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

004969

3/6/1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 31-Month Rat Feeding Study with Maneb

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

FROM: Byron T. Backus Byron T. Backus 03/06/86
Toxicologist
Toxicology Branch

THROUGH: Clint Skinner, Ph.D. 3-6-86
Head, Section III
and
Theodore Farber, Ph.D. 3/6/86
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

Chemical no. 539 Report No. 160215

Project No. 692 EPA ID No. 014504

Action Requested:

The Special Review Branch has requested a review of this study in order to determine its acceptability.

Comments and Conclusions:

1. This study has been classified as Core Supplementary Data because of a considerable number of problems, including the presentation of some of the data and the way some calculations were made. Sufficient histology data must be given for lower-dose rats in order to determine a NOEL based on bladder pathology findings and other possible effects.

2. One immediately obvious error within the report involves body weight data at 12 months. The means on which statistical analyses were performed are presented on p. 48 and indicate that there was no significant difference then between rats in the 1000 ppm group and their controls. However, the values on p. 48 for 12 months differ considerably from those on p. 47; from the information presented on p. 61 it appears that the values on p. 48 are derived from

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only the 5 rats/sex/group which were sacrificed at 12 months, and not on all the rats which had survived to that date, and that, in fact, the 1000 ppm rats had significantly less mean body weight at 12 months than their controls. This error was not detected and so as a result there are such statements in the summary presentation as (p. 1): "at the end of the the first year of the study all weights were within the normal range again." and (p. 16): "At the examinations after one year the body weight gain had normalised and showed similar results as those of the untreated animals." Both statements are erroneous.

3. A considerable number of absolute organ weights from rats which survived to termination are presented in parenthesis (p. 369-383). For controls, 24 males and 30 females survived to termination; 0 and 3 respectively had absolute thyroid weights parenthesized. For 1000 ppm rats, 22 males and 33 females survived; thyroid weights were parenthesized for 4 and 9 respectively. All parenthesized thyroid weights were elevated with respect to the mean of each group (presumably this is why they have been set off from the others, although no explanation appears to be present).

   Calculations from thyroid weight data for 1000 ppm males (p. 381) indicate that without the parenthesized data from 4 males the mean thyroid weight for this group would be 0.041 grams with a S.D. of 0.016. With the parenthesized data it would be 0.072 grams with a S.D. of 0.090. As presented the value is 0.046 grams with a S.D. of 0.021; the only "obvious" way that this value could be obtained is by averaging in the parenthesized thyroid weights of two subjects (21 and 65) with the values from the other 18 rats, without using (and without indicating they had not been used, or giving a justification for it) data from rats 9 and 43 (which had the highest thyroid weights).

   A similar situation probably exists with respect to the thyroid weights for females at 1000 ppm.

4. The numbers of the individual rats surviving to termination which were assigned parenthesized weight values for thyroids are given below:

   Control males: none
   Control females: 16, 45, 63

   1000 ppm males: 9, 21, 43, 65
   1000 ppm females: 7, 10, 21, 25, 32, 36, 38, 64, 67

   For 1000 ppm rats, female 32 is reported (p. 23) as having an adenoma involving the parathyroid. No pathological findings (either tumorous or non-tumorous) for the thyroid are reported

for any of the other animals (control or 1000 ppm) listed above. Considering the reported weights of some of their thyroids (i.e., 1000 ppm male 9 had a thyroid weighing 0.255 grams, 5X the mean value for the group; 1000 ppm male had a thyroid weighing 0.413 grams, approximately 8X the mean for the group) some sort of explanation is required (even if the cause of these increased weights was hypertrophy).

5. Histology was not done on lower-dose groups at termination. While the overall tumor incidences for the control and high-dose groups are reported as similar (p. 20) there were some site differences between the groups. Liver tumors occurred only at 1000 ppm (4 males, 1 female), and kidney tumors only at 1000 ppm (2 males, both also with liver tumors). There was also a slight (probably not statistically significant) increased incidence of urinary bladder tumors in 1000 ppm males relative to their controls. In connection with possible bladder effects ten 1000 ppm males had non-tumorous pathological urinary bladder findings (7 had increased or moderate epithelial dysplasia), while no non-tumor findings for the urinary bladder are reported for control males. Without histology data from lower Maneb dose groups it is not known whether these possible effects are part of dose-related trends, or are isolated occurrences involving 1000 ppm rats only. Some histology data from lower-dose rats should be submitted so that NOEL's for urinary bladder pathology findings and other possible effects can be established.

