2-28-92

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

009338

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Maneb - 2-Generation Reproduction Study

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

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/27/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 2/27/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 2/25/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: ATOCHEM North America (Pennwalt Corporation)
Chemical: manganese ethylene-1,2-bisdithiocarbamate; ethylene thiourea
Synonym: Maneb
Project No.: 2-0318
Caswell No.: 539
Record No.: none; Case: 818618; Submission: S405829
Identifying No.: 014505
DP Barcode: D170395
MRID No.: 420494-01
Action Requested: For Immediate Review - MANEB "CORT" data. Please review the Maneb 2-gen. repro. rat study (MRID 42049401). Also, if possible, please give me the current status for the tox. requirements for MANEB and ETU (i.e., outstanding data requirements, new data requirements, GLNS that are satisfied, etc.).

Comment: In response to the Maneb Comprehensive Data Call-In of April 1, 1987, the Registrant has submitted a report: " A Study of the Effect of Maneb (technical) on the Reproductive Function of Two Generations in the Rat". This study has been reviewed, and the DER is attached.

Under the conditions of the study, exposure to Maneb via the d

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during the pre-mating period and through gestation and lactation for two generations, at dose levels of 0, 75, 300 and 1200 ppm, produced some maternal toxicity at the high-dose level. Food consumption and body weight were decreased at the high-dose level in both sexes of both generations. Overall body-weight gain was decreased at the high dose in both generations (both sexes) and in the mid-dose F0 females. There was no effect demonstrated on pregnancy rate, gestation times, or the ability to rear young to weaning, but the pre-coital time was slightly longer in the high-dose F1 dams compared to the concurrent control value and all other groups in both generations. Various organ weights (thyroid, liver, kidney, lung, testes) were affected by treatment, but a no-effect level was demonstrated for each except for the F0 testes weight. There was no effect of treatment on the implantation rate, litter size, or pup mortality in either generation. Litter and pup weights were comparable at birth among the groups in both generations, but there was a decrease observed in both in the F1 generation at Day 12 post partum and in both generations at Day 21 post partum. In both generations, there was a slight delay in the startle response of the offspring at the mid- and high-dose levels, and the onset of vaginal opening was delayed in the F1 high-dose female offspring. There were various organ-weight effects, which were similar to those observed in the parental animals, although no no-effect level was observed for the increase in liver weight for either sex in the F1 offspring.

The NOEL for maternal toxicity can be set at 75 ppm (≈ 6 mg/kg/day), the LEL at 300 ppm (≈ 25 mg/kg/day), based on decreased body weight/body-weight gain and food consumption. A NOEL for reproductive effects can be set at 75 ppm, the LEL at 300 ppm, based on a slight delay in the startle response in the offspring at the mid- and high-dose levels in both generations. The NOEL for the effect of Maneb on the testes weight of the adult males would be assessed more appropriately in a 90-day or chronic toxicity study. The NOEL for effects on the offspring can be set at 75 ppm, the LEL at 300 ppm, based on the delay of the startle response. Although there were increased liver weights at all dose levels in the F1 offspring of both sexes, the magnitude of the response between the dose levels was not comparable to the magnitude of the increase in the dose and is not viewed as biologically significant.

Classification: Core: minimum. This study satisfies the guideline requirements (83-4) for a 2-generation reproduction study.

With regard to the current status for the toxicology data requirements for Maneb, the toxicological data available on Maneb and the outstanding data requirements were outlined in the Revised Toxicology Chapter of the Registration Standard on Maneb (dated 4/13/88) and the PD 2/3, published in the federal Register of December 20, 1989 (54 FR 52158). To date, the 2-generation-reproduction study (subject of the current action) and the rat developmental toxicity study (DER dated 1/15/92) have been

submitted and have been reviewed by TB II. The latter study is classified core supplementary. A 21-day dermal study was submitted, reviewed (DER dated 3/8/89), and classified core minimum. Additional data have been submitted on the mutagenicity studies and these have been upgraded (TB II memo dated 9/27/88 and 10/19/88) to acceptable. Although a 4-week inhalation study was submitted recently (TB II memo dated 11/26/91) to upgrade the 90-day inhalation study, additional data are required, and the study remains core supplementary. NOTE: Recently, a chronic dog study (MRID # 42133101) on Metiram was submitted to TB II for review (DP Barcode D172771), although the "Bean Sheet" refers to this study as a Maneb Study. To date, no chronic dog study on Maneb has been submitted to TB II for review. Other data requirements include: (1) a mouse carcinogenicity study; (2) adequate additional data to upgrade the 31-month rat chronic toxicity/carcinogenicity study; and (3) adequate data to upgrade the rabbit developmental toxicity study.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/27/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 2/27/92
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 2-generation reproduction - rat TOX. CHEM. NO.: 539

MRID NO.: 420494-01

TEST MATERIAL: Maneb technical

SYNONYMS: manganese ethylene-1,2-bisdithiocarbamate

STUDY NUMBER: MNB 1/9072

SPONSOR: Maneb Registration Group/NPC Incorporated

TESTING FACILITY: Huntingdon Research Centre Ltd.

