6-15-89

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

JUN 15 1989 007251

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Maneb - Studies Submitted in Response to the Data Call-In of April 1, 1987

TO: Susan Lewis
Product Manager (21)
Registration Division (H7505C)

FROM: Linda L. Taylor, Ph.D. 6/7/89
Toxicology Branch I, Section II
Health Effects Division (H7509C)

THRU: K. Clark Swentzel 6/9/89
Acting Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. 6/9/89
Acting Chief, Toxicology Branch/HFAS/HED (H7509C)

Registrant: Penwalt Corporation
Chemical: Manganese ethylene-1,2-bisdithiocarbamate
Project: 9-0857
Caswell No.: 539
Record No.: 239779; 239780; 239781
Identifying No.: 4581-255; 4581-359; 4581-355
MRID No.: 40982401; 40982701; 40982601
Action Requested: Review data.

Comment: As requested by the Comprehensive Data Call-In of April 1, 1987, a report of the follow-up phase of a previously submitted subchronic inhalation study (MRID # 00162084) has been provided (cover letter dated January 31, 1989). This report has been reviewed, and the DER is attached. Additionally, a subchronic oral toxicity study in rats and a developmental toxicity study in rabbits were received, which have been reviewed by Dynamac. These latter two DER's are also attached. A summary of each is provided below.

1. Subchronic inhalation study - With the exception of an initial decrease in body weight in the high-dose females, which was comparable to the decrease observed at the end of the exposure period, no differences were observed among the groups at the end of the recovery period that could be attributed to test material exposure. ETU was not detected in any of the tissues/body fluids measured, nor were residues of Maneb detected.

As indicated in the original DER, this study can be upgraded to core minimum if residues of ETU and Maneb can be measured in lung tissue (if available) from the rats sacrificed at 13 weeks. Otherwise, the Registrant must justify limiting the high dose to 100 mg/m^3, in order for the study to be upgraded to core minimum. To date, no such data have been submitted to TB II for review. Therefore, the classification of this study remains: Core Supplementary.

2. Subchronic oral toxicity study - Dietary administration of 80, 400, and 1300 ppm maneb technical produced effects on body weight (decreased), food consumption (decreased), and thyroid function (thyroxine was decreased; thyroid stimulating hormone and triiodothyronine were increased) at 1300 ppm, and thyroid/parathyroid and kidney weights (increased) and histologic changes of the thyroid and kidneys at 400 and 1300 ppm. These weight changes were accompanied by an increased incidence of thyroid (follicular cell hyperplasia and increased colloid) and kidney (granular pigment) histopathology primarily in the mid- and high-dose animals. Reduced but persistent histopathological kidney changes occurred following the 4-week recovery period. No NOEL can be determined based on the renal changes observed at the lowest dose level in males.

This study is classified as Core supplementary, pending (1) clarification of dose levels tested; (2) submission of (a) data/information to support the contention that the discrepancy noted between the target dose level and that achieved for the mid-dose level at weeks 4 and 13 will not adversely affect the interpretation of the results and (b) documentation to assure that adequate doses were administered during the intervening weeks to the mid-dose group; and (3) identification of the granular pigment found in the kidneys of the treated animals.

3. Developmental toxicity study in rabbits - Administration of the test compound to pregnant rabbits (gavage) from gestational days 6 through 18 resulted in decreases in both maternal body-weight gain and food consumption at the high-dose. Additionally, there was a significant decrease in the number of viable fetuses and a significant increase in the number of resorptions in the high-dose does compared to control does. No compound-related abnormalities were observed in the external, visceral, or skeletal examinations of the fetuses.

The study is classified as Core Supplementary, pending submission of the following data:

1) analytical chemistry data and handling, preparation, and analytical procedures on concentration analysis of the dosing material;
2) individual animal data for maternal food consumption, clinical observations, necropsy findings, and animal assignment for the three phases of the study;
3) copies of the fetal x-rays employed in the skeletal examinations; and
4) data on the sperm used for insemination (including source, age, and collection procedures)].

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007251

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 4/12/89
Tox. Branch II, Section II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 4/12/89
Tox. Branch II, Acting Head Section II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 13-Week Inhalation - Rat (Addendum) TOX. CHEM. NO.: 539

MRID NO.: 40982401; 40982701; 40982601

TEST MATERIAL: Maneb

SYNONYMS: Manganese bisdithiocarbamate

STUDY NUMBER: 550-001

SPONSOR: Maneb Data Task Force

TESTING FACILITY: International Research and Development Corporation
Mattawan, MI

TITLE OF REPORT: Thirteen Week Subchronic Inhalation Toxicity Study on Maneb in Rats - Addendum to the Final Report Covering Recovery Phase

AUTHOR: Charles E. Ulrich

REPORT ISSUED: April 16, 1987

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: With the exception of an initial decrease in body weight in the high-dose females, which was comparable to the decrease observed at the end of the exposure period, no differences were observed among the groups at the end of the recovery period that could be attributed to test material exposure. ETU was not detected in any of the tissues/body fluids measured, nor were residues of Maneb detected.

As indicated in the original DER, this study can be upgraded to core minimum if residues of ETU and Maneb can be measured in lung tissue (if available) from the rats sacrificed at 13 weeks. Otherwise, the Registrant must justify limiting the high dose to 100 mg/m^3, in order for the study to be upgraded to core minimum. To date, no such data have been submitted to TB II for review.

Classification: core supplemental, pending submission of the previously requested data.

A. MATERIALS:

1. Test Compound: Maneb, technical; Description: yellowish powder; Batch #: 32-1671; Purity: not stated in previous DER.

2. Test Animals: Species: rat; Strain: Sprague-Dawley (Charles River CD®); Age: 49; Weight: not stated in previously DER; Source: Charles River Breeding Laboratories, Inc., Portage, MI.

B. STUDY DESIGN: A summary of the Experimental Design was provided in the form of a table (Table 1 and previous TB II DER are attached).

C. RESULTS:

Clinical Observations and Surivial

There were no apparent compound-related effects on appearance and behavior during the recovery phase of the study. No deaths occurred.

Body Weight and Food Consumption

The high-dose females displayed decreased body weight during the first two weeks of the recovery phase, compared to controls.

FEMALES	Body Weight - % of Control		
week of recovery	Low	Mid	High
1	96	92	88*
2	97	94	90*
3	97	94	92
4	96	93	91
13	97	95	95

*$p<0.05$

At the end of the exposure period, the mid-dose females were 91% of control, and the high-dose females were 86% of control. At week 1 of recovery, the high-dose males were 99% of control; at week 13, they were 104% of control.

Ophthalmoscopic Examination

At the end of the recovery period, chorioretinal hypoplasia (right eye) was observed in one mid-dose female, and keratitis was observed in one high-dose male (left eye). Following the exposure period, the incidence of these lesions was as follows (sexes combined in DER).

	chorioretinal hypoplasia	keratitis
Control	0/39	0/39
Low	0/40	0/40
Mid	2/40	0/40
High	3/40	2/40

The original reviewer stated that these findings were tentatively considered dose-related since they occurred only in the mid- and high-dose groups. Although there were only two animals with these findings after the 14-week recovery period, they too were mid- and high-dose animals, which lends support to the contention that the findings may be treatment-related.

Hematology and Clinical Chemistry

The only statistically significant differences noted following the 14-day recovery period were increases in platelet count and aspartate aminotransferase levels in the high-dose females, and in urea nitrogen levels in the high-dose males.

Tissue Residue Analysis

ETU

No ETU residues were reported in any biological fluid or tissue sample following the 13-week recovery period. ETU had been detected in urine and in the thyroids following the 13-week exposure period.

Maneb

Maneb residues were not detected in any urine or plasma sample. In liver, apparent residues of Maneb were detected, but the amounts were similar in all groups (including controls), suggesting that what was detected was not Maneb but an interferring compound.

Gross Pathology

There were no differences noted among the groups with respect to macroscopic findings. The low- and mid-dose females displayed decreases in mean kidney weight, absolute and relative to brain liver weight, and mean relative to brain lung weight; however, the high-dose females did not show a similar response. These differences are not considered to be treatment-related.

Histopathology

None of the microscopic changes observed could be attributed to test material exposure.

CONCLUSION

With the exception of an initial decrease in body weight in the high-dose females, which was comparable to the decrease observed at the end of the exposure period, no differences were observed among the groups at the end of the recovery period that could be attributed to test material exposure. ETU was not detected in any of the tissues/body fluids measured, nor were residues of Maneb detected.

As indicated in the original DER, this study can be upgraded to core minimum if residues of ETU and Maneb can be measured in lung tissue (if available) from the rats sacrificed at 13 weeks. Otherwise, the Registrant must justify limiting the high dose to 100 mg/m^3, in order for the study to be upgraded to core minimum. To date, no such data have been submitted to TB II for review.

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Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

AUG - 4 1986

005413

MEMORANDUM

SUBJECT: 90-day Subchronic inhalation study on Maneb
Caswell #539 Project No. 2138

TO: Ms. Jodi Bakst
SPRD

FROM: Byron T. Backus
Toxicology Branch
HED (TS-769C)

THROUGH: Marcia van Gemert, Ph.D., Section Head
Review Section III
Toxicology Branch, HED (TS-769C)

Judith Hauswirth, Ph.D., EBDC Coordinator
Mission Support Staff
Toxicology Branch, HED (TS-769C)

and
Theodore M. Farber, Ph.D., Chief
Toxicology Branch, HED (TS-769C)

Compound: Maneb

Action Requested:

The Toxicology Branch has been asked to review, on an expedited basis, a subchronic 90-day inhalation study. The conclusions of this review are also to be incorporated in the Maneb registration standard.

Comments and Recommendations:

1. The study has been classified as supplementary, partly because of lack of evidence that 100 mg/m^3 (the highest exposure level tested) is in fact a maximally tolerated dose (MTD) or is sufficiently close to it. Additional histopathology and residue data (from rats sacrificed after a 13-week recovery period) have yet to be received, and it is possible that some of this information will establish that 100 mg/m^3 is either an MTD or is sufficiently close to it.

2. An additional concern is that no residue data for ETU

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and/or Maneb were submitted for lung tissue. It would be significant if accumulation of Maneb occurs in the lungs and serves as a source of ETU. Also, manganese by inhalation is a potential hazard, as humans exposed to manganese salts or ores by this route may develop symptoms similar to those of Parkinsonism. For this reason, some analytical determination of manganese in the lung tissue of exposed rats is appropriate.