6. Additional problems with this study include the reporting of epithelial papillomatosis of the prostrate as a tumor finding in one control male (#35), but as a non-tumor finding in two 1000 ppm males (#3 - see p. 455, and #4 - see p. 456); use of $p \leq 0.01$ rather than $p \leq 0.05$ for level of significance, and lack of analytical data for Maneb content of the diet (despite a statement on p. 5 that samples of all diets were sent to the sponsor for analysis every 13 weeks).

7. There may be additional problems with this report and study that would become evident with further review of the data.

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

Data Evaluation Report (attached):

Leuschner, F., Leuschner, A., Klie, R., Dontenwill, W., and Rogulja, P. Chronic Oral Toxicity of Manganese-Ethylene-1,2-Bis-Dithiocarbamate, 90% - Called for Short 'Maneb' - in Sprague-Dawley (SIV 50) Rats. Study dated April 9, 1979. Study conducted at the Laboratorium für Pharmakologie und Toxikologie. Study submitted by Rohm and Haas; received at EPA 9-13-85; in Acc. 259628.

Data Evaluation Report

Compound:

Maneb

Study type:

Chronic feeding - rat

Citation:

Leuschner, F., Leuschner, A., Klie, R., Dontenwill, W., and Rogulja, P. Chronic Oral Toxicity of Manganese-Ethylene-1,2-Bis-Dithiocarbamate, 90% - Called for Short 'Maneb' - in Sprague-Dawley (SIV 50) Rats. Study dated April 9, 1979. Study conducted at the Laboratorium für Pharmakologie und Toxikologie. Study submitted by Rohm and Haas; received at EPA 9-13-85 and in Acc. 259628.

Reviewed by:

Byron T. Backus
Toxicologist
Toxicology Branch

Approved by:

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

Core Classification: Core Supplementary

Conclusions:

1. While the study demonstrates such effects as decrease in mean body weight and increases in thyroid-to-body weight ratio and absolute mean thyroid weight (as well as changes in thyroid functions) at the HDT (1000 ppm), there are considerable problems in this report in terms of its presentation and quality.

2. In the summary findings (p. 1) it is stated (for the 1000 ppm group) that "at the end of the first year of the study all weights were within the normal range again." On p. 16 it is stated that "At the examinations after one year the body weight gain had normalised and showed similar results as those of the untreated animals." Both are erroneous statements. The statistical analyses for body weights at 12 months were based only on the 5 rats/sex/group that were sacrificed at that time (refer to p. 61), rather than all of the rats which had survived to that date.

3. A considerable number of absolute organ weights from rats which survived to termination are presented in parenthesis (p. 369-383). For controls, 24 males and 30 females survived to termination; 0 and 3 respectively had absolute thyroid weights parenthesized. For 1000

ppm rats, 22 males and 33 females survived; 4 and 9 respectively had thyroid weights parenthesized. All parenthesized thyroid weights were elevated with respect to the mean of the group (presumably this is why they have been set off from the others, although no explanation is given). Calculations from thyroid weight data for 1000 ppm males (p. 381) indicate that without the parenthesized data from 4 males the mean thyroid weight for this group would be 0.041 grams with a S.D. of 0.016. With all parenthesized data it would be 0.072 grams with a S.D. of 0.090. As presented the value given is 0.046 grams with a S.D. of 0.021; the only obvious way that this value could be obtained is by averaging in the parenthesized thyroid weights of two subjects (21 and 65) with the values from the other 18 rats, without using (and without indicating that they had not been used, or giving the justification for it) data from rats 9 and 43 (which had the highest thyroid weights).

A similar situation probably exists with respect to the females at 1000 ppm.

4. The numbers of the individual rats surviving to termination which were assigned parenthesized weight values for thyroids are given below:

   Males, control: none
   Females, control: 16, 45, 63

   Males, 1000 ppm: 9, 21, 43, 65
   Females, 1000 ppm: 7, 10, 21, 25, 32, 36, 38, 64, 67

   For the 1000 ppm rats, female 32 is reported (p. 23) as having an adenoma involving the parathyroid. No pathological findings (either tumorous or non-tumorous) for the thyroid are reported for any of the other animals (control or 1000 ppm) listed above. Considering the weights of some of these thyroids (i.e., male #9 at 1000 ppm had a thyroid weighing 0.255 grams, five times the mean value for the group; male #43 at 1000 ppm had a thyroid weighing 0.413 grams, more than eight times the mean value for the group) this is rather surprising, and some sort of explanation (even if hypertrophy was the cause of the increased weights, or if these values on necropsy weights were errors; but were included in the report and identified by parenthesis) is required.

