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

004920

2/4/1986.

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 6-Month Monkey Feeding Study with Maneb

TO: Mr. Mike Branagan, PM 65
Special Review Branch (TS-767C)

FROM: Byron T. Backus Byron T. Backus 02-03-86
Toxicologist
Toxicology Branch

THROUGH: Clint Skinner, Ph.D. Alan Clark 2/4/86 (for CS)
Head, Section III
and
Theodore Farber, Ph.D. [illegible] 2/5/86
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

Chemical no. 539

Project No. 1078

Action Requested:

The Special Review Branch has requested a review of this study in order to determine whether it is an acceptable substitute for a 90-day non-rodent subchronic feeding study.

Comments and Conclusions:

1. The study has been classified as supplementary as a non-rodent subchronic feeding study. It can be upgraded to acceptable if information is provided as to how (and when) the Maneb was mixed with the diet along with analytical results demonstrating that the Maneb content remained reasonably stable until the preparation was fed to the monkeys. If no analytical work was done during the actual study, then samples of "Altromin 6021" with Maneb should be prepared, stored for whatever period of time they were stored during the study, and analyzed.

2. Tentatively (pending analytical results) the NOEL is set at 100 ppm, and the LEL (increase in thyroid weight among males) at 300 ppm. Effects noted at 3000 ppm were increase (statistically significant at $p < 0.01$) in thyroid weight associated with an enlargement of this organ, along with significantly reduced ^{131}I-

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absorption in the thyroid and lower mean percentage of protein-bound ^{131}I at 26 weeks. Reduced weight gain for monkeys at 3000 ppm and somewhat lower food consumption for the males of this group, while not statistically significant at $p < 0.01$, may have resulted from thyroid effects. Another possible effect (perhaps not statistically significant at $p < 0.01$) at 3000 ppm was an increase in mean absolute liver weights, particularly among males.

3. A copy of the data evaluation report should be provided to the registrant.

Data Evaluation Report (attached):

1. Leuschner, F., Leuschner, A., Schneider, C., Schwerdtfeger, W., and Dontenwill, W. Oral Toxicity of Manganese Ethylene-1,2-Bis-Dithiocarbamate, 90%, Internal No. WF 1172 - Called for short "Maneb" - in the Rhesus Monkey (Repeated Dosage for six Months). Study conducted at the Laboratorium fur Pharmakologie und Toxikologie, D-2104 Hamburg. Report dated January 31, 1977.

Data Evaluation Report

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Compound:

Maneb

Study type:

6-Month feeding study - rhesus monkey

Citation:

Lauschner, F., Leuschner, A., Schneider, C., Schwerdtfeger, W., and Dontenwill, W. Oral Toxicity of Manganese Ethylene-1,2-Bis-Dithiocarbamate, 90%, Internal No. WF 1172 - Called for short "Maneb" - in the Rhesus Monkey (Repeated Dosage for six Months). Study conducted at the Laboratorium fur Pharmakologie und Toxikologie, D-2104 Hamburg. Report dated January 31, 1977. Received at EPA 9-13-85; in Acc. 259627.

Reviewed by:

Byron T. Backus
Toxicologist
Toxicology Branch

Byron T. Backus 02-07-86

Approved by:

Clint Skinner, Ph.D.
Section Head
Review Section III
Toxicology Branch

Core Classification: Core Supplementary Data (as a subchronic feeding study)

Conclusions:

1. While the study is currently classified as core supplementary data as a non-rodent subchronic feeding study, it can be upgraded to acceptable if information is provided as to how (and when) the Maneb was mixed with the diet and some analytical results are provided demonstrating that the Maneb content remained reasonably stable until the preparation was fed to the monkeys. If no analytical work was done during the actual study, then samples of "Altromin 6021" with Maneb should be prepared and analyzed.