3. Since chorioretinal hypoplasia occurred only in groups III and IV (incidences of 2/40 and 3/40 respectively) and keratitis was present only in group IV (2/40), these ophthalmological findings are tentatively considered to be dose-related.

4. Additional findings also tentatively considered related to exposure were reduced body weights of females at 30 and 100 mg/m^3, and the indications of reduced (although not to a statistically significant level) T_3 and T_4 in group IV (100 mg/m^3) females. The significantly increased combined lung+trachea-to-body-weight ratio in group IV males and females is somewhat equivocal.

5. "Statistically significant differences" in mean body weights for group III and IV males sacrificed for histopathology and organ weights at 13 weeks relative to their controls were primarily a consequence of selection - inadvertant or otherwise - for lower weight males used for tissue residue analyses. While the 13 control males sacrificed at 13 weeks probably did not have a significantly higher mean body weight than group III or IV males, after the 4 lower weight males were taken from this group the remaining 9 males did have a significantly higher mean body weight. Some explanation should be provided as to why this was done.

6. The major value of this study is that it provides some ETU thyroid residue data following subchronic inhalation exposure (6 hrs/day, 5 days/wk for 13 weeks) to Maneb at 100 mg/m^3. Paradoxically, it was the high-dose males which showed the higher mean concentration of ETU associated with the thyroid (23.6 mcg/ml, vs. 8.4 mcg/ml for females), while females showed possible decreases in T_3 and T_4. Regrettably, serum T_3 and T_4 were not measured for the 4 animals/sex/dosage level which were used for tissue residue analysis, so it is not known whether there was a tendency for those rats with the higher concentrations of ETU in the thyroid to have lower T_3 and T_4 levels.

Data Evaluation Report (attached)

Wirth, M. J., Chandra, M., Geil, R. G., Goldenthal, E. I., Cutler, R. N., Schaefer, C. M., Drummond, J. G., Kalra,

R. K. and Blanchard, G. L. Thirteen week subchronic inhalation toxicity study on Maneb in rats - Final Report (unpublished study no. 550-001 prepared by International Research and Development Corp. for the Maneb Data Task Force; report issued July 18, 1986).

Reviewed by: Byron T. Backus, Toxicologist Byron T. Backus 8/19/86
Section III, Tox. Branch (TS-769C)

Secondary Reviewer: Marcia van Gemert, Ph.D., Section Head
Section III, Tox. Branch (TS-769C) Marcia van Gemert 8-21-86

DATA EVALUATION REPORT

STUDY TYPE: Subchronic inhalation - rat TOX. CHEM. NO.: 539

ACCESSION NUMBER: 263889 MRID NO.:

TEST MATERIAL: Maneb

SYNONYMS: Manganese bisdithiocarbamate

STUDY NUMBER(S): 550-001

SPONSOR: Maneb Data Task Force

TESTING FACILITY: International Research and Development Corp.
Mattawan, MI 49071

TITLE OF REPORT: Thirteen Week Subchronic Inhalation Toxicity Study on Maneb in Rats - Final Report.

AUTHOR(S): Wirth, M. J., Chandra, M., Geil, R. G., Goldenthal, E. I., Cutler, R. N., Schaefer, C. M., Drummond, J. G., Kalra, R. K. and Blanchard, G. L.

REPORT ISSUED: July 18, 1986

STUDY CLASSIFICATION: Core Supplementary Data

CONCLUSIONS:

1. The study has been classified as supplementary, partly because of lack of evidence that 100 mg/m^3 (the highest exposure level tested) is in fact a maximally tolerated dose (MTD) or is sufficiently close to it. Additional histopathology and residue data (from rats sacrificed after a 13-week recovery period) have yet to be submitted, and it is possible that some of this information will establish that 100 mg/m^3 is either an MTD or is sufficiently close to it.

2. An additional concern is that no residue data for ETU and/or Maneb were submitted for lung tissue. It would be significant if accumulation of Maneb occurs in the lungs, and serves as a source of ETU. Also, manganese by inhalation is a potential hazard, as humans exposed to manganese salts

or ores by this route may develop symptoms similar to those of Parkinsonism. For this reason, some analytical determination of manganese in the lung tissue of exposed rats is appropriate.

3. Since chorioretinal hypoplasia occurred only in groups III and IV (incidences of 2/40 and 3/40 respectively) and keratitis was present only in group IV (2/40), these ophthalmological findings are tentatively considered dose-related.

4. Additional findings considered related to exposure were reduced body weights of females at 30 and 100 mg/m^3, and the indications of reduced (although not to a statistically significant level) T_3 and T_4 in group IV (100 mg/m^3) females. An equivocal finding is the significantly increased combined lung-trachea organ-to-body weight ratio in group IV males and females.

5 "Statistically significant differences" in mean body weights for group III and IV males sacrificed for histopathology and organ weights at 13 weeks relative to their controls were primarily a consequence of selection - inadvertant or otherwise - for lower weight control males used in residue analyses. The remaining 9 male controls had a significantly higher mean weight than the 10 males of groups III and IV. Some explanation should be provided as to why this was done.

A. MATERIALS:

1. Test compound: Maneb Technical Grade 32-1671, identified as a yellowish powder.

2. Test animals: Sprague-Dawley derived albino rats (Charles River CD®) from Charles River Breeding Laboratories, Inc. Portage, MI. At initiation of exposure both male and female rats were 49 days of age.

B. STUDY DESIGN:

1. Exposure levels (0, 10, 30 and 100 mg/m^3) were selected on the basis of results from a five day range-finding study in which groups of five male and five female rats were exposed (nose only) six hours/day for five consecutive days to gravimetrically measured concentrations of 0, 96, 290 and 1000 mg/m^3 of the test material. Some mortalities occurred on day 5 at the two highest exposure levels (1/5 males and 1/5 females at 290 mg/m^3, and 1/5 males and 2/5 females at 1000 mg/m^3). As a result, 100 mg/m^3 was selected as the highest exposure level for the subsequent 13-week study.

2. 28 males and 28 females were randomly assigned to each of four dosage groups by use of a computer-generated random

number table. Body weight variances for the groups were statistically tested and found to be homogeneous.

3. Each group was exposed 6 hours/day, 5 days/week for 13 consecutive weeks in one of four nose-only exposure chambers.

4. Following the 13 week exposure, 14 animals/sex/dosage level were sacrificed. Organ weights were determined and tissues were histologically examined from 10/14 rats/sex/dosage group. Tissues from the remaining 4 rats/sex/group were used for ETU and/or Maneb residue analysis. The remaining 14 rats/sex/dosage group were scheduled to be sacrificed following a 90-day recovery period; and the data from these rats have yet to be received.

5. Nominal exposure concentrations were determined once a week by dividing the total amount of test material used that week by the total volume of air which passed through the chambers. Actual exposure concentrations were determined daily by taking a sampling over almost the entire exposure period and measuring the weight gain. A light-scattering aerosol monitor was used to qualitatively assess exposure concentrations.

The mean nominal and actual exposure concentrations over the entire exposure period were (from p. 29):

	Exposure Concentrations - mg/m^3 (with S.D.)	
Group	Nominal	Gravimetric
II	19 (9.6)	10 (0.8)
III	67 (20.0)	32 (3.1)
IV	187 (25.9)	96 (2.9)

6. Three times during the study, samples of the exposure atmospheres from each chamber were analyzed for Maneb and ethylenethiourea (ETU). The following means were obtained (calculated from the data on p. 30):

	Maneb (mg/m^3)		ETU
Group	Analytical	Gravimetric	Analytical
I	<0.28	-	
II	8.9	8.7	0.12
III	39.7	36	0.71
IV	111.7	106	2.50

7. Particle size distribution of the test material aerosol was determined 3 times/week for each exposure group with an Andersen® 8-stage cascade impactor. The following are the means for the entire exposure period (from p. 30):

Group	Particle Size Distribution: Equivalent Aerodynamic Diameter (mcm)	Geometric Standard Deviation
II	3.6	2.00
III	3.6	2.01
IV	3.5	2.02

8. Quality Assurance: There is a copy of a quality assurance statement signed by Margery J. Wirth, Acting Director of Quality Assurance on page 2, along with a listing of when on-site inspections were conducted (p. 3).

C. METHODS AND RESULTS:

1. Observations: Rats were examined twice a day for mortality and signs of toxicity.

Results

There were no evident exposure-related effects on appearance or behavior. Some rats showed red material around the nose and scabbed or missing distal tails, but there was no exposure-related trend and these were considered incidental findings. One control male died during study week 10.

2. Body Weight: Individual body weights were recorded at approximately weekly intervals, beginning prior to the first exposure.

Males at the highest exposure level (100 mg/m^3) had significantly ($p < 0.05$) lower mean body weights than their controls at weeks 1, 2 and 3. Males at 30 mg/m^3 had initial body weights somewhat greater than their controls, but after week 2 they were consistently (but not significantly) lower. Group 3 (30 mg/m^3) and 4 (100 mg/m^3) females usually had mean body weights significantly lower than those of their controls.

Representative mean body weights - males

Week	Group I 0 mg/m^3	Group II 10 mg/m^3	Group III 30 mg/m^3	Group IV 100 mg/m^3
0	224	229 102	230 103	227 101
1	274	273 100	276 101	259* 95
2	305	303 99	303 99	290* 95
3	333	331 99	331 99	316* 95
8	419	430 103	416 99	400 95
13	477	497 104	464 97	450 94
14	494	521 105	492 100	489 99

*Significantly different from controls at $p < 0.05$.

Representative mean body weights - females

Week	Group I 0 mg/m³	Group II 10 mg/m³	Group III 30 mg/m³	Group IV 100 mg/m³
0	163	163 100	161 99	160 98
1	190	188 99	184 97	175* 92
2	205	204 100	198 97	194* 95
3	221	215 97	211* 95	204* 92
8	264	260 98	246* 93	234* 89
13	288	284 99	261* 91	249* 86
14	302	289 96	278 92	267* 88
Gain	139	126	117	107

*Significantly different from controls at $p < 0.05$.

3. Food consumption: Not measured.

4. Ophthalmological examinations: All rats were examined before exposure. After 13 weeks of exposure and 13 weeks of post-exposure, all survivors of each group, except those animals used for tissue residue level determinations, were examined ophthalmologically.

   Results: The only occurrences of chorioretinal hypoplasia were in groups III and IV, and keratitis was observed only in group IV. From appendix I:

Group	Number of animals examined	Incidence of chorioretinal hypoplasia*	Incidence of keratitis*
I	39	0/39	0/39
II	40	0/40	0/40
III	40	2/40	0/40
IV	40	3/40	2/40

*in one eye only; none of the animals had more than one eye affected.