5. Histology was not done for lower-dose groups at termination. While the overall tumor incidences for the control and high-dose are reported as similar (see p. 20) there were some differences between the groups. Liver tumors occurred only at 1000 ppm (4 males, 1 female), and kidney tumors only at 1000 ppm (2 males, both of which also had liver tumors). There was also a slight increased incidence in urinary bladder tumors in 1000 ppm males relative to their controls. In connection with possible bladder effects ten 1000 ppm males had non-tumorous pathological urinary bladder findings (7 had increased or moderate epithelial dysplasia), while no non-tumor findings for the urinary bladder are reported for control males. Without histology data from lower Maneb dose groups it is not known whether these possible effects involve dose-related trends, or are isolated occurrences in 1000 ppm rats only.

6. Additional problems with this study include the reporting of epithelial papillomatosis of the prostrate as a tumor finding in one control male (#35), but as a non-tumor finding in two 1000 ppm males (#3 - refer to p. 455, and #4 - refer to p. 456); use of $p < 0.01$ rather than $p < 0.05$ for level of significance; and lack of analytical data (despite the statement made on p. 5 that samples of all diets were sent to the sponsor for analysis every 13 weeks).

7. There may be further problems with this study that would develop with further review of the data.

8. Because of these deficiencies, and because of uncertainties resulting from the way the data are presented (including mean organ weights), the study is classified as Core Supplementary Data. In order to upgrade the study classification the deficiencies indicated above must be corrected, and sufficient histology data from lower dose rats should be supplied to define a NOEL in terms of pathology findings for the bladder and other possible effects.

Materials:

Sprague-Dawley (SIV 50) rats, with initial ages of 38-39 days for the males, and 42-43 days for the females. Initial weights ranged from 100 to 104 g.

Test compound: WF 1172 (technical), identified as Manganese-ethylene-1,2-bis dithiocarbamate, 90%.

Procedure:

The test material was added (as a "constant admixture") to the diet at concentrations of 0, 30, 100, 300 and 1000 ppm. The diet is identified as "Altromin 1321." At 13-week intervals during the study samples of all diets were sent to the sponsor for analysis of the test compound, and the stability of the test compound in the food over a 7-day period was also examined. Food was offered ad libitum, as was drinking water.

Rats were randomly assigned to groups composed of 90 males and 90 females. They were caged singly. Behavior and external appearance were observed twice daily. Faeces were observed. Food and drinking water intake were monitored daily, and were recorded at 1 and 4 week intervals respectively. Beginning at week 26 all rats were palpated once a week for tumors. Time of recognition, location and dimensions of palpable tumors were recorded.

Individual body weights were determined weekly until test week 26, and once a month thereafter.

Blood was drawn from the retrobulbar venous plexus under light ether anesthesia from 10 rats/sex/group at 0, 3, 6, 12, 18 and 24 test months. The following haematology measurements were made on the individual samples:

Haemoglobin content (mmol/l blood)
Erythrocytes
Differential blood count (in %)
Hematocrit value (% in v/v of total blood)
Thromboplastin time (sec)
Blood clotting time (sec)
Platelets (10^9/l blood)
Reticulocytes (% of the erythrocytes)

The following clinical biochemistry measurements were made on the individual samples:

Alanine aminotransferase [Glutamic pyruvic transaminase] (SGPT)
Alkaline phosphatase (AP)
Blood urea
Glucose
Sodium
Potassium
Calcium
Chloride
Uric acid
Phosphate
Creatinine

At 3, 6, 12 and 24 test months 10 animals/sex/group were given (on an empty stomach) 10 uCi of ^{131}I (as iodide in a 2 ml aqueous solution). Subsequently, the following determinations were made:

Loss of ^{131}I from the thyroid (1, 2.5, 4 and 24 h as well as 3, 5, 7 and 10 days after administration; the biological half-life in days was calculated)
^{131}I-activity of serum (% of total activity in serum)
Protein-bound ^{131}I in serum (% of total activity in serum)
Serum thyroxine -T_4 (ug/100 ml of serum)
Bond index of triiodinethyroxine ("binding power of the serum proteins for triiodinethyroxine, referrring to a normal serum. The index is increasing if the thyroxine level of serum is decreasing").

Urinalyses measurements were carried out at 0, 3, 6, 12, 18 and 24 months. Each rat received 40 ml 0.3% saline solution/kg body weight by stomach tube; urine was collected in a metabolic cage. The urine samples were analyzed for:

Color
Specific gravity
Protein
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, and other abnormal constituents such as sperm, casts and crystals.