2. Tentatively (pending analytical results) the NOEL is set at 100 ppm, and the LEL (increase in thyroid weight in males) at 300 ppm. Effects noted at 3000 ppm were increase (statistically significant at $p < 0.01$) in thyroid weight associated with an abnormal enlargement of this organ, along with significantly reduced ^{131}I absorption in the thyroid and lower mean percentage of protein-bound ^{131}I at 26 weeks. Reduced weight gain for monkeys at 3000 ppm and somewhat lower food consumption for the males of this group, while not statisticaly significant at $p < 0.01$, may also have involved thyroid effects. Another possible effect (not statistically significant at $p < 0.01$) at 3000 ppm was an increase in mean liver weight (along with an increase in liver-to-body weight ratio). Effects

were more pronounced among the males than females.

Materials and Methods:

32 Cross (random?) bred Rhesus monkeys, equal numbers of both sexes, were obtained from Primelabs Inc., Farmingdale, NJ 07727. Estimated initial ages were 5-6 yrs; initial weights were 4.8-7.0 kg. Monkeys were quarantined (where is not specified) in Germany for 12 weeks; during this period or subsequently animals were found to be negative for tuberculosis exposure. There was no sign of disease in any of the animals and all had normal ECG-findings and a normal prothrombin time.

Diet: Altromin 6021 (manufactured by Messrs. ALTROMIN GmbH, P.O. Box 285, D-4937 Lage/Lippe); analyzed every 3 months to check for presence of aflatoxins (limit of detection: 2 ppb). This diet was mixed with tap water (1:1 proportion) and "the taste was improved by banana pap."

"Drinking water was given unrestricted."

Test material: Manganese ethylene-1,2-bis-dithiocarbamage, 90%. (presumably 90% purity). Internal no. (batch no?) WF 1172.

Procedure:

Groups of 4M, 4F rhesus monkeys, received 0, 100, 300 or 3000 ppm of Maneb in their diets over a period of 6 months.

Monkeys were observed daily for appearance and behavior. 80 grams of food admixture/kg b.w. was offered to each monkey for 24 hours. Uneaten food was removed at the end of this time and weighed. Food consumption was apparently recorded on a daily basis (although reported weekly), although it is noted (p. 17) that some accuracy was lost because monkeys tended to lose some of their food while playing with it (p. 17). Body weight was determined on a weekly basis.

The following haematology measurements were made at 0, 6, 13, 18 and 26 weeks for all animals (it is not reported whether animals were fasted and/or anesthetized before blood was taken):

Haemoglobin content (g/100 ml blood)
erythrocyte and leucocyte counts
differential count
haematocrit value
prothrombin time
erythrocyte sedimentation rate (mm/hour and mm/2 hrs)
blood clotting time (sec)
platelets (100,000/mm^3 blood)
reticulocytes (% of the erythrocytes)

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The following clinical biochemistry measurements were made at 0, 6, 13, 18 and 26 weeks for all animals (althouqh it is not certain animals were fasted before these determinations were made):

Glutamic pyruvic transaminase (SGPT)
Glutamic oxalacetic transaminase (SGOT)
Glucose
Blood urea nitrogen (BUN)
Total protein
Total bilirubin
Alkaline phosphatase (SAP)
Sodium
Potassium
Calcium
Chloride
Uric acid
Liver function (bromsulphthalein test according to Rosenthal, White, Hofman and Oettel).
Albumin
Globulin

At 6, 13 and 26 weeks each animal was given, on an empty stomach, 10 uCi of ^{131}I (as iodide in an aqueous solution, volume 2 ml). Subsequently, the following determinations were made:

^{131}I-absorption of the thyroid (ipm)
(Radioactivity of thyroid was measured at 2 and 48 hrs after administration of the radioactive iodine).
Serum thyroxine (T_4)
Binding power of T_3 in %.
Protein-bound ^{131}I in serum (% of total ^{131}I).
Loss of ^{131}I from the thyroid ("half-life time" in days).