According to report text (p. 35): "The chorioretinal hypoplasia noted in group III and IV was of congenital origin; thus no obvious trends in pathology suggestive of test material-related reactions were observed."

5. Bleeding (from the orbital sinus plexus of 10 overnight fasted mice/sex/exposure group) was done after 13 weeks of exposure. The CHECKED (X) parameters were examined:

   a. Hematology

X	Hematocrit (HCT)		Total plasma protein
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Total leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)

Results: From p. 34 of the report: "After approximately thirteen weeks of exposure, there were no biologically meaningful or statistically significant differences for any parameter between the control group and any of the exposed groups." Examination of the means (table 7) for each of these parameters gives no indication of any dose-related trends.

b. Clinical Chemistry

The CHECKED (X) parameters were examined:

	Electrolytes		Other
X	Calcium	X	Albumin
X	Chlorides	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine
X	Phosphorus	X	Blood urea nitrogen
X	Potassium	X	Total cholesterol
X	Sodium	X	Globulin (calculated)
	Enzymes	X	Glucose
X	Alkaline phosphatase	X	Total bilirubin
	Cholinesterase	X	Total serum protein
	Creatinine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		
	Thyroid function		
X	Triiodothyronine (T_3), plasma		
X	Thyroxine (T_4), plasma		
X	Thyroid stimulating hormone (TSH), plasma		

Results: From p. 34 of the report: "After approximately thirteen weeks of exposure, there were no biologically meaningful differences for any parameter between the control group and any of the exposed groups. Serum sodium levels were statistically elevated in group IV males, but the differences were not considered toxicologically meaningful since a dose relationship was not evident."

Relative to their respective controls females of group IV had a greater reduction in mean body weight than group IV males. However, there was no significant difference between group IV females and their controls with respect to mean serum sodium levels. From table 8, p. 64 & 67:

Sodium levels in mEq/l (with S.D.) at week 13:

Group	I	II	III	IV
Males	145 (1.2)	146 (1.6)	145 (1.3)	147*(2.0)
Females	146 (4.8)	145 (1.9)	146 (1.9)	145 (2.5)

*Significantly different from controls at $p < 0.05$

Group IV females showed somewhat lower mean values for T_3 and T_4 than their controls and the other two exposure groups; however, differences were not statistically

significant:

T_3 and T_4 serum levels (with S.D.) in females at 13 weeks

Parameter	Group I	Group II	Group III	Group IV
T_3(ng/dl)	158 (31.4)	164 (42.3)	158 (20.3)	144 (15.1)
T_4(ug/dl)	5.27(1.186)	5.45(0.940)	5.28(0.847)	5.02(1.016)

This reviewer has taken the individual scores for each of the females in groups I and IV for these parameters, and has compared them by the Wilcoxon Rank Sum Test. The results are 123-87 for T_4 (thyroxine) and 109-101 for T_3 (triiodothyronine). Statistical significance ($p < 0.05$) would be reached at 128-82.

Group IV males showed a slightly less mean thyroxine level from that of their controls (6.04 to 6.33 ug/dl), but there was no statistical significance to this. Group IV males actually had a slightly higher mean T_3 level than their controls.

6. Urinalysis: Not done (urine was collected from 4 rats/sex/group for analyses for Maneb and ETU at 13 weeks).

7. Tissue Residue Analysis: Samples of urine, plasma, liver and thyroid were collected from 4 rats/sex/group after 13 weeks of exposure, and were stored frozen until analyzed. Urine, plasma and liver were analyzed for (p. 24) "EDRC" (EBDC?) - in the findings this is reported as Maneb - and ETU, while thyroids, because of their small size, were analyzed for ETU only.

Results:

ETU was detected in the urine of all exposed rats, in the plasma of only one rat (from group III), in the livers of two group IV females, and only in thyroids of (all) group IV rats. The concentration of ETU in urine from females was greater than that for males, but the concentration in thyroids (group IV rats only) was higher in males than females. From p. 70-71:

Group	Maneb Exposure (mg/m^3)	Mean values of ETU present in: Urine (mcg/ml) males	Urine (mcg/ml) females	Thyroid (mcg/ml) males	Thyroid (mcg/ml) females
I	0	<0.7	<0.7	-	-
II	10	1.2	2.9	-	-
III	30	4.3	10.7	-	-
IV	100	16.0	23.7	23.6	8.4

The mean amount of ETU present in the urine was, for a given sex, roughly proportional to the Maneb exposure.

The range of ETU concentration in the thyroid for group IV males was from 11.3 to 36.8 mcg/ml, and for females, from 3.8 to 13.6 mcg/ml.

Maneb was detected in the urine of 4 group II, 4 group III and 6 group IV rats, but not in the urine from any of the controls. What appeared to be Maneb was also detected in the livers of all group IV rats (mean of 0.21 mcg/ml), but this was a somewhat equivocal finding as it also seemed to be present (at about the same concentration) in the livers of two control rats, and the highest "level" (0.48 mcg/ml) was from a group II male. Concentrations of Maneb in the urine were lower than those of ETU from the same rats. From p. 72-73:

Mean values of Maneb present in:

Group	Urine (mcg/ml)	
	males	females
I	<0.4	<0.4
II	0.63†	0.74*
III	1.25	-
IV	4.64*	1.69*

† value from 1/4 rats at this exposure level
* mean value from 3/4 rats at this exposure level

The mean values given above for Maneb are "high" as they do not include data from rats in which levels of Maneb were below limits of detection.

8. Sacrifice and Pathology: Ten of the 14 rats/sex/group (tissues from the 4 other rats/sex/group were used in residue analysis, and were not histologically examined) sacrificed at 13 weeks received a complete post-mortem examination. The trachea of each animal was exposed and clamped; the thoracic cavity was opened, and the lungs were removed and examined while inflated. Representative sections of the CHECKED (X) tissues were preserved in phosphate-buffered neutral formalin for histological examination. Double-checked (XX) organs were also weighed.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta	XX	Brain (3 levels)
X	Salivary glands	XX	Heart	X	Sciatic nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Eyes
X	Duodenum	XX	Spleen		Glandular
X	Jejunum	X	Thymic region	X	Pituitary
X	Ileum		Urogenital	XX	Adrenals
X	Cecum	XX	Kidneys	X	Lacrimal gland
X	Rectum	X	Urinary bladder	X	Mammary gland
XX	Liver	XX	Testes	XX	Parathyroids*
	Gall bladder		Epididymides	XX	Thyroid*
X	Pancreas	X	Prostate		Other
	Respiratory	X	Seminal vesicle	X	Bone (femur)
XX	Tracheat	XX	Ovaries	X	Skeletal muscle
XX	Lungst	X	Uterus	X	Skin
		X	Vagina	X	Nasal tissue
				X	Gross lesions

†Trachea and lungs were weighed together
*Thyroids and parathyroids were weighed together
Representative samples of tissues were used to prepare hematoxylin and eosin-stained paraffin sections. A full tissue complement was prepared for all rats of the control and high-dose groups. The lung, trachea, nasal tissues, adrenal, liver, thyroid-parathyroid, pituitary and gross lesions were examined from all rats in the low and mid-dosage groups.

Results:

a. Organ weights at termination:

Although mean body weights for the 28 males/group in groups III and IV were not significantly different from their control group, the mean body weights for the 10 males/group in groups III and IV sacrificed at 13 weeks for organ weights and histopathology were significantly lower than the mean of the 9 control males sacrificed for organ weights and histopathology at 13 weeks.

Mean body weights (grams) at 13 weeks for males (number of rats in parenthesis) - these values do not appear in the report but have been calculated from individual weights on p. 164, 166, 168 and 170:

	Group I 0 mg/m^3	Group II 10 mg/m^3	Group III 30 mg/m^3	Group IV 100 mg/m^3
All males at 13 weeks	477 (27)	497 (28)	464 (28)	450 (28)
Males sacrificed at 13 weeks & used for residue analysis	428 (4)	498 (4)	477 (4)	441 (4)
Males sacrificed at 13 weeks for organ wts & histopathology	509 (9)	493 (10)	459 (10)	440 (10)
Males not sacrificed at 13 weeks	471 (14)	499 (14)	464 (14)	460 (14)

The mean body weights (grams) for males which were sacrificed at 13 weeks are reported as the following (p. 76):

	Group I 0 mg/m^3	Group II 10 mg/m^3	Group III 30 mg/m^3	Group IV 100 mg/m^3
Sacrificed for Organ wts & Histo.	466	450	420†	398†

†Significantly different from controls at $p < 0.05$

Since these values are 39-43 grams less than the corresponding values in the previous table, they presumably represent post-sacrifice (after exsanguination?) weights.

The "statistically significant differences" in mean body weights for group III and IV males sacrificed for histopathology and organ weights relative to the control value are primarily a consequence of selection - inadvertant or otherwise - of four lower weight males (mean of 428 grams when weighed at 13 weeks) for tissue residue analysis among the 13 controls sacrificed at 13 weeks. The remaining 9 control males, used for organ weight determinations and histopathology, had a mean body body weight of 509 grams (calculated from the individual weights at 13 weeks as reported on p. 164), which was higher than the 13-week mean weights (459 and 440 grams respectively, as calculated from individual weights reported on p. 168 and 170) for the group III and IV males sacrificed for organ weight determinations and histopathology at 13 weeks.

In group III and IV males organ-to-body weight ratios were significantly elevated for brain, adrenal and (group IV males only) kidney, lung + trachea, and testis. In each case the mean organ weight in group III and/or IV males was about the same as that for controls. In the case of the significantly elevated combined lung+trachea-to-body weight ratio in group IV males, a similar finding was present in females (see p. 11 of this DER):

Mean lung+trachea weights and body wt ratios in males:

	Group I 0 mg/m^3	Group II 10 mg/m^3	Group III 30 mg/m^3	Group IV 100 mg/m^3
Lung+trachea wt (grams)	1.88(0.226)	1.79(0.256)	1.82(0.215)	1.88(0.268)
Lung+trachea to body wt ratio % x 10	4.04(0.265)	3.97(0.477)	4.34(0.248)	4.73†(0.542)

†Significantly different from controls at $p < 0.05$

No significant differences (or evidence for dose-related trends) were observed for mean combined thyroid-parathyroid weights or the combined thyroid-parathyroid-to-body weight ratio.