Eyes were examined at 6 weeks, and again at 3, 6, 12 and 24 months, using an ophthalmoscope, with particular attention paid to development of cataracts. At the same time, hearing was checked with a simple noise test and the teeth were inspected.

Animals which died "or were killed prematurely" (sacrificed in extremis?) were dissected and inspected macroscopically. After 70% mortality was reached in controls (31 months) all surviving rats were sacrificed by decapitation and exsanguination. Interim sacrifices of 5 rats/sex/group were carried out at 3, 6 and 12 months.

The following organ weights were determined for all animals:

Heart	Kidney(s)	Gonads
Liver	Adrenal(s)	Thyroid
Lungs	Thymus	Brain
Spleen	Pituitary	

After haematoxylin-eosin staining (presumably of slide preparations) the following organs were examined histologically "in all animals with the highest Maneb-concentration and the control rats as well as those of the interim dissections:"

Heart	Brain	Urinary bladder
Lungs	Prostate/Uterus	Bone marrow
Liver	Stomach	Trachea
Spleen	Duodenum	Aorta
Kidney	Jejunum	Esophagus
Adrenal	Ileum	Pancreas
Thymus	Colon	Peripheral nerve
Pituitary	Rectum	Skeletal muscle
Gonads	Salivary gland	Bone
Thyroid	M. (mediastinal?) lymph node	Eye
		Mammary gland

Statistical evaluation: "An analysis of variance of Student's-t-test were carried out on body weight, organ weights and the results of haematology; limit for significance was $p \leq .01$."

Results:

Although it is stated (p. 5) that samples of all diets were regularly sent to the sponsor for analysis every 13 weeks, no analytical results (or even the methodology used) are reported. It is also stated (p. 5) that the stability of the test compound in food over at least 7 days was examined; however, no data are reported.

There was no indication of any Maneb dose-related effects on behavior or external appearance. It is reported (p. 13) that "faeces were normal" and

"intermittent disturbances were not observed."

Mortalities: Summary mortality data for the complete 31-month study are presented on p. 13. Table 17 (p. 408-417) reports when spontaneous mortalities and sacrifices in extremis occurred:

CUMULATIVE MORTALITY

END OF WEEK	GROUP I 0 PPM		GROUP II 30 PPM		GROUP III 100 PPM		GROUP IV 300 PPM		GROUP V 1000 PPM	
	M	F	M	F	M	F	M	F	M	F
52	3	1	1	1	0	0	1	1	0	2
65	4	3	6	5	3	1	3	4	1	2
78	5	5	8	9	9	4	6	5	2	6
91	13	11	9	12	17	7	11	9	8	10
104	17	15	16	18	23	11	19	13	13	15
117	25	19	25	23	29	14	26	20	20	21
130	33	30	35	31	39	24	34	28	28	31
132	35	31	37	32	41	26	36	30	29	33

There are no statistically significant differences between groups with respect either to mortalities or when these mortalities occurred. Males in the 1000 ppm group showed slightly less mortality than males of the other groups, possibly as a result of initially retarded growth and/or lower body weights. However, despite lower mean body weight, group V females showed slightly more mortality.

Causes of death: These are reported (as part of the pathological findings on p. 418-491) only for the control and group V rats which died (not the ones which were sacrificed in extremis). No summary table is presented for this information.

Food intake: What are apparently the means by sex and dosage level are presented in table 1 (p. 41-42), reported in g/kg b.w. (with no standard deviations). There is no evidence of any dose-related trend. Absolute values for food consumption are not reported. On p. 14 it is stated that at the 30, 100 and 300 ppm levels food consumption was "normal" both in terms of relative and absolute values, while at 1000 ppm it was "slightly decreased" in terms of absolute value while it was "normal" in terms of body weight. There is no evidence that any statistical analysis was done on absolute food consumptions.

Body weights: Striking inconsistencies exist with respect to mean body weights as presented in table 3 on p. 47 and 48 for test month 12. These include a female control mean reported (p. 48) as 275.4 grams, while the value as reported on p. 47 for test month 12 is 324 grams; and a group V male mean reported (p. 48) as 540.0, but which is reported on p. 47 as 382 grams (the highest group V male mean weight is reported on p. 47 as occurring in test month 25 when a value of 428 grams was reached). This reviewer believes the reason for these discrepancies is because the 12-month values used in statistical analyses were calculated only from the 5 animals/sex/dosage group (refer to p. 61) which were sacrificed at 12 months.

The remainder of the table on p. 48 appears to be correct:

Mean body weights at 0, 3, 6, 12, 24 and 31 months (from p. 47 & 48).