Urinalyses measurements were carried out at 0, 6, 13, 18 and 26 weeks. Each animal received 25 ml tap water/kg body weight by stomach tube; urine was collected one hour later by means of a catheter. Urine specimens were analyzed for:

Color
Specific weight (specific gravity?)
Protein (acetic acid heating test)
Glucose)
Bilirubin)
Hemoglobin) Using Bili-Labstix
Ketone bodies)
pH)

Urinary sediment was examined for epithelial cells, leucocyctes, erythrocytes, organisms such as worm eggs and bacteria, casts and crystals.

Electrocardiographic measurements were made on each animal at 0, 6, 13, 18 and 26 weeks. The heart rate, P-Q and Q-T times were determined and "the functional picture evaluated."

Eyes, hearing and teeth were evaluated at 0, 6, 13, 18 and 26 weeks. Eyes were examined opthalmoscopically. Hearing "was tested by means of a simple noise test."

After 26 weeks of treatment the animals were sacrificed by an IV dose of 0.3 ml T 61/kg (T61 is identified, p. 14, as 200 mg N-[2-ethyl-2-(m-methoxyphenyl)butyl-1-]-gamma-hydroxybutyramide, 50 mg methylenebis [(4-cyclohexyl)trimethylammonium iodide] and 5 mg 4'-butylaminobenzoyl-2-dimethylaminoethanol hydrochloride), exsanguinated, and complete autopsies were performed. The weights of the following internal organs were determined for each of the monkeys, paraffin sections were prepared, stained with hematoxylineosin, and were examined histologically:

Heart	Kidney	Gonads
Liver	Adrenal	Thyroid
Lungs	Thymus	Brain
Spleen	Pituitary	

Paraffin sections of the following organs were also stained with hematoxylin-eosin and examined histologically:

prostate/uterus	eye	peripheral nerve
stomach	urinary bladder	skeletal muscle
duodenum	bone marrow	skin
jejunum	trachea	tongue
ileum	aorta	spinal cord
colon	esophagus	gall bladder
rectum	pancreas	bone
salvary gland	mes. lymph node	

Additionally, frozen sections of heart, liver and kidney were prepared and stained with "sudan" (Sudan black?). The bone marrow was sectioned "undecalcified."

Statistical evaluation:

All determined values were evaluated using analysis of variance and Student's t-test. Limit for significance was $p \leq 0.01$ (p. 15), rather than the usual $p \leq 0.05$.

Results:

All animals survived to study termination. Behavior and external appearance of the individual monkeys was unremarkable during the 6 months. Feces were normal in all groups. All of the females had normal menstrual discharges during the study.

One of the males in the 3000 ppm group showed an actual weight loss (0.6 kg) during the 26 week study, while another male in this group showed only a slight (0.1 kg) weight gain. All the other males showed individual weight gains of at least 0.5 kg (Table 3, p. 52).

One of the females in the 100 ppm group showed no weight gain during

the 26-week period (remaining at 5.7 kg), as did one female in the 3000 ppm group which remained at 5.6 kg. Overall, mean body weight gains in the 3000 ppm monkeys were somewhat lower than those of controls and the other two groups. From table 3, p. 51:

	GROUPS			
	Mean weights in kg + S.D.			
Week	I	II	III	IV
	0 ppm	100 ppm	300 ppm	3000 ppm
0	5.2 ± 0.4	5.5 ± 0.5	5.5 ± 0.5	5.5 ± 0.7
6	5.4 ± 0.4	5.7 ± 0.5	5.7 ± 0.5	5.4 ± 0.8
13	5.6 ± 0.4	5.7 ± 0.4	6.0 ± 0.4	5.7 ± 0.9
18	5.7 ± 0.5	5.9 ± 0.4	6.1 ± 0.4	5.7 ± 0.9
26	6.0 ± 0.5	6.2 ± 0.5	6.6 ± 0.4	5.9 ± 1.0

The following mean (percentage) changes in weights are calculated from the values given above:

	GROUPS			
Week	I	II	*III	IV
	0 ppm	100 ppm	300 ppm	3000 ppm
6	0.2 (4%)	0.2 (4%)	0.2 (4%)	-0.1 (-2%)
13	0.4 (8%)	0.2 (4%)	0.5 (9%)	0.2 (4%)
18	0.5 (10%)	0.4 (7%)	0.6 (11%)	0.2 (4%)
26	0.8 (15%)	0.7 (13%)	1.1 (20%)	0.4 (7%)