For the 10 females/group sacrificed at 13 weeks, mean body

weight in group IV was significantly lower than that of controls (note that the mean body weight for all females in group III was significantly lower than that of controls at week 13 - refer to p. 4 of this review). From p. 81: Mean post-sacrifice body weights (grams) for females used for organ weights and histopathology:

	Group I 0 mg/m^3	Group II 10 mg/m^3	Group III 30 mg/m^3	Group IV 100 mg/m^3
Post-sacrifice weights	250 (10)	259 (10)	235 (10)	219†(10)

†Significantly different from controls at $p < 0.05$

Among the females the following findings were noteworthy:

The mean combined lung-trachea weight was slightly elevated (not significantly so) in group IV females, while the combined lung and trachea to body weight ratio was significantly elevated. The spleen-to-body and combined thyroid-parathyroid-to-body-weight ratios were somewhat lower in group IV females (but not significantly so) as compared with their controls. From p. 81-85:

Organ weights and organ-to-body weight ratios (with standard deviations) in females at 13 weeks:

	Group I 0 mg/m^3	Group II 10 mg/m^3	Group III 30 mg/m^3	Group IV 100 mg/m^3
Lung+trachea wt (grams)	1.33(0.099)	1.38(0.155)	1.35(0.177)	1.42(0.195)
Lung+trachea to body wt ratio % x 10	5.35(0.231)	5.36(0.364)	5.75(0.422)	6.46†(0.577)
Spleen wt (gms)	0.54(0.102)	0.59(0.095)	0.50(0.117)	0.43(0.108)
Spleen to body wt ratio % x 10	2.14(0.348)	2.28(0.261)	2.11(0.405)	1.94(0.316)
Thyroid+parathyroid (mg)	22 (6.1)	19 (6.1)	20 (5.7)	17 (5.1)
Thyroid+parathyroid to body wt ratio % x 1000	8.92(2.070)	7.23(1.998)	8.43(2.270)	7.39(2.282)

†Significantly different from controls at $p < 0.05$

b. Gross pathology

There were no test article related changes in any of the 10 rats/sex/group that were sacrificed at 13 weeks.

c. Microscopic pathology

There was no evidence of any dose-related microscopic changes in the 10 rats/sex/group that were sacrificed and examined after 13 weeks.

C. DISCUSSION:

The major value of this study is that it provides some ETU thyroid residue data following subchronic inhalation exposure (6 hrs/day, 5 days/wk for 13 weeks) to Maneb at 100 mg/m^3. Additionally, there is a suggestion in females at this dose level (but not males) of a slight - but not statistically significant - decrease in T_3 and T_4. Paradoxically, it was the high-dose males which showed the higher mean concentration of ETU associated with the thyroid (23.6 mcg/ml, vs. 8.4 mcg/ml for females).

Regrettably, serum T_3 and T_4 were not measured for the 4 animals/sex/dosage group which were used for tissue residue analysis, so it is not known whether there was a tendency for those animals with the higher concentrations of ETU in the thyroid to have lower T_3 and/or T_4 levels.

Since chorioretinal hypoplasia occurred only in groups III and IV, and keratitis was present only in group IV, these low incidence ophthalmological findings are tentatively considered dose-related.

The lack of any histological changes for the thyroid in high dose rats sacrificed at 13 weeks is disturbing, as is the lack of evidence (other than reduced body weights in group III and IV females, an equivocal increased combined lung-trachea to body weight ratio in both sexes, and possibly eye effects) of toxicity. One of the concerns this reviewer has is whether 100 mg/m^3 is in fact an MTD, despite the occurrence of 20% mortality in a preliminary 5-day study at 290 mg/m^3. The major symptom (labored respiration) occurring in the preliminary study at 290 and 1000 mg/m^3 was apparently subsequently absent in rats exposed to 100 mg/m^3 over the 13-week period.

Although this is designated a final report, there will be an addendum which will include histology data on 10 rats/sex/exposure group sacrificed after a 13-week recovery period, and presumably some residue data on an additional 4 rats/sex/exposure group sacrificed at the same time. It is possible then that there will be findings that will

establish 100 mg/m^3 as either an MTD or sufficiently close to it.

Another concern is the lack of ETU and/or Maneb residue data from lung tissue and/or the trachea. It would be significant if accumulation of Maneb occurs in the lungs, and if this could then be a source of ETU. Also, manganese is a potential hazard by the inhalation exposure route. According to one reference (Gosselin, Hodge & Smith, Clinical Toxicology of Commercial Products, 5th ed.) "Symptoms of workers exposed to manganese dusts include masklike facial expression, spastic gait, tremors, slurred speech... Rat studies indicate that manganese gradually accumulates in brain tissue to produce a lesion similar to that of Parkinsonism."

Because of these considerations, the current classification of this study is core supplementary. If lung tissues are still available from the rats which were sacrificed at 13 weeks, and residues of ETU, Maneb and Manganese can be determined from these tissues, and if a suitable justification can be given for 100 mg/m^3 as an MTD, then the study can be upgraded to core minimum.

007251

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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DYNAMAC No.: 159-B
TASK No.: 1-59B
May 12, 1989

DATA EVALUATION RECORD

MANEB

Subchronic Oral Toxicity in Rats

APPROVED BY:

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

Signature: Roman J Prenta for

Date: May 12-1989

007251

EPA No.: 68D80056
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May 12, 1989

DATA EVALUATION RECORD

MANEB

Subchronic Oral Toxicity in Rats

REVIEWED BY:

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity in rats. GUIDELINE § 82-1

MRID NUMBER: 409826-01.

TEST MATERIAL: Maneb technical.

SYNONYM(S): Manganese ethylene-1,2-bisdithiocarbamate.

STUDY NUMBER(S): 153-140.

SPONSOR: Pennwalt Corporation, Philadelphia, PA.

TESTING FACILITY: Hazleton Laboratories America, Rockville, MD.

TITLE OF REPORT: Subchronic toxicity study with Maneb technical in the rat.

AUTHOR(S): Trutter, J. A.

REPORT ISSUED: December 20, 1988.

CONCLUSIONS:

When 80, 400, or 1300 ppm maneb technical was fed to Sprague-Dawley rats for 13 weeks followed by a 4-week recovery period, there were no overt signs of toxicity or dose-related effects on mortality, ophthalmology, or hematology. Body weights and food consumption of high-dose males and females were decreased during the dosing period; these parameters remained decreased during the recovery period in high-dose females. Thyroxine was decreased in dosed males and females following the dosing period while thyroid stimulating hormone and triiodothyronine were increased during this time; females were affected to a greater degree than males. These changes regressed following the recovery period. Similarly, thyroid/parathyroid and kidney weights were increased during the dosing period. These weight changes were accompanied by an increased incidence of thyroid (follicular cell hyperplasia and increase in colloid) and kidney (granular pigment) histopathology primarily in mid- and high-dose males and females. Reduced but persistent histopathological kidney changes occurred following the 4-week recovery period; organ weights of dosed animals were similar to that of controls. The No-Observed-Effect Level (NOEL) cannot be determined based on the renal changes observed at the lowest dose level in males.

Classification: Core Supplementary, pending clarification of whether the values cited as the amount of test material consumed have been corrected for the difference noted between the percentage of active ingredient provided by the Sponsor and that found by the analysis performed at the testing facility. Because of the noted discrepancy between the target dose level and that achieved for the mid-dose group at weeks 4 and 13, some data/information should be provided to explain/justify why this will not adversely affect the interpretation of the results. Since the diets were prepared on a weekly basis, documentation should be provided to assure that adequate doses were administered during the intervening weeks (between weeks 4 and 8 and 8 and 13). Additionally, the glandular pigment found in the kidneys of the treated animals should be identified.

A. MATERIALS:

1. Test Compound: Maneb technical; Description: yellow powder; Batch No.: 8607-584/25; Purity: 77.9% (reported to be 86% purity by study sponsor).

2. Test Animals: Species: rat; Strain: Sprague-Dawley derived CrL:CD®BR; Age: 43 days at study initiation; Weight: males--207.2 to 234.1 g; females--143.2 to 169.3 g; Source: Charles River Laboratories, Raleigh, NC.

B. STUDY DESIGN:

1. Animal Assignment: Following 2 weeks of acclimation, animals were assigned to the following test groups using a computerized randomization procedure designed to ensure homogeneity of body weight:

Test Group	Dose in Diet (ppm)	Main Study[a] (14 weeks) Males	Main Study[a] (14 weeks) Females	Recovery (4 weeks) Males	Recovery (4 weeks) Females
1 Control	0	15	15	5	5
2 Low (LDT)	80	15	15	5	5
3 Mid (MDT)	400	15	15	5	5
4 High (HDT)	1300	15	15	5	5

[a]10 animals/sex/dose were sacrificed at 14 weeks; 5 animals/sex/dose were placed on a 4-week recovery phase following the dosing period.

Animals were housed individually in an environmentally controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: Compound purity was reported to be 86% by the study sponsor; the amount of test material required for the nominal doses were calculated to adjust the compound activity to 100%. Diets were prepared on a weekly basis. Samples of treated food were analyzed for homogeneity at week 1; concentration analyses were performed at weeks 2, 4, 8, and 13. Stability of the test compound in the diet was analyzed on days 0, 4, 7, and 10. The diet mixture was stored at room temperature. A reserve sample of the test compound was collected at study initiation, and reserve samples of the mixed diets were collected from each batch for future analysis.

Results: Analysis of the test compound (which was reported by the study sponsor to be 86.0% active ingredient) was found to be 77.9% active by the study laboratory. Test diet assay values were corrected to reflect this change. The diets were found to be homogenous within a range of 93 to 98% for the 80-, 400-, and 1300-ppm diets and from 83 to 88% stable over a 10-day period at room temperature. Recovery values of the diet are presented in Table 1. Diet concentrations ranged from 85 to 95%, 74 to 98%, and 82 to 93% of the nominal concentration for the 80, 400, and

TABLE 1. Recovery of Maneb from Test Diets at Representative Intervals

Week	Percent of Target concentration (ppm)		
	80	400	1300
1	94[a]	98	93
2	85	96	93
4	90	76	82
8	85	94	91
13	95	74	89
Overall Mean ± S.D.	89.8 ± 4.76	87.6 ± 11.61	89.6 ± 4.56

[a]Percentage of target concentration; week 1 concentration recovery based on homogeneity data.

1300 ppm diets, respectively. The analysis of control diets was not reported.