	GROUP I 0 PPM		GROUP II 30 PPM		GROUP III 100 PPM		GROUP IV 300 PPM		GROUP V 1000 PPM	
	M	F	M	F	M	F	M	F	M	F
START										
Mean	102.5	102.6	102.2	102.2	102.7	102.4	102.3	102.5	102.4	102.3
S.D.	1.4	1.3	1.3	1.4	1.4	1.5	1.3	1.3	1.6	1.4
3 MONTHS										
Mean	398.8	261.3	410.0	26 .6	389.2	232.9*	401.4	252.8*	388.5	241.6*
S.D.	41.0	20.6	34.1	21.6	48.1	32.7	38.9	22.0	39.4	18.7
6 MONTHS										
Mean	454.6	279.4	465.9	292.8*	474.3	286.0	461.3	289.8	366.2*	254.2*
S.D.	51.5	25.2	48.4	30.8	56.0	33.2	52.6	31.6	67.5	33.0
12 MONTHS†										
Mean	506†	324†	505†	321†	510†	324†	511†	321†	382†	279†
S.D.	-	-	-	-	-	-	-	-	-	-
24 MONTHS¶										
Mean	546¶	367¶	544¶	379¶	551¶	370¶	549¶	368¶	421¶	332¶
S.D.	-	-	-	-	-	-	-	-	-	-
31 MONTHS										
Mean	424.3	301.0	433.9	338.4*	485.5*	355.3*	460.8	353.8*	420.5	330.2*
S.D.	61.5	47.0	52.5	57.6	55.1	79.6	55.6	65.3	38.2	39.1

*Reported as significantly different from controls at $p \leq 0.01$
†Values as reported on p. 47; values reported on p. 48 considered to be erroneous. Not evaluated in terms of statistical significance.
¶Values as reported on p. 47; no values reported for this date on p. 48. Not evaluated in terms of statistical significance.

It is not certain (individual body weight data aren't given) whether the lowering of many of the mean body weights at 31 months was due to a higher mortality rate of heavier rats in preceding months or to weight losses in many rats during this period (or to a combination of these factors).

Hematology: There were no significant differences between groups, or any evidence for dose-related trends in any of the parameters measured.

Clinical biochemistry: There were no effects on any of the measured parameters normally considered as thyroid-unrelated.

At 1000 ppm there were statistically significant ($p \leq 0.01$) increases in the half life period for ^{131}I-retention in the thyroid for males (months 6 and 12) and females (month 6). Additionally, males showed an elevated value for this function at 24 months, and females at 12 and 24 months, but these were not statistically significant at $p \leq 0.01$. From table 10, p. 223-228:

Mean ^{131}I-retention time in the thyroid (half-life in days):

	GROUP I 0 PPM		GROUP II 30 PPM		GROUP III 100 PPM		GROUP IV 300 PPM		GROUP V 1000 PPM	
	M	F	M	F	M	F	M	F	M	F
3 MONTHS										
Mean	5.1	5.2	5.0	5.1	5.1	4.7	5.0	4.9	5.1	5.0
S.D.	0.6	0.6	0.9	0.6	0.9	0.7	1.1	0.8	0.7	0.6
6 MONTHS										
Mean	5.2	5.1	5.2	5.2	4.8	4.8	4.8	5.0	6.5*	6.3*
S.D.	0.8	0.7	0.5	0.9	0.6	0.5	0.7	0.6	1.0	1.1
12 MONTHS										
Mean	5.3	5.3	5.0	5.2	5.2	4.8	4.8	4.9	6.3*	5.8
S.D.	0.5	0.7	0.8	0.8	0.7	1.0	1.0	0.8	0.9	0.9
24 MONTHS										
Mean	5.1	5.1	5.1	5.0	5.0	5.0	4.6	4.9	5.5	5.5
S.D.	0.6	0.6	0.7	0.7	0.8	0.5	0.6	0.6	0.9	0.9

Mean T_4 (Thyroxine) content of serum was somewhat depressed (but not to $p \leq 0.01$) in 1000 ppm males and females at 6 and 12 months relative to controls. From table 11, p. 249-254:

Thyroxine content of serum (ug/100 ml serum)

