary glands♣
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
X	Liver
X	Gall bladder
X	Pancreas
	Respiratory
X	Trachea
X	Lung
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
X	Aorta
X	Heart
X	Bone marrow♥
X	Lymph nodes♣
X	Spleen
X	Thymus
	Urogenital
X	Kidneys
X	Urinary bladder
X	Testes
X	Epididymides
X	Prostate
	Seminal vesicle
X	Ovaries
X	Uterus
X	Vagina

X	Neurologic
X	Brain♦
X	Periph. nerve (sciatic)
X	Spinal cord□
X	Pituitary
X	Eyes (optic n.)
	Glandular
X	Adrenal gland
	Lacrimal gland
X	Mammary gland area
X	Parathyroids
X	Thyroids
	Other
X	Bone (femur)
X	Skeletal muscle
X	Skin
X	All gross lesions and masses

♥femur & sternum; ♦medulla/pons, cerebellar & cerebral cortex; ♣mesenteric & retropharyngeal; ♣parotid & mandibular; □cervical, midthoracic, & lumbar

Bone marrow smears from one sternebrum from all dogs were taken for possible further investigation. Samples of sciatic nerve, after perfusion fixation with glutaraldehyde, were embedded in epoxy resin blocks for possible future examination.

a. Organ Weight: The weight (absolute, relative to body and brain weight) of the thyroid gland was increased in both sexes at the 2200 ppm dose level. Additionally, the right thyroid (relative to body) weight was increased in males at the 1000 ppm dose level, but statistical significance was not attained for the increase observed in the left thyroid. Adrenal (absolute/relative-to-body/to brain) weight was increased at the 2200 ppm dose level in both sexes, but only the relative-to-body weight in the males attained statistical significance. No other organ weights were affected by treatment.

Organ-Weight Data (grams)

Group/Organ	0 ppm	50 ppm	200 ppm	1000 ppm	2200 ppm
MALES					
Thyroid					
(absolute)					
left	0.320	0.400	0.363	0.512	0.937**
right	0.284	0.400	0.353	0.524	0.881**
total	0.604	0.800	0.716	1.036	1.818
(relative) to:					
body wt.					
left	0.0035	0.0042	0.0040	0.0066	0.0116**
right	0.0031	0.0042	0.0039	0.0067*	0.0107**
brain wt.					
left	0.3916	0.4882	0.4213	0.6764	1.2215**
right	0.3453	0.4872	0.4111	0.6968	1.1415**
Adrenals					
(absolute)					
left	0.508	0.502	0.603	0.525	0.620
right	0.539	0.559	0.621	0.529	0.634
(relative to):					
body wt.					
left	0.0056	0.0052	0.0068	0.0065	0.0077*
right	0.0059	0.0058	0.0069	0.0067	0.0079*
brain wt.					
left	0.6191	0.6054	0.7087	0.6945	0.7992
right	0.6545	0.6716	0.7259	0.7002	0.8187
FEMALES					
Thyroid					
(absolute)					
left	0.337	0.304	0.368	0.485	1.037**
right	0.335	0.329	0.389	0.459	0.952**
total	0.672	0.633	0.757	0.944	1.989
(relative to):					
body wt.					
left	0.0044	0.0040	0.0049	0.0063	0.0165**
right	0.0043	0.0043	0.0052	0.0060	0.0151**
brain wt.					
left	0.4551	0.3511	0.4889	0.6103	1.4386**
right	0.4524	0.3802	0.5231	0.5788	1.3217**
Adrenals					
(absolute)					
left	0.534	0.531	0.523	0.520	0.589
right	0.564	0.585	0.585	0.560	0.644
(relative to):					
body wt.					
left	0.0070	0.0070	0.0072	0.0068	0.0091
right	0.0075	0.0077	0.0080	0.0073	0.0099
brain wt.					
left	0.7172	0.6173	0.6893	0.6498	0.8213
right	0.7505	0.6791	0.7736	0.6999	0.8938

* $p<0.05$; ** $p<0.01$

b. Gross Pathology: Thyroid enlargement and/or thickening were observed macroscopically in all dogs at the 2200 ppm dose level, in most (3/5 ♂♂;4/5 ♀♀) of the 1000 ppm dose level dogs, and in one dog at each of the other two dose levels. The incidence of salivary gland enlargement was increased in a dose-related manner in males at 200, 1000, and 2200 ppm and in one female at the 2200 ppm dose level. Retropharyngeal lymph node discoloration was observed in several animals, but there was no clear dose response.

c. Microscopic Pathology: Thyroid follicular hyperplasia was displayed in all dogs of both sexes at the two highest dose levels, with severity increasing with dose.