3. Food and Water Consumption: Animals received food (Purina Certified Rodent Laboratory Chow #5002) and water ad libitum, except during food fast initiated prior to collection of blood and prior to necropsy.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Body weight, food consumption, hematology, clinical chemistry, thyroid function tests, and organ weight data were analyzed using analysis of variance with transformations where necessary as indicated by Levene's test for homogeneity of variance. Dunnett's t-test was used to assess the significance of intergroup differences. Trends were evaluated using the Terpstra-Jonckheere test and/or by linear regression.

5. Quality Assurance: A quality assurance statement was signed and dated December 29, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of morbidity and mortality. Detailed physical examinations were performed weekly.

Results: No deaths occurred during the study. Tremors were observed in 2 of 15 mid-dose females and 2 of 15 high-dose females during study weeks 5, 8, 9, and 12; the study author reported the relationship of this observation to dosing to be unknown. Tremors were not observed in dosed males. The incidence of alopecia was slightly increased in high-dose males and females when compared to concurrent controls; however, this finding was generally limited to the forepaws and legs and did not increase over the duration of the study.

2. Body Weight: Rats were weighed at study initiation and weekly thereafter.

Results: Body weights of high-dose males and females decreased consistently from study weeks 2 to 13 when compared with concurrent controls; this effect was most pronounced in females (Table 2). At 12 and 13 weeks of study, the body weights of high-dose females were 16% lower than controls; the decrease was significant ($p<0.05$) at 13 weeks. The body weights of high-dose males were 7 and 6% lower than concurrent controls at 12 and 13 weeks, respectively. Body weight gains calculated over 13 weeks were significantly ($p<0.05$) decreased for high-dose males

TABLE 2. Representative Mean Body Weights and Body Weight Gains of Rats Fed Maneb for 13 Weeks[a]

Dose (ppm)	Mean Body Weights (g ± S.D.) at Week 0	4	8	13	Body Weight Gains (g ± S.D.) Weeks 0-13
			Males		
0	221.4 ± 6.15	403.6 ± 20.89	496.7 ± 31.08	567.8 ± 38.86	346.4 ± 34.69
80	220.2 ± 5.81	412.9 ± 19.72	509.5 ± 29.23	577.3 ± 45.55	357.2 ± 42.99
400	220.2 ± 5.84	407.3 ± 23.57	503.0 ± 35.91	583.9 ± 38.65	363.6 ± 36.09
1300	224.4 ± 5.44	375.0 ± 22.89	462.4 ± 31.21	534.4 ± 37.48	310.0 ± 37.32*
			Females		
0	155.0 ± 6.74	242.5 ± 13.35	284.5 ± 18.17	318.1 ± 22.88	163.1 ± 23.40
80	157.7 ± 5.78	249.4 ± 20.54	294.5 ± 23.52	322.5 ± 25.96	164.8 ± 25.89
400	157.1 ± 6.85	248.5 ± 20.42	279.1 ± 21.90	313.9 ± 29.58	156.8 ± 24.74
1300	159.1 ± 5.60	212.3 ± 15.50	240.5 ± 19.11	266.2 ± 26.99*	107.1 ± 24.19*

[a]Based on 15 animals/sex/dose.

*Significantly different from control values at $p < 0.05$.

(11%) and females (34%) when compared with controls. During the 4-week recovery period, body weights of males and females receiving 1300 ppm maneb recovered; body weight gains over the recovery period were 1.7 times and 2.3 times that of concurrent controls for males and females, respectively (Table 3). At week 17, body weights of high-dose males were equivalent to those of concurrent controls; the body weights of high-dose females remained 10.8% lower than concurrent controls at this time. Body weights of low- and mid-dose males and females were similar to concurrent controls throughout the study period.

3. Food Consumption and Compound Intake: Consumption was determined, and mean daily diet consumption was calculated weekly at the time of body weight determinations. Efficiency and compound intake were calculated from the consumption and body weight gain data.

   Results: Representative food and compound consumption data are summarized in Table 4. The food consumption of high-dose females was slightly decreased when compared with concurrent controls (8% decrease from controls for weeks 1-13); food consumption for these animals remained slightly decreased through the recovery period to week 17. The food consumption of high-dose males was slightly decreased at many intervals throughout the dosing period when compared with concurrent controls; food consumption of these animals recovered during weeks 14 through 17. The food consumption of low-and mid-dose animals was similar to concurrent controls. Compared with nominal daily dosage levels, compound intake was 5, 24, and 77 mg/kg for low-, mid-, and high-dose males, respectively, and 6, 30, and 103 for low-, mid-, and high-dose females, respectively. Variability was noted in food efficiency data; however, there were no discernible dose-related trends or patterns.

4. Ophthalmological Examinations: Ophthalmological examinations were performed prior to study initiation and at week 13.

   Results: No ocular lesions were reported in control or dosed males or females.

5. Hematology and Clinical Chemistry: Blood was collected from the abdominal aorta at week 14 for hematology and clinical analysis from 10 fasted animals/sex/group. Thyroid function analyses were performed on recovery animals (5 animals/sex/group) at weeks 14 and 18; blood was collected from the orbital sinus. The CHECKED (X) parameters were examined:

TABLE 3. Representative Mean Body Weights and Body Weight Gains of Rats Following 13 Weeks of Dosing With Maneb[a]

Dose (ppm)	Mean Body Weights (g ± S.D.) During Recovery at Study Week 15	Mean Body Weights (g ± S.D.) During Recovery at Study Week 17	Body Weight Gains (g ± S.D.) Weeks 13-17
		Males	
0	590.9 ± 58.81	624.1 ± 65.48	35.5 ± 13.88
80	569.6 ± 52.13	588.1 ± 70.56	30.9 ± 28.37
400	601.0 ± 41.00	634.9 ± 47.13	49.6 ± 6.86
1300	587.7 ± 45.85	628.9 ± 48.53	62.1 ± 13.96*
		Females	
0	330.4 ± 26.93	340.5 ± 23.35	17.1 ± 16.79
80	325.6 ± 23.49	338.8 ± 21.84	21.9 ± 7.46
400	320.6 ± 31.55	341.9 ± 30.51	28.4 ± 5.24
1300	283.6 ± 16.80	303.5 ± 22.22	39.0 ± 8.98*

[a]Based on 5 animals/sex/dose.

*Significantly different from control values at $p < 0.05$.

TABLE 4. Representative Mean Food and Compound Consumption of Rats Fed Maneb for 13 Weeks

Dose (ppm)	Mean Food Consumption (g/week) at Week 1	7	13	Mean Compound Consumption (mg/kg body weight/day)
		Males		
0	174.3 ± 9.5	181.1 ± 15.8	172.4 ± 19.1	--[a]
80	177.3 ± 11.7	185.5 ± 13.2	177.7 ± 17.2	5 ± 1.1
400	178.0 ± 10.9	181.4 ± 13.2	183.0 ± 15.1	24 ± 5.6
1300	170.3 ± 8.8	173.2 ± 11.4	171.8 ± 10.0	77 ± 16.3
		Females		
0	138.8 ± 11.3	140.5 ± 14.7	130.5 ± 14.9	--[a]
80	133.6 ± 10.1	139.9 ± 16.6	122.8 ± 8.8	6 ± 1.2
400	140.1 ± 11.9	135.1 ± 10.0	128.7 ± 15.4	30 ± 5.8
1300	133.0 ± 19.1	123.2 ± 18.9	123.5 ± 20.5	103 ± 15.9

[a]The analysis of control diets was not reported.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
X	Platelet count*		Coagulation:thromboplastin time (PT)
	Reticulocyte count (RETIC)		
X	Red cell morphology		

Results: It was reported that there were no changes of toxicological significance in hematology parameters. Hemoglobin and hematocrit values were slightly decreased in females receiving 400 and 1300 ppm, with a significant negative trend ($p < 0.05$); however, the values were reported to be within the normal range of the laboratory.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium*	X	Albumin*
X	Chloride*	X	Albumin/globulin ratio
	Magnesium*	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*		Cholesterol*
X	Sodium*	X	Globulins
		X	Glucose*
	Enzymes	X	Total bilirubin*
	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein*
	Creatinine phosphokinase*		Triglycerides
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)*		Thyroid Function tests
X	Serum aspartate aminotransferase (SGOT)*	X	Triiodothyronine (T_3)
	Gamma glutamyltransferase (GGT)	X	Thyroxine (T_4)
		X	Thyroid stimulating hormone (TSH)

Results: Representative results of thyroid function tests are summarized in Table 5. A dose-related decrease in T_4 levels and a concomitant increase in TSH levels were exhibited in dosed females at 14 weeks; the decrease in T_4 was significant ($p<0.05$) at the high dose when compared with concurrent controls. T_4 levels were slightly but not

*Recommended by Subdivision F (October 1982) Guidelines.

TABLE 5. Representative Thyroid Function Data of Rats Following Maneb Dosing for 13 Weeks and 4 Weeks of Recovery[a]

Dose (ppm)	Thyroid Activity					
	T_3 at Week (ng/dL)		T_4 at Week (µg/dL)		TSH at Week (ng/dL)	
	14	18	14	18	14	18
			Males			
0	67.0 ± 8.11	79.4 ± 21.03	4.2 ± 1.03	5.3 ± 1.80	1.9 ± 0.51	4.0 ± 1.63
80	69.0 ± 28.70	78.9 ± 26.45	3.6 ± 1.39	5.2 ± 1.39	2.2 ± 0.96	3.7 ± 1.94
400	65.4 ± 15.40	70.4 ± 9.06	3.7 ± 0.91	5.6 ± 1.15	2.1 ± 1.25	2.8 ± 0.86
1300	99.1 ± 26.32	77.1 ± 13.41	3.3 ± 1.24	5.9 ± 1.68	6.4 ± 4.29	2.8 ± 0.58
			Females			
0	101.1 ± 29.08	84.6 ± 20.73	3.2 ± 0.49	2.9 ± 0.53	1.0 ± 0.15	1.3 ± 0.22
80	100.6 ± 11.30	87.5 ± 21.58	3.2 ± 0.88	3.4 ± 1.04	1.2 ± 0.17	1.6 ± 0.58
400	99.7 ± 10.94	87.5 ± 18.08	2.5 ± 0.68	3.7 ± 0.89	1.4 ± 0.49	1.6 ± 0.56
1300	107.5 ± 20.16	94.3 ± 19.22	1.8 ± 0.26*	3.9 ± 0.86	1.6 ± 0.40	1.4 ± 0.40

[a]Based on five rats/sex/dose with the exception of four females measured for TSH in control group at 14 weeks.