Mean cumulative food consumption (in g/kg) can be calculated from the data given on p. 42-49. The males in the highest dose level group ate slightly less cumulatively on a body weight basis than the males of the control and lower dose groups, although differences are probably not statistically significant:

Mean food consumption (g/kg/day x 7 days/week x 26 weeks)

Group	Males	Females
I	9772	9569
II	9653	9660
III	9779	9772
IV	9149	9562

Hematology: There was no indication of any meaningful differences between groups, dose-related trends or time-related changes with respect to such parameters as hemoglobin, RBC and/or WBC counts, differential counts, hematocrit values, prothrombin times, erythrocyte sedimentation rates, blood clotting times, platelet counts or reticulocytes as a percentage of the erythrocytes.

Clinical biochemistry: Mean SGPT in monkeys at 3000 ppm was somewhat depressed (by about 19.3%) from the control value at 26 weeks only, but, given the standard deviations, it is doubtful whether this was statistically significant even at $p < 0.05$. There were no indications of any possibly significant differences at any time between groups with respect to glucose, BUN, SAP, SGOT, total bilirubin, serum proteins (albumin, globulins), or uric acid. At 26 weeks (but not at weeks 6 or 13) the monkeys at 3000 ppm showed significantly ($p < 0.01$) less ^{131}I absorption by the thyroid than did the controls; from p. 136:

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^{131}I-absorption of the thyroid (ipm = cpm?) ± S.D.

Group	Week 6	Week 13	Week 26
I	716.8 ± 127.0	774.6 ± 194.7	872.6 ± 87.8
II	724.9 ± 159.9	742.1 ± 174.2	793.5 ± 157.5
III	676.3 ± 103.4	736.6 ± 98.2	845.9 ± 143.1
IV	663.8 ± 202.4	712.9 ± 164.4	551.4 ± 188.8 †

†significantly different from control with $p \leq 0.01$

There was no indication of any sex-related differences:

^{131}I-absorption of the thyroid (ipm = cpm?) ± S.D.

Group	Week 6	Week 13	Week 26
I (males)	714.5 ± 162.6	895.3 ± 210	914.5 ± 94
I (females)	719 ± 105.8	654 ± 74.6	830.8 ± 67
II (males)	822 ± 169.5	683.8 ± 129.9	788.3 ± 128.3
II (females)	627.8 ± 75.9	800.5 ± 211.8	798.8 ± 203.3
III (males)	677.3 ± 145.3	761 ± 105.6	879.5 ± 100.7
III (females)	675.3 ± 61.7	712.3 ± 98.9	812.3 ± 186.2
IV (males)	644 ± 232.3	689.5 ± 139.1	548.3 ± 231.9
IV (females)	683.5 ± 201.5	736.3 ± 205.5	554.5 ± 171.4

Although not statistically significant at $p < 0.01$, 3000 ppm monkeys at 26 weeks showed a shorter half-life (18.2 days to the controls' 20.3 days) with respect to loss of ^{131}I, and their thyroxin levels tended (again, without statistical significance) to be somewhat lower than those of the controls and other two dosage groups. The mean percentage of protein-bound ^{131}I in the 3000 ppm monkeys was significantly lower ($p \leq 0.01$) at 26 weeks than the control value; from p. 141:

Protein-bound ^{131}I
% Bound

Group	Week 6	Week 13	Week 26
I (0 ppm)	4.3 ± 0.7	4.7 ± 1.1	5.0 ± 1.0
II (100 ppm)	4.5 ± 0.8	4.2 ± 0.8	4.1 ± 0.9
III (300 ppm)	4.4 ± 1.3	4.6 ± 1.2	4.0 ± 1.2
IV (3000 ppm)	3.8 ± 1.1	3.8 ± 1.2	3.3 ± 1.0 †