	GROUP I 0 PPM		GROUP II 30 PPM		GROUP III 100 PPM		GROUP IV 300 PPM		GROUP V 1000 PPM	
	M	F	M	F	M	F	M	F	M	F
3 MONTHS										
Mean	6.3	6.1	5.2	6.4	6.4	6.1	6.3	6.2	6.6	6.3
S.D.	0.8	0.7	0.9	0.9	0.7	0.9	1.0	0.8	0.8	0.6
6 MONTHS										
Mean	6.4	6.3	6.5	6.3	6.0	6.2	6.7	6.4	5.8	5.1
S.D.	0.7	0.9	0.8	1.0	0.7	0.6	0.9	0.7	0.9	0.6
12 MONTHS										
Mean	6.4	6.2	6.2	6.5	6.0	6.3	6.1	6.1	5.7	5.2
S.D.	0.8	0.9	0.9	0.8	0.9	0.7	0.7	0.9	1.2	1.0
24 MONTHS										
Mean	6.3	6.4	5.9	6.5	6.3	6.2	6.2	6.3	6.0	6.4
S.D.	1.0	1.1	0.8	0.6	0.7	1.1	0.8	0.7	1.0	1.0

Urinalysis: There were no evident differences between groups, or any evidence for dose-related trends in any of the parameters measured (color, specific gravity, protein, glucose, bilirubin, hemoglobin, ketone bodies, pH, urinary sediment).

Eyes, hearing and dentition: According to the text (p. 17-18) an ophthalmoscopic examinations at the end of the study, as well as before the interim examinations, showed no pathological changes. Hearing tests, conducted on the same dates as the ophthalmoscopic examinations, indicated no impairment of hearing had occurred. The dentition was free of pathological changes.

Organ weights-interim sacrifices: Mean thyroid weights were significantly (at $p \leq 0.01$) elevated in 1000 ppm males at 26 weeks. Thyroid weights were elevated in 1000 ppm males relative to their controls at week 52, and in 1000 ppm females relative to their controls at week 26; but these elevations were not statistically significant at $p \leq 0.01$ (although they may have been at $p \leq 0.05$); from pages 305, 306, 314, 315, 323 and 324:

Absolute mean thyroid weights (in g) - interim sacrifices:

	GROUP I 0 PPM		GROUP II 30 PPM		GROUP III 100 PPM		GROUP IV 300 PPM		GROUP V 1000 PPM	
	M	F	M	F	M	F	M	F	M	F
13 WEEKS										
Mean	0.033	0.026	0.026	0.020	0.033	0.030	0.040	0.036	0.034	0.024
S.D.	0.005	0.008	0.005	0.004	0.003	0.008	0.006	0.010	0.012	0.004
26 WEEKS										
Mean	0.022	0.024	0.033	0.027	0.020	0.016	0.025	0.025	0.040*	0.029
S.D.	0.005	0.008	0.011	0.004	0.004	0.003	0.007	0.006	0.003	0.006
52 WEEKS										
Mean	0.028	0.020	0.029	0.027	0.028	0.023	0.033	0.027	0.043	0.021
S.D.	0.005	0.007	0.005	0.004	0.003	0.008	0.005	0.005	0.012	0.003

At week 26 group IV (300 ppm) males showed significantly (again, $p \leq 0.01$) elevated mean testicular weights relative to their controls, but there was essentially no difference between group V (1000 ppm) males and controls.

Organ weights - terminal sacrifice: Mean thyroid weights were significantly ($p \leq 0.01$) elevated in group V (1000 ppm) males and females relative to their controls; from p. 367 and 368:

Absolute mean thyroid weights (in grams) - terminal sacrifice:

	GROUP I 0 PPM		GROUP II 30 PPM		GROUP III 100 PPM		GROUP IV 300 PPM		GROUP V 1000 PPM	
	M	F	M	F	M	F	M	F	M	F
TERMINAL SACRIFICE										
Mean	0.030	0.027	0.029	0.025	0.033	0.026	0.031	0.028	0.046*	0.040*
S.D.	0.010	0.007	0.008	0.006	0.010	0.007	0.009	0.006	0.021	0.01[illegible]

*Significantly different from control at $p \leq 0.01$

On p. 368 mean heart weight is reported for high-dose females as 3.82 grams, but on p. 383 it is reported as 1.26 g. From the individual animal data for this group it appears that the latter value is correct, and there is no real difference between 1000 ppm females and their controls (reported value: 1.33 g) in this respect.

Mean heart weight was significantly less in group II males (30 ppm) relative to control males, but there was no evidence from other group means that this one occurrence was dose-related.

Non-tumor findings: There were noticeably higher (but no indications of statistical significance, lack thereof, or whether these calculations were made, are reported) incidences of pathological findings in group V (HDT) rats as compared with controls for the following (from p. 25-31):

	Controls		High-dose (1000 ppm)	
	Males	Females	Males	Females
Prostate/uterus	11	2	25	5
Urinary bladder	0	1	10	1
Peripheral nerve	2	0	5	0
Skeletal muscle	49	20	47	41

Twenty-two males at 1000 ppm had prostatitis and/or abscesses of the prostate, as compared to 11 control males reported (p. 418-437) as having abscesses of the prostate.