*Significantly different from control values at $p<0.05$.

significantly decreased, and TSH levels were slightly but not significantly increased in dosed males. The marked increase in mean TSH in high-dose males was the result of high TSH levels in two outliers (two of five animals measured). T_4 levels of males and females were similar to or higher than those of concurrent controls at the end of the recovery period; TSH levels were similar to or lower than controls at this time. T_3 levels were slightly increased in high-dose males following the dosing period and slightly increased in high-dose females at the end of the recovery period. The study author considered these T_3 changes to be inconsistent.

There were no changes of toxicological significance in other clinical chemistry parameters. All data were within the limits of normal expected variability.

6. Urinalysis: Urinalyses were not performed.

7. 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. In addition, the (XX) organs were weighed:

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue	X Aorta*+	XX Brain*
X Salivary glands*+	X Heart*+	X Peripheral nerve (sciatic nerve)*+
X Esophagus*+	X Bone marrow+	X Spinal cord (3 levels)
X Stomach*+	X Lymph node (mesenteric)*+	X Pituitary*+
X Duodenum*+	X Spleen*+	X Eyes (optic nerve)+
X Jejunum*+	X Thymus*+	
X Ileum*+		
X Cecum*+		
X Colon*+		
X Rectum*+		
XX Liver*+		
Gallbladder+		
X Pancreas*+		

+Recommended by Subdivision F (October 1982) Guidelines.

*Tissues indicated with an asterisk were examined histologically from control and high-dose animals.

Respiratory	Urogenital	Glandular
X Trachea+*	XX Kidneys+*	X Adrenals+*
X Lung+*	X Urinary bladder+*	X Lacrimal gland (exorbital)
	XX Testes+*	X Mammary gland+
	XX Epididymides*	XX Thyroids+*
	Prostate	XX Parathyroids+*
	Seminal vesicle	Harderian glands
	X Ovaries*	
	X Uterus+*	

Other
X Bone (sternum, femur)+*
X Skeletal muscle+
Skin
X All gross lesions and masses*

Kidneys from 5 representative high-dose animals were examined for pigment deposition. Liver, lung, kidney, thyroid tissues, and gross lesions were examined histologically for low- and mid-dose animals.

Results:

a. Organ Weights: Representative results of mean thyroid/parathyroid and kidney weights are presented in Table 6. A dose-related increase was found in the thyroid/parathyroid weights of dosed males and females; absolute weights were significantly ($p < 0.05$) increased in mid- and high-dose males, and relative weights were significantly ($p<0.05$) increased in high-dose males. Thyroid/parathyroid weights of dosed and control animals were similar following the 4-week recovery. Slight increases in absolute and relative kidney weights of dosed males and females were considered by the study author to be the result of renal pigment deposition and lowered body weights.

b. Gross Pathology: There were no compound-related changes in macroscopic pathology in dosed animals at either the 14-week or 18-week sacrifice.

+Recommended by Subdivision F (October 1982) Guidelines.

TABLE 6. Selected Organ Weights and Organ-to-Body Weight Ratios in Rats Fed Maneb for 13 Weeks[a]

Dose (ppm)	Thyroid/Parathyroid Absolute (g)	Thyroid/Parathyroid Relative (%)	Kidney Absolute (g)	Kidney Relative (%)
		Males		
0	0.036 ± 0.005	0.007 ± 0.001	3.44 ± 0.26	0.66 ± 0.06
80	0.042 ± 0.010	0.008 ± 0.002	3.68 ± 0.39	0.66 ± 0.08
400	0.045 ± 0.010*	0.008 ± 0.002	3.69 ± 0.23	0.67 ± 0.04
1300	0.048 ± 0.012*	0.010 ± 0.002*	3.54 ± 0.29	0.72 ± 0.06
		Females		
0	0.032 ± 0.11	0.011 ± 0.004	2.02 ± 0.13	0.69 ± 0.06
80	0.035 ± 0.006	0.011 ± 0.001	2.10 ± 0.13	0.69 ± 0.07
400	0.035 ± 0.008	0.012 ± 0.003	2.38 ± 0.60	0.81 ± 0.27
1300	0.036 ± 0.009	0.014 ± 0.003	2.14 ± 0.22	0.85 ± 0.09*

[a]Based on 10 animals/sex/group sacrificed at study week 14 with the exception of 9 males in the control group.

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c. Microscopic Pathology: Selected histologic findings are listed in Tables 7 and 8. Follicular cell hyperplasia of the thyroid was exhibited in 1/10 mid-dose males, 10/10 high-dose males, and 2/10 high-dose females compared with 0/10 control males and 0/10 control females. Increased colloid of the thyroid was also exhibited in 4/10 high-dose males. Thyroid changes regressed following the recovery phase. A follicular cell adenoma of the thyroid was observed in one high-dose male; this was considered to be a spontaneous incidental neoplasm and not compound related. Two of ten low-dose males, all mid-dose males and females, and all high-dose males and females exhibited a granular renal pigment which was considered compound related. The incidence of chronic progressive nephropathy was similar in dosed and control males and females at 14 weeks.

At the end of the recovery period, a reduction in the incidence and amount of renal pigment was reported in 3/5 mid- and high-dose males and 4/5 mid- and high-dose females; the pigment was not considered by the study author to be associated with degenerative or inflammatory changes in the kidney. At the end of the recovery period, the incidence of chronic progressive neuropathy was slightly increased in mid- and high-dose males only when compared with concurrent controls.

D. STUDY AUTHOR'S CONCLUSIONS:

The author concluded that dietary administration of maneb produced effects on body weight, food consumption, and thyroid function at 1300 ppm, and thyroid/parathyroid weights and histologic changes of the thyroid and kidneys at 400 and 1300 ppm. Following a 4-week recovery period, complete regression of effects occurred with the exception of decreased body weights in females and histologic renal effects in males and females.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete, and the conduct of the study and reporting of the data were acceptable. However, the calculations of the overall mean test diet concentrations (text Table 1 of the study report) were incorrect; the correct overall mean concentration calculations are presented in Table 1 of this review. Purity of the test compound was reported to

TABLE 7. Representative Histopathological Findings in Rats Fed Maneb for 13 Weeks

Organ/Finding	Males Dose (ppm) 0	80	400	1300	Females Dose (ppm) 0	80	400	1300
Thyroid	(10)[a]	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Follicular cell hyperplasia	0	0	1	10	0	0	0	[illegible]
Increased colloid	0	0	0	4	0	0	0	0
Kidney	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy, chronic progressive	5	4	5	6	4	3	5	2
Tubular pigment	0	2	10	10	0	0	10	10

[a]Number of tissues examined are in parentheses.

TABLE 8. Representative Histopathological Findings in Rats Fed Maneb Following the 4-Week Recovery Period

Organ/Finding	Males Dose (ppm) 0	80	400	1300	Females Dose (ppm) 0	80	400	1300
Thyroid	(5)[a]	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Follicular cell adenoma-benign	0	0	0	1	0	0	0	0
Kidney	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Nephropathy, chronic progressive	2	3	4	4	0	0	0	0
Tubular pigment	0	0	3	3	0	0	4	4

[a]Number of tissues examined are in parentheses.

be 86% by the study sponsor and 77.9% by the study laboratory. It is not clear whether the test diets were corrected to reflect this difference. On page 33 of the final report, the Table Text 5 lists the values for the compound consumed by each group. It is unclear whether these values have been corrected to reflect the difference noted between the percentage of active ingredient (86%) accorded to the sponsor (the percentage on which the diets were presumably prepared) and the percentage (77.9%) found in the analysis performed at the testing laboratory. This aspect should be addressed by the study author. Additionally, an explanation of the analytical procedures used should be provided, detailing whether duplicate samples were run, etc. The 400-ppm diet concentration was reported to vary more than 20% from target at weeks 4 and 13; this is outside the range of acceptability. Because of this discrepancy between the target dose level and that achieved, some data/information should be provided to explain/justify why this will not adversely affect the interpretation of the results. Since the diets were prepared on a weekly basis, the mid-dose group received these lower dose levels for two entire weeks. Documentation should be provided to assure that adequate doses were administered during the intervening weeks (between weeks 4 and 8 and weeks 8 and 13).

Although the author stated that the relationship of the tremors observed in the mid- and high-dose females to test material exposure was unknown, it is to be noted that this is a manganese compound and the tremors may be due to the manganese content. This effect was noted only in the females, was of low incidence, did not occur at the low-dose level and, therefore, further follow-up on this aspect is not necessary at this time.

We agree with the author's assessment that the principal changes in body weights, thyroid function, organ weights, and histopathology occurred in mid- and high-dose animals; however, slight changes in thyroid function (T_4 and TSH) did occur in low-dose males at study week 13. Similar changes in thyroid function were dose related in mid- and high-dose females at this time. Historical laboratory data for thyroid function indices were not provided and should be requested. The incidence of renal pigment in low-dose males (2/10 as compared with 0/10 in concurrent controls) cannot be ignored even though the study author did not consider the presence of this pigment to be associated with degenerative or inflammatory changes of the kidney. Although glandular pigment was observed at the low-dose, it was only in males and its occurrence at this level was shown to be reversible; therefore, this dose may be approaching the no-effect level for this change. The author should be requested to provide data/information on the identity

of the renal pigment. It is of interest that the incidence of chronic progressive nephropathy, which was not discussed by the study author, was slightly higher in mid- and high-dose males at the end of the recovery period.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA No.: 68D80056
DYNAMAC No.: 159-A
TASK No.: 1-59A
June 1, 1989

DATA EVALUATION RECORD

MANEB

Developmental Toxicity Study in Rabbits

STUDY IDENTIFICATION: Merkle, J. Study to determine the prenatal toxicity of manganous ethylenebis (dithiocarbamate) in rabbits. (Unpublished study No. 83/094 conducted by BASF AG, Ludwigshafen Rhein, Federal Republic of Germany and submitted by Maneb Registration Group, NPC, Inc., Sterling, VA; dated May 24, 1983.) MRID No. 409824-01.

APPROVED BY:

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

Signature:

Date: 5/3/89

1. CHEMICAL: Manganous ethylenebis dithiocarbamate.

2. TEST MATERIAL: Maneb, lot No. 81/310, was 90.6% pure and described as a yellow solid.

3. STUDY/ACTION TYPE: Developmental toxicity study in rabbits.

4. STUDY IDENTIFICATION: Merkle, J. Study to determine the prenatal toxicity of manganous ethylenebis (dithiocarbamate) in rabbits. (Unpublished study No. 83/094 conducted by BASF AG, Ludwigshafen Rhein, Federal Republic of Germany and submitted by Maneb Registration Group, NPC, Inc., Sterling, VA; dated May 24, 1983.) MRID No. 409824-01.