Severity of Follicular Hyperplasia in the Thyroid

Group (PPM) Parameter	MALES					FEMALES				
	0	50	200	1000	2200	0	50	200	1000	2200
THYROID n=	5	5	5	5	5	5	5	5	5	5
Follicular hyperplasia										
Grade 1	0	0	0	2	0	0	0	0	3	0
Grade 2	0	0	0	3	0	0	0	0	1	0
Grade 3	0	0	0	0	3	0	0	0	1	2
Grade 4	0	0	0	0	2	0	0	0	0	2
Grade 5	0	0	0	0	0	0	0	0	0	1

No histopathological findings were reported in the thyroid for either female at the 50 and 200 ppm dose levels that displayed thyroid enlargement/thickening at necropsy. Additionally, no histological changes in the salivary glands were observed to explain the necropsy findings. There were no other findings suggestive of any treatment-related effect.

D. DISCUSSION

The thyroid is the target organ for Maneb. Effects on the thyroid in the current study include increased organ weight, enlargement and thickening (macroscopically), and follicular hyperplasia (microscopically). There was a slight, but consistent, decrease in plasma thyroxine concentration, which the authors contend may have lead to an increased release of thyroid stimulating hormone from the pituitary gland, via a feedback mechanism, and the increase in the size of the thyroid gland. TB II notes that increases in lipid levels are indicative of metabolic adaptation following thyroid disturbances, and the changes observed in the clinical chemistry parameters (increased triglycerides and cholesterol) are consistent with decreased thyroid function. The authors stated that the increase in adrenal gland weight at the highest dose level, which was not associated with any pathology, was considered to be a stress-related hypertrophy commonly observed in dogs administered a wide range of xenobiotics. The lower incidence of females at the highest

dose level displaying signs of estrus during the study cannot be excluded as a treatment-related effect, although the age at first estrus in beagles is known to vary considerably. In the absence of any pathological change, it may be considered a secondary response to the other effects observed at this dose level, rather than to a direct effect on the reproductive system. TB II notes that a similar finding of fewer females displaying estrus changes was noted in the chronic dog study on Metiram (DER dated 4/13/92), although no clear dose response was displayed.

The treated dogs displayed anemia, which the authors contend had the characteristics of a hemolytic type anemia; i.e., a reduction of red cell values followed by compensatory hemopoiesis, which was indicated by the increased reticulocyte count and an increase in red cell size. The authors also stated that the increase in platelet count and polychromasia at the 2200 ppm dose level indicates a generalized bone marrow response, although TB II notes that no effects were reported in the bone marrow. In the Methods' section of the report, it is stated that bone marrow smears from one sternebrum from all dogs were taken for possible further investigation, but no data were provided.

Although none of the neurological examinations (performed to assess gait, postural reactions, spinal reflexes, and cranial nerves) was affected by treatment, no microscopic examination of the sciatic nerve was performed, other than routine histological examination. Since microscopic lesions can occur in the absence of any clinical signs, neuropathological examination of the sciatic nerve (including its extensions, sural, tibial, and peroneal nerves, as well as the interosseus muscle) should be performed and the results submitted.

E. CONCLUSION

Under the conditions of the study, exposure to Maneb via the diet at dose levels of 50, 200, 1000, and 2200 ppm for one year resulted in decreased body weight in the 2200 ppm dose level females throughout most of the study (from week 3 on the range was 92-87% of control value). Male body weight was comparable among the groups throughout the study. Body weight gain and food consumption were decreased at the 2200 ppm dose level in both sexes, with statistical significance being attained throughout most of the study in the females and for the first 13 weeks in males. Several hematology parameters, suggestive of a hemolytic anemia, were affected by treatment at the two highest dose levels. Additionally, several clinical chemistry parameters were altered, which are consistent with hypothyroidism. Thyroid (absolute and relative to body/brain) weight was increased in both sexes at the 1000 and 2200 ppm dose levels, but statistical significance was attained only at

the highest dose level. Adrenal gland weight was slightly increased in both sexes at the 2200 ppm dose level, although statistical significance was attained only in males (relative to body weight). Follicular hyperplasia was displayed in all dogs (both sexes) at the two highest dose levels, with the severity increasing with dose.

The NOEL for effects other than neuropathological effects was 50 ppm (1.53 ♂♂/1.71 ♀♀ mg/kg) and the LEL was 200 ppm (6.36 ♂♂/7.18 ♀♀ mg/kg), based on decreased body-weight gain/food consumption, changes in hematology/clinical chemistry parameters indicative of thyroid toxicity/anemia, increased thyroid weight, and follicular (thyroid) hyperplasia. Although none of the neurological parameters examined was affected by treatment, microscopic lesions can occur in the absence of clinical signs. Therefore, neuropathological examination of the sciatic nerve is required before a final determination regarding the NOEL can be made.

This study is classified core supplementary, pending submission of the neuropathological examination of the sciatic nerve, including its extensions, sural, tibial, and peroneal nerves, as well as the interosseus muscle.