Of ten 1000 ppm males with non-tumor findings for the urinary bladder, 7 had increased or moderate epithelial dysplasia (no non-tumor findings for the urinary bladder occurred in control males).

With respect to the peripheral nerve findings, the two males (I-22, I-65) in the control group both had calcareous deposits (see p. 424 and 436); all five males (V-19, V-23, V-35, V-47, V-53) in the 1000 ppm group had neuritis and/or perineural inflammation (refer to p. 460-469).

The only pathological finding for skeletal muscle in control males was myopathy, which apparently occurred in 48/49 control males (the remaining male is #36, reported as having a pathological finding for skeletal muscle on p. 30; however, no such finding is reported for this rat on p. 427). Of the 47 1000 ppm males reported as having some pathological finding for skeletal muscle, all had myopathy (refer to p. 455-474), and one (#35) also had myositis. Among the 20 control females with muscle pathology 18 had myopathy and 2 (#32, 60) had abscesses (p. 440-454). Among the 41 group V (1000 ppm) females with muscle pathology all had myopathy as the only finding for this tissue.

Liver findings:

	Controls		High-dose (1000 ppm)	
	Males	Females	Males	Females
fatty degeneration	29	17	32	16
hepatic cell hyperplasia	1			
necrotic foci	9	1	5	3
nodular hyperplasia	1			
cirrhosis		2		
hepatitis & fibrosis		2		
cysts		2		
hyperplasia of the bile ducts	1			4
hemorrhagic necrosis				1

Despite the statistically significant increased mean thyroid weights in 1000 ppm rats (both males and females) at termination, the listing on p. 27 reports non-tumorous pathological findings from only two males (#34, and 59). For both of these rats the pathological finding with respect to the thyroid is "signs of hyperfunction" with no further information (refer to p. 464 and 470).

On p. 370 the weight of the thyroid for control female #16 is elevated, and is reported in parenthesis; this also occurs on p. 371 for thyroid weights of control females 45 and 63, on page 381 for 1000 ppm males 9, 21, 43 and 65, and on p. 382 for 1000 ppm females 7, 10, 21, 25, 32, 36 and 38.

Tumor incidences: Overall incidences are given on p. 20 and 22 of the report for controls and high-dose animals:

	Controls		High-dose (1000 ppm)	
	Males	Females	Males	Females
Number of rats examined	75	75	75	75
Number of rats with tumors	58	50	51	51
Number of tumors	107	68	83	75

Although overall incidences in controls and high-dose rats were similar, there were some tumors which were more prevalent in one group than the other:

	Controls		High-dose (1000 ppm)	
	Males	Females	Males	Females
Leukemias	3	12	8	12
Lymphomas	3	3	0	0
Thyroid				
adenoma	3	0	2	1(parathyroid)
carcinoma	6	4	0	0
Follicular carcinoma	1	0	1	0
Urinary bladder				
precancerous dysplasia	7	0	11*	0
carcinoma	1	0	0	0
cysto-papillary tumor	1	0	0	0
cysto-papillary carcinoma	0	1	0	0
papilloma	0	0	3	0
pavement carcinoma	0	0	1	0
carcinoma of the squamous epithelium	0	0	2	0

*reported as 12 in table 20 on p. 502.

Epithelial papillomatosis of the prostate is reported (p. 22) as a tumor finding in one control male (#35), but the occurrence of the same condition in two 1000 ppm males (#3 - refer to p. 455, and #4 - refer to p. 456) is

not reported in the tumor findings for this group (p. 22-24) although it is reported in non-tumor findings (p. 28). Papillomatosis is defined (Dorland's Illustrated Medical Dictionary) as the development of multiple papillomas, so it must be considered a tumorous condition.

"Possible" tumors and non-tumorous pathological changes are combined together by sites for the other 3 groups on pages 32-39. Most of the "findings" in these groups were for animals which died or were sacrificed in extremis. It is stated (p. 31) "Histological examination was not made in all animals at the lower Maneb-concencentrations (30, 100 and 300 ppm in the food)."

Discussion:

This reviewer finds this report misleading in terms of presentation, and deficient in terms of quality and protocol.

In the summary findings (p. 1) it is stated (for the 1000 ppm group) that "at the end of the first year of the study all weights were within the normal range again." As part of the results section it is stated on p. 16 that "At the examinations after one year the body weight gain had normalised and showed similar results as those of the untreated animals." In fact, these are erroneous statements. The statistical analyses for body weights at 12 months were based only on the 5 rats/sex/group that were sacrificed at that time (refer to p. 61), rather than all of the rats which had survived to that date.