5. REVIEWED BY:

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DATA EVALUATION RECORD

STUDY TYPE: Developmental toxicity. GUIDELINE § 83-3

ACCESSION/MRID NUMBER: 409824-01.

TEST MATERIAL: Maneb.

SYNONYM(S): Manganese ethylenebis; dithiocarbamate.

STUDY NUMBER(S): 83/094.

SPONSOR: Maneb Registration Group, NPC, Inc., Sterling, VA (Pennwalt Corporation).

TESTING FACILITY: BASF AG, Ludwigshafen Rhein, Federal Republic of Germany.

TITLE OF REPORT: Study to determine the prenatal toxicity of manganous ethylenebis (dithiocarbamate) in rabbits.

AUTHOR(S): J. Merkle.

REPORT ISSUED: May 24, 1983.

CONCLUSIONS:

An assessment of the maternal and developmental toxicity of maneb was precluded due to the lack of essential information on analytical chemistry procedures and individual animal data. The classification of this study may be upgraded upon submission of the following data.

a. Analytical chemistry data and sample storage, preparation, and analysis procedures for the dosing material.

b. Individual animal data for maternal food consumption, clinical observations, necropsy findings, and animal assignment for the three phases of the study.

c. Copies of the fetal x-rays employed in the skeletal examination.

d. Data on the sperm used for insemination (including source, age, and collection procedures).

CLASSIFICATION: Supplementary data.

A. MATERIALS:

Test Compound: Purity: 90.6% active ingredient.
Description: Yellow solid.
Lot No.: 81/310.
Contaminants: 2.0% ethylene thiourea.

Vehicle(s): Sodium carboxymethyl cellulose (CMC, Tylose®); supplied by Hoechst AG; lot No. CB 30 000.

Test Animals: Species: Rabbit.
Strain: Himalayan Chbb: HM.
Source: Dr. K. Thomae GmbH Breeding Facility, Biberach, FRG.
Age: 35 to 45 weeks.
Weight: Mean weight, 2.41 kg.

B. STUDY DESIGN:

This study was designed to assess the developmental toxicity potential of maneb, when administered by oral gavage from gestational days 6 through 18, inclusive. The study was divided into three phases during which equal numbers of animals from each group were inseminated, dosed, and necropsied. The first phase was initiated on November 9, 1981; the second on November 16, 1981; and the last on January 11, 1982.

Mating: Artificial insemination was employed. One hour prior to insemination, each female was injected intravenously with 40 I.U. of chorionic gonadotrophin (Primogonyl® in saline) supplied by Schering AG, Berlin, FRG. The day of insemination was designated as day 0 of gestation. No other information was presented.

Group Arrangement:

Test group	Dose level (mg/kg/day)	Number assigned[a]
Control (CMC)	0	15
Low (LDT)	5	15
Mid (MDT)	20	15
High (HDT)	80	15

[a]Does were randomly assigned to dose groups.

Dosing:

All doses were in a volume of 5 mL/kg of body weight prepared daily during the dosing period. The dosing solutions were analyzed for concentration but not for homogeneity and stability. Dosing was based on body weights measured on gestational day 6.

Selection of dose levels for this study was based on the findings of a preliminary study in which doses of 200 or 500 mg/kg resulted in maternal toxicity and a dose of 80 mg/kg was tolerated.

Observations:

The animals were checked daily for mortality and abnormal condition throughout the gestation period. Body weights were measured 7, 5, and 3 days prior to insemination and on days 0, 2, 4, 6, 7, 9, 11, 14, 16, 18, 21, 23, 25, 28, and 29 of gestation. Food consumption was measured and recorded daily starting on day 1 of gestation. Dams were sacrificed on day 29 of gestation, and fetuses were delivered by cesarean section. Examinations at sacrifice consisted of:

- gross necropsy of dams;
- measurement of number of corpora lutea;

- measurement of uterine and placental weight;
- measurement of number of implantations;
- measurement of number of live and dead fetuses; and
- measurement of the length of the umbilical cord.

The fetuses were examined in the following manner:

- an external examination was performed;
- fetal weights were recorded;
- crown-to-rump measurements were recorded;
- sex was determined;
- viscera of all fetuses were examined in situ by ventral dissection with sectioning of the heart and kidneys, and abnormalities were recorded prior to evisceration;
- skeletal structure of all fetuses (heads included) using x-rays; and
- after x-rays were completed, heads were removed, fixed in Bouin's solution, processed, and examined using Wilson's method.

Statistical Analysis:

The following statistical analysis methods were employed: the Williams test, U-test, and Fisher test were used. Body weight gains were calculated, and differences between means were analyzed for the following gestational day intervals: 0-2, 2-4, 4-6, 6-9, 9-11, 11-14, 14-16, 16-18, 18-21, 21-23, 23-25, and 25-29. Food consumption values were calculated daily.

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated December 16, 1988, was provided.
- A signed Statement of Compliance with EPA GLP's, not dated, was provided.
- A signed Quality Assurance Statement, dated April 22, 1988, was provided.

C. RESULTS:

1. Test Material: Results of the concentration analyses indicated that actual mean concentrations ranged from 70-81.5% for the low dose, 94-98% for the middle dose, and 46-104% for the high dose of target doses.

2. Maternal Toxicity:

Mortality: One female (No. 53) from the high-dose group died on day 8 of the study. This doe apparently was not pregnant.

In addition to spontaneous deaths, four does were sacrificed during the study. One female (No. 41) from the mid-dose group was sacrificed on day 16 of the study because of a dosing error (diagnosed at necropsy). She apparently was not pregnant. Three females from the high-dose group were sacrificed during the study. Two does aborted on days 21 and 28 of gestation (Nos. 52 and 56, respectively), and the third was sacrificed moribund (No. 54). All three were pregnant. No other deaths were observed.

Abortions: In the high-dose group, one female (No. 52) aborted on day 21 of gestation. Another high-dose female (No. 56) delivered prematurely on day 28 of gestation. One control female (No. 13) also delivered prematurely on day 29 of gestation.

Clinical Observations: No compound-related clinical signs of toxicity were observed during the study. One high-dose female (No. 49) had a bloody vaginal discharge on day 28 of gestation. Prior to being sacrificed, a mid-dose female (No. 41) exhibited increased respiration rate and cyanosis from an apparent gavage error. No other unusual observations were reported.

Body Weight: The investigator supplied the following data: significant decreases ($p \leq 0.05$) in body weight were observed in females from the high-dose group on days 11, 23, and 25 of gestation when compared with controls. Body weight gains for high-dose dams were significantly less ($p \leq 0.01$) during gestational days 6-9 and 21-23 and significantly greater ($p \leq 0.05$) at the day 11-14 interval when compared with controls. No other significant differences were observed.

Food Consumption: The investigator supplied the following data: significant decreases ($p \leq 0.01$) in food consumption were observed in high-dose females on days 7-9 and 10-11 of gestation when compared with controls. No other significant differences were reported.

Gross Pathological Observations: No compound-related abnormalities were found at terminal sacrifice. Two females from the low-dose group and one female from the high-dose group had parasitic cysts, described as approximately 1 to 2 mm in diameter and filled with a watery white fluid and located on or in the kidneys and/or liver. One high-dose female that died during the study had fibrin deposits on the pericardium and sternum as well as cardiac dilation. These were not considered to be related to administration of the test material.

Cesarean Section Observations: Preimplantation loss and pregnancy rates were comparable between control and test groups (Table 1). The author observed no differences in the number of corpora lutea or implantations among control and test groups.

Postimplantation loss was nonsignificantly increased in does administered 80 mg/kg/day when compared with controls. In addition, a significant decrease ($p \leq 0.01$) in the number of viable fetuses and a significant increase ($p \leq 0.01$) in the number of resorptions were observed in high-dose dams when compared with control dams. No significant differences in postimplantation loss, number of resorptions, or number of viable fetuses were observed between the control, low-, and mid-dose groups. For one female (No. 49 from the high-dose group, all seven fetuses (i.e., the entire litter) were resorbed, and one female (No. 2) from the control group had seven dead fetuses (the entire litter). Fetal body weights, crown-to-rump length, and sex ratio were comparable between control and test groups. The placental weight of female fetuses only was significantly ($p \leq 0.01$) greater at the high-dose level than for controls. Uterine weight was significantly reduced ($p < 0.01$) in does administered 80 mg/kg/day when compared with controls. Placental and uterine weights were comparable between controls and animals in the low- and mid-dose groups.

3. Developmental Toxicity:

External Examinations: No abnormalities except for a single incidence of pseudoankylosis in one control fetus were found upon external examination of the fetuses.

TABLE 1. Cesarean Section Observations[a]

Dose (mg/kg/day)	0	5	20	80
No. animals assigned	15	15	15	15
No. animals mated/ inseminated	15	15	15	15
Pregnancy rate (%)	93.3	86.7	100.0	93.3
Maternal wastage				
No. died	0	0	0	2[b]
No. died/pregnant	0	0	0	1
No. nonpregnant	1	2	0	1
No. aborted	0	0	0	1
No. premature delivery	1	0	0	1
Total corpora lutea	124	116	122	90
Corpora lutea/dam[c]	9.54	8.92	8.71	8.18
Total implantations	91	85	85	63
Implantations/dam	7.00	6.54	6.07	5.73
Total live fetuses	79	76	81	42
Live fetuses/dam	6.08	5.85	5.79	3.82**
Total resorptions	5	9	4	21
Early[c]	4	7	3	21
Late	1	2	1	0
Resorptions/dam	0.26	0.69	0.29	1.91**
Total dead fetuses	7[d]	0	0	0
Dead fetuses/dam	0.5	0	0	0
Mean fetal weight (g)[e]	40.9 ± 3.33	42.8 ± 2.32	41.5 ± 5.03	43.0 ± 4.79
Preimplantation loss (%)	26.2	26.3	29.3	27.2
Postimplantation loss (%)	14.4	10.1	4.3	30.6
Sex ratio (% male)	55.7	53.9	50.6	50.0

[a]Data extracted from Study No. 83/094, Table No. 030, 121 through 124, and 141 through 144.
[b]This value includes one female that was moribund and sacrificed on day 23 of gestation.
[c]Includes the early and intermediate resorptions reported by the author.
[d]One control female had seven dead fetuses.
[e]Mean ± S.D.
**Significantly different from control value at $p \leq 0.01$.