†significantly different from control with $p \leq 0.01$

The significant difference existing for group IV in protein-bound ^{131}I at 26 weeks was largely due to the males:

Protein-bound ^{131}I
% Bound

Group	Week 6	Week 13	Week 26
I (males)	4.0 ± 0.7	5.0 ± 1.2	4.7 ± 0.9
I (females)	4.5 ± 0.7	4.4 ± 1.0	5.3 ± 1.1
II (males)	4.5 ± 0.9	4.3 ± 1.1	3.8 ± 0.9
II (females)	4.5 ± 0.7	4.1 ± 0.4	4.4 ± 0.9
III (males)	5.0 ± 1.6	5.0 ± 1.5	4.3 ± 1.2
III (females)	3.8 ± 0.6	4.3 ± 1.0	3.8 ± 1.3
IV (males)	4.0 ± 1.4	4.6 ± 1.0	2.7 ± 0.9
IV (females)	3.6 ± 0.7	3.0 ± 0.7	3.9 ± 0.6

Urinalysis: There was no evidence of any significant differences between groups, possible dose-related trends or any suggestive time-related changes with respect to such parameters as specific gravity, pH, color, protein, glucose, bilirubins, ketones, hemoglobin or urinary sediment.

Electrocardiography: No electrocardiographic changes or differences between groups were noted.

Sight, hearing and dentition: From p. 21: Ophthalmoscopic examination "revealed no pathological findings." There was no evidence of impairment of hearing. "The dentition was free from pathological changes."

Gross autopsies: Nothing remarkable was found in any of the monkeys which could be ascribed to exposure to Maneb.

Organ weight data: Absolute weights for thyroids were increased for monkeys in the 300 and 3000 ppm groups, and this was statistically significant at 3000 ppm; from p. 171 and 172:

Thyroid mean organ-to-body weight ratios and group mean wts.

Group	Organ-to-body weight ratio		Actual wts ± S.D.	
	L	R	L	R
I (0 ppm)	0.09	0.09	0.51 ± 0.14	0.55 ± 0.14
II (100 ppm)	0.09	0.09	0.56 ± 0.19	0.53 ± 0.13
III (300 ppm)	0.10	0.09	0.66 ± 0.16	0.61 ± 0.13
IV (3000 ppm)	0.17	0.16	0.98† ± 0.37	0.96† ± 0.35

†significantly different from control with $p \leq 0.01$

Breaking down thyroid-to-body weight ratios and actual thyroid weights by sex, the effect is more pronounced in males:

Group	Organ-to-body weight ratio		Actual wts ± S.D.	
	L	R	L	R
I (males)	0.084 ± 0.042	0.096 ± 0.043	0.50 ± 0.20	0.58 ± 0.20
I (females)	0.090 ± 0.016	0.089 ± 0.011	0.53 ± 0.10	0.52 ± 0.06
II (males)	0.098 ± 0.021	0.084 ± 0.019	0.59 ± 0.11	0.51 ± 0.09
II (females)	0.083 ± 0.038	0.089 ± 0.028	0.52 ± 0.26	0.55 ± 0.17
III (males)	0.120 ± 0.023	0.098 ± 0.025	0.78 ± 0.11	0.65 ± 0.18
III (females)	0.082 ± 0.018	0.087 ± 0.011	0.54 ± 0.10	0.58 ± 0.07
IV (males)	0.230 ± 0.034	0.238 ± 0.052	1.26 ± 0.28	1.28 ± 0.13
IV (females)	0.113 ± 0.026	0.106 ± 0.024	0.69 ± 0.11	0.65 ± 0.10

Although not statistically significant at $p < 0.01$, monkeys at 3000 ppm also showed, relative to controls and the two lower dose groups, increase in mean liver weight, and decreases in mean weights for spleen and brain; from p. 172:

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Group	Mean liver wt ± S.D.	Mean spleen wt ± S.D.	Mean brain wt ± S.D.
I (0 ppm)	120.4 ± 11.9	6.6 ± 1.2	83.3 ± 6.9
II (100 ppm)	121.0 ± 16.5	6.7 ± 1.0	83.0 ± 6.5
III (300 ppm)	122.9 ± 15.2	6.2 ± 1.4	85.1 ± 9.5
IV (3000 ppm)	133.0 ± 13.3	5.7 ± 1.3	79.6 ± 7.6

Changing these to organ-to-body weight ratios and keeping data from the sexes separate:

Group	Liver-to-body wt ratio x 1000 ± S.D.	Spleen-to-body wt ratio x 1000 ± S.D.	Brain-to-body wt ratio x 1000 ± S.D.
I (males)	20.20 ± 0.95	1.02 ± 0.22	13.81 ± 0.67
I (females)	19.87 ± 1.32	1.21 ± 0.30	13.98 ± 1.41
II (males)	19.32 ± 1.75	1.12 ± 0.30	13.97 ± 1.19
II (females)	19.85 ± 1.90	1.10 ± 0.18	13.02 ± 0.31
III (males)	18.06 ± 1.52	0.93 ± 0.32	13.16 ± 1.96
III (females)	19.27 ± 1.46	0.96 ± 0.10	12.79 ± 1.35
IV (males)	24.83 ± 3.23	1.09 ± 0.19	15.26 ± 1.30
IV (females)	21.17 ± 0.92	0.92 ± 0.30	12.39 ± 1.71

The increase in liver-to-body weight is noticeable, particularly for the males, although it also seems to be somewhat present in the females. The lower mean spleen and brain weights in the group IV monkeys were due to lower than usual values from a single female (#29).

The only consistent abnormal histology findings on microscopic examination involved the thyroids of 7/8 of the group IV (3000 ppm) monkeys. The seven monkeys all had what is described (p. 208) as: "enlarged thyroid with large follicles, epithelium rather flat, colloid somewhat darker than usual." In addition, two of these 7 subjects also had: "moderate proliferation of the epithelium." Only one of the monkeys at 3000 ppm had no thyroid-associated pathological findings.

Discussion:

One concern of this reviewer is that no analytical work was done on any food samples that were fed to the monkeys. According to the text (p. 6) "Maneb was added to the food homogeneously (constant admixture) and was given on all seven days of the week." It is uncertain whether the "constant admixture" means that the Maneb was added to the food on a daily basis (just before it was given to the monkeys) or whether it was mixed with the food on a weekly, monthly or some other basis. Because of this uncertainty, the study has been classified as supplementary.

In order to upgrade this study information should be provided as to how (and when) the Maneb was mixed with the diet, and some analytical results should be provided demonstrating that the Maneb content remained reasonably stable until the diet was fed to the monkeys. If no analytical work was done during the actual study, then samples of "Altromin 6021" with Maneb should be prepared and analyzed.

Otherwise, the study adequately demonstrates a number of dose-related effects of Maneb at 3000 ppm on the thyroid and its functions. These include the statistically significant increase in mean absolute thyroid weight, the associated abnormal enlargement of the thyroid occurring in 7/8 animals of this dose group, along with the significantly reduced ^{131}I-absorption and lower mean percentage of protein-bound ^{131}I at 26 weeks. The reduced weight gain of the monkeys at 3000 ppm and somewhat lower food consumption for the males of this group, while not statistically significant at $p \leq 0.01$, may also have been a result of effects on the thyroids of these animals.

In terms of study acceptance, there was an adequate number of subjects (4/sex/dosage group). However, given this relatively low number of subjects some possible effects (such as increase in both mean absolute liver weight and liver-to-body weight ratios) may not have been statistically significant, particularly as the study utilized a $p \leq 0.01$ for the "resolution" of significant effects. It is difficult to imagine that the liver weight increases could have resulted from effects originating in the thyroid.

Although probably not statistically significant at $p < 0.01$, there is a noticeable elevation in mean absolute thyroid weights for the male monkeys at 300 ppm, and this is part of what appears to be a dose-related trend. On this basis, the NOEL is set at 100 ppm, and the LEL at 300 ppm.