During the course of routine review, it was noted that a considerable number of absolute organ weights from rats which survived to termination are presented in parenthesis (p. 369-383). The number of rats in the control and high-dose groups which survived to termination, and the number which have thyroid weights in parenthesis are given below:

	Controls (p. 369-371)		High-dose (p. 381-383)	
	Survived	Thyroid wts in parenthesis	Survived	Thyroid wts in parenthesis
Males	24	0	22	4
Females	30	3	33	9

All of these parenthesized thyroid weights were elevated with respect to the mean for the group. No explanation has been found in this submission as to why these particular values have been set off from the others, but presumably it was because they were elevated. Calculations from thyroid weight data for 1000 ppm males (p. 381) indicate that without the parenthesized data from 4 males the mean thyroid weight for this group would be 0.041 grams with a S.D. of 0.016. With the parenthesized data it would be 0.072 grams with a S.D. of 0.090. As presented the value given is 0.046 grams with a S.D. of 0.021; the only way that this value could be obtained is by averaging in the parenthesized thyroid weights of two subjects (21 and 65) with the values from the other 18 rats, without using (and without indicating that they had not been used) the data from rats 9 and 43 (which had the most elevated thyroid weights) of this group.

A similar situation probably exists with respect to the females at 1000 ppm. Without data from the 9 females whose thyroid weights are parenthesized, the mean thyroid weight for the remaining females is 0.032 grams, with a S.D. of 0.008. With data from all these 9 females included with the others the mean is 0.049 grams, with a S.D. of 0.043. As actually presented the value is 0.040 grams, with a S.D. of 0.019.

The numbers of the individual rats surviving to termination which were assigned parenthesized weight values for thyroids are given below:

Males, control: none
Females, control: 16, 45, 63

Males, 1000 ppm: 9, 21, 43, 65
Females, 1000 ppm: 7, 10, 21, 25, 32, 36, 38, 64, 67

For the 1000 ppm rats, female 32 is reported (p. 23) as having an adenoma involving the parathyroid. No pathological findings (either tumorous or non-tumorous) for the thyroid are reported for any of the other animals (control or 1000 ppm) listed above. Considering the weights of some of these thyroids (i.e., male #9 at 1000 ppm had a thyroid weighing 0.255 grams, five times the mean value for the group; male #43 at 1000 ppm had a thyroid weighing 0.413 grams, more than eight time the mean value for the group) this is rather surprising.

While other organ weights from specific rats also appear in parenthesis, this reviewer has examined only those relating to the thyroid.

While the overall tumor incidences for the control and high-dose groups are reported as similar (see p. 20) there were some differences between the groups. Liver tumors occurred only at 1000 ppm (4 males, 1 female), and kidney tumors only at 1000 ppm (2 males, both of which also had liver tumors). There was also a slight increased incidence in urinary bladder tumors in 1000 ppm males relative to their controls:

	Controls		High-dose (1000 ppm)	
	Males	Females	Males	Females
Urinary bladder				
precancerous dysplasia	7	0	11*	0
carcinoma	1	0	0	0
cysto-papillary tumor	1	0	0	0
cysto-papillary carcinoma	0	1	0	0
papilloma	0	0	3	0
pavement carcinoma	0	0	1	0
carcinoma of the squamous epithelium	0	0	2	0

*reported as 12 in table 20 on p. 502.

In connection with possible bladder effects it is noteworthy that ten 1000 ppm males had non-tumorous pathological urinary bladder findings (7 had increased or moderate epithelial dysplasia), while no non-tumor

findings for the urinary bladder are reported for control males. Without histology data from lower Maneb dose groups it is not known whether these possible effects are part of a dose-related trend, or are isolated occurrences involving the 1000 ppm male rats only.

Additional problems with this study include the reporting of epithelial papillomatosis of the prostrate as a tumor finding in one control male (#35), but as a non-tumor finding in two 1000 ppm males (#3 - refer to p. 455, and #4 - refer to p. 456); use of $p \leq 0.01$ rather than $p \leq 0.05$ for level of significance; and lack of analytical data (despite the statement made on p. 5 that samples of all diets were sent to the sponsor for analysis every 13 weeks).

Because of these deficiencies, and because of uncertainties resulting from the way the data are presented (including mean organ weights), the study is classified as Core Supplementary Data. In order to upgrade the study classification the considerable number of deficiencies must be corrected, and sufficient histology data from lower dose rats must be supplied to define a NOEL in terms of pathology findings for the bladder and other possible effects.