Visceral Examinations: No compound-related abnormalities were found (Table 2).

Skeletal Examinations: No compound-related abnormalities were found (Table 3).

C. DISCUSSION/CONCLUSIONS:

a. Maternal Toxicity: Body weight gains for the predosing, dosing, postdosing, and entire gestation intervals are presented in Table 4. These values were recalculated and statistically analyzed using Analysis of Variance by the reviewers based on individual animal data. Nonpregnant animals and pregnant animals that aborted or delivered prematurely were excluded from the means. In Table 5, the food consumption values for the predosing, dosing, and postdosing periods as well as total food consumed for the entire gestation period are presented. These were calculated by the reviewers and were based on group mean values because no individual food consumption data were presented. For this reason statistical analysis was not performed.

Results indicated that the body weight gain of high-dose females was decreased during the predosing and postdosing periods and for the entire gestation period. However, a statistically significant decrease ($p \leq 0.05$) was observed only for the postdosing interval. These decreases were not reflected in the corrected body weight gain, which was slightly higher for high-dose rabbits than for controls. However, high-dose rabbits consumed approximately 25 and 10% less food per day than controls during the dosing interval and for the entire gestation period, respectively. The decrease in food consumption during the dosing period appeared to result in a delayed decrease in body weight gain during the postdosing period. This difference may have been due to smaller litter size, and, hence, a smaller weight gain in high-dose animals than in controls. However, the effects, although marginal, were considered to be compound related.

b. Developmental Toxicity:

i. Deaths/Resorptions: A significant, compound-related decrease in the number of viable fetuses and a significant compound-related increase in the number of resorptions were observed in the high-dose animals when compared with controls. This was associated with a nonsignificant increase in postimplantation loss at 30 mg/kg/day. A slight increase in the number of resorptions, observed in dams administered 5 but not 20 mg/kg/day, was considered to be incidental.

TABLE 2. Summary of Visceral Findings[a]

Observation	Dose level (mg/kg/day) 0	5	20	80
No. litters (fetuses) examined	12(79)	13(76)	14(81)	10(42)
Anophthalmia (unilateral)				
No. (%) fetuses	0	0	0	1(2.4)
No. (%) litters	0	0	0	1(10.0)
Coloboma (unilateral)				
No. (%) fetuses	1 (1.3)	0	0	0
No. (%) litters	1 (8.3)	0	0	0
Microphthalmia (unilateral)				
No. (%) fetuses	0	1(1.3)	0	0
No. (%) litters	0	1(7.7)	0	0
Agenesis-gallbladder				
No. (%) fetuses	1(1.3)	3(3.9)	1(1.2)	1(2.4)
No. (%) litters	1(8.3)	3(23.1)	1(7.1)	1(10.0)

[a]Data extracted from study No. 83/094, Tables No. 042 through 046 and 143 through 156.

TABLE 3. Summary of Skeletal Findings[a]

Observation	Dose level (mg/kg/day) 0	5	20	80
No. litters (fetuses) examined	12(79)	13(76)	14(81)	10(42)
Fused sternebrae				
No. (%) fetuses	0	1(1.3)	2(2.5)	0
No. (%) litters	0	1(7.7)	2(14.3)	0
Asymmetrical sternebrae				
No. (%) fetuses	2(2.5)	2(2.6)	1(1.2)	1(2.4)
No. (%) litters	2(16.7)	2(2.0)	1(7.1)	1(10.0)
Single sternebra absent				
No. (%) fetuses	21(26.6)	27(35.5)	21(25.9)	6(14.3)
No. (%) litters	9(75.0)	7(53.8)	9(64.3)	5(50.0)
Talus bilateral-incomplete ossification				
No. (%) fetuses	6(7.6)	0	6(7.4)	0
No. (%) litters	3(25.0)	0	3(21.4)	0

[a]Data extracted from Study No. 83/094, Tables No. 049 through 052 and 157 through 168.

TABLE 4. Body Weight Gains and Corrected Body Weight Gain (g)[a]

Dose (mg/kg/day)	Prior to dosing period (0-6)	Dosing period (6-18)	Post-dosing period (18-29)	Entire gestation period (0-29)	Corrected BW gain entire gestation period[b]
0	22.0 ± 42.0[c]	14.5 ± 88.2	181.1 ± 78.1	217.5 ± 68.2	-144.7 ± 109.3
5	13.8 ± 28.4	28.5 ± 43.6	175.1 ± 82.6	217.4 ± 113.3	-124.4 ± 77.5
20	22.6 ± 47.4	29.4 ± 91.6	166.0 ± 73.5	218.0 ± 154.6	- 96.4 ± 112.0
80	0.36 ± 18.1*	37.8 ± 77.7	89.1 ± 82.5*	127.3 ± 112.2	-107.0 ± 76.8

[a]Data extracted from Study No. 83/094, Tables No. 065 through 076 and 113 through 116. Statistical analysis (analysis of variance) was calculated by the reviewers for these intervals. Nonpregnant animals and pregnant animals aborting or delivering prematurely were excluded from calculations.
[b]Calculated as the body weight gain for the entire gestation period minus the gravid uterine weight.
[c]Mean ± S.D.
*Significantly different from controls at p≤0.05.

TABLE 5. Food Consumption Data (g/animal/day)[a]

Dose (mg/kg/day)	Prior to dosing period (1-6)	Dosing period (6-18)	Postdosing period (18-29)	Entire gestation Period (1-29)
0	83.9 ± 4.12[b]	71.3 ± 11.87	88.4 ± 8.07	80.1 ± 12.51
5	89.7 ± 1.51	71.6 ± 8.10	92.3 ± 13.14	83.0 ± 14.23
20	87.5 ± 2.34	70.2 ± 9.68	89.2 ± 11.93	80.9 ± 13.62
80	86.9 ± 3.28	55.5 ± 20.24	83.7 ± 14.47	72.1 ± 21.85

[a]Data extracted from Study No. 83/094, Table No. 055.
[b]Mean ± S.D.

ii. Altered Growth: Fetal body weights were not adversely affected by administration of the test material. In fact, the mean fetal weights were slightly higher than that of controls. This was probably due to smaller litters.

iii. Developmental Anomalies: No compound-related effects were observed. Incidences of developmental anomalies were comparable between control and test groups.

iv. Malformations: A slight, nonsignificant, increase in the incidence of fetuses and litters (three fetuses from three different litters) with agenesis of the gallbladder was observed in the 5-mg/kg/day dose group when compared with controls (one fetus was affected). However, since a similar increase was not observed at the mid- and high-dose levels, and the incidence for this abnormality was within the historical control range, this finding was considered to be incidental.

c. Study Deficiencies: The following deficiencies were noted.

1. Analytical chemistry data and a description of the analytical methods used to determine dose concentrations were not presented. In addition, analyses of the stability or homogeneity of the test material in the vehicle apparently were not conducted.

Analyses of the dosing suspensions indicated that actual concentrations varied considerably from aliquot to aliquot for each sample and from sampling period to sampling period. These data suggest that either the homogeneity of the dose suspensions was unacceptable or the extraction of the test material from the vehicle prior to analysis was incomplete. Furthermore, extraction or stability problems were indicated during the last analysis. The concentration for the 80-mg/kg/day dose was extremely low (57% of target concentration), and, therefore, the suspension was reanalyzed twice on consecutive days. Each subsequent analysis produced a lower average concentration. Fortunately, the dosing suspensions were prepared daily, and, therefore, the possible stability problem may not have affected animal dosages. However, administration of a nonhomogeneous suspension could have caused an inconsistent pattern of test material dosages to the animals within the group which in turn could have impacted negatively on the results of the study. Submission of analytical data and sample handling, preparation, and analysis procedures would aid in

determining whether the problems are due to incorrect analysis procedures or instability or nonhomogeneity of the test material.

2. Individual animal data for maternal food consumption, clinical observations, and gross necropsy findings were not presented, and, therefore, summary tables and results could not be verified. Furthermore, no information on the sperm used for the insemination (source, age, collection method, dilution factors) was presented.

3. The author reported that the study was divided into three phases during which equal numbers of animals from each group were inseminated, dosed, and necropsied. The data were then pooled and analyzed. The first phase was initiated on November 9, 1981; the second on November 16, 1981, and the last on January 11, 1982. Consequently, approximately 2 months separated the first and the last phases, during which changes in environmental and other conditions probably occurred. No information on animal assignments to each phase was presented. Therefore, the data from each phase could not be separated to determine whether the time difference had an effect. In addition, the possible use of more than one shipment of animals was not reported. This also may have affected the results.

4. Analyses to determine actual concentration of the dosing suspensions were conducted three times during the study to coincide with the initiation of dosing for the three phases. However, the number of analyses was not adequate since the dose material was prepared daily, and consequently, approximately 36 mixes were prepared. In addition, actual concentrations (70-82%) for the low dose were outside the acceptable range of target concentration throughout the study. Therefore, low-dose animals actually received approximately 3.8 and not 5 mg/kg/day as was reported. Lastly, concentration analysis indicated that the dose suspension for the high-dose group was extremely low for the last of the three analyses. The aliquot was reanalyzed twice to check analysis procedures, and the resulting concentrations were less than that of the original analysis. These differences, as mentioned earlier, may have been due to poor extraction procedures or stability problems. In any case, the subsequent days' mixings should have been analyzed to verify dose preparation procedures, but they were not. Approximately five animals per group were affected, but these

could not be identified, and their data thus could not be separated. Consequently, any effects cannot be assessed.

5. Fetal skeletal examinations were performed using x-rays. This method of skeletal examination is not commonly used and may not have been adequate to visualize all of the abnormalities that may have been found upon direct observation of the stained skeletons. Submission of copies of the x-rays may aid in assessing the adequacy of the method.

E. CLASSIFICATION: Supplementary data.

The No-Observed-Effect Levels (NOELs) and Lowest-Observed-Effect Levels (LOELs) for maternal and developmental toxicity were not established because of the deficiencies listed above. The classification may be reassessed upon submission of the following data:

- analytical chemistry data and handling, preparation, and analytical procedures on concentration analysis of the dosing material;
- individual animal data for maternal food consumption, clinical observations, necropsy findings, and animal assignment for the three phases of the study;
- copies of the fetal x-rays employed in the skeletal exami--nation; and
- data on the sperm used for insemination (including source, age, and collection procedures).

F. RISK ASSESSMENT: Not appropriate.