TITLE OF REPORT: A Study of the Effect of Maneb (Technical) on Reproductive Function of Two Generations in the Rat

AUTHORS: PR Ryle, PF Bell, C Parker, H Farmer, JM Offer, A Anderson, and IS Dawe

REPORT ISSUED: May 2, 1991

QUALITY ASSURANCE: A quality assurance statement was provided

CONCLUSIONS: Under the conditions of the study, exposure to Maneb via the diet during pre-mating (10 weeks F0, 12 weeks F1) and through gestation and lactation, at dose levels of 0, 75, 300 and 1200 ppm, produced some maternal toxicity at the high-dose level. Food consumption and body weight were decreased at the high-dose level in both sexes of both generations. Overall body-weight gain was decreased at the high dose in both generations (both sexes) and in the mid-dose F0 females. There was no effect demonstrated on pregnancy rate, gestation times, or the ability to rear young to weaning, but the pre-coital time was slightly longer in the high-dose F1 dams compared to the concurrent control value and all other groups in both generations. Various organ weights (thyroid, liver, kidney, lung, testes) were affected by treatment, but a no-effect level was demonstrated for each except for the F0 testes weight. There was no effect of treatment on the implantation rate, litter size, or pup mortality in either generation. Litter and pup weights were comparable at birth among the groups in both generations, but there was a decrease observed in both in the F1 generation at Day 12 post partum and in both generations at Day 21 post partum. In both generations, there was a slight delay in the startle response of the offspring at the mid- and high-dose levels, and the onset of vaginal opening was delayed in the F1 high-dose female offspring.

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There were various organ-weight effects, which were similar to those observed in the parental animals, although no no-effect level was observed for the increase in liver weight for either sex in the F1 offspring.

The NOEL for maternal toxicity can be set at 75 ppm (≈ 6 mg/kg/day), the LEL at 300 ppm (≈ 25 mg/kg/day), based on decreased body weight/body-weight gain and food consumption. A NOEL for reproductive effects can be set at 75 ppm, the LEL at 300 ppm, based on a slight delay in the startle response in the offspring at the mid- and high-dose levels in both generations. The NOEL for the effect of Maneb on the testes weight of the adult males would be assessed more appropriately in a 90-day or chronic toxicity study. The NOEL for effects on the offspring can be set at 75 ppm, the LEL at 300 ppm, based on the delay of the startle response. Although there were increased liver weights at all dose levels in the F1 offspring of both sexes, the magnitude of the response between the dose levels was not comparable to the magnitude of the increase in the dose and is not viewed as biologically significant.

Classification: Core: This study satisfies the guideline requirements (83-4) for a 2-generation reproduction study.

A. MATERIALS

1. Test Compound: Maneb technical; Description: yellow/buff powder; Batch #: 8704-286/15 (weeks 1-5), BI 070788 (week 6 to end); Purity: 87.3%, which decreased to 80.0% and was replaced; new batch was 89.6%.

2. Test Animals: Species: rat; Strain: Crl: CD®(SD) BR VAF/Plus; Age: 6 weeks old; Weight: males: ≈23 g, females: ≈35 g, on arrival; Source: Charles River, Portage, MI USA.

3. Statistics: Food/water consumption and body-weight gain - analysis of variance, followed by Williams' test; Litter data [mean values of litter size, cumulative loss (pup mortality), litter weight, mean pup weight, sex ratio within litters, and age of attainment of pre-weaning development markers] - non-parametric tests (Jonckheere and Kruskal-Wallis); where 75% of the values for a given variable consisted of one value, Fisher's Exact Test was used and where appropriate, Mantel's test for trend in proportions was performed also; Organ weight - analysis of variance or covariance (final body weight as covariant when the within-group relationship between organ weight and body weight was significant at the 10% level. A log (x+1) transformation of organ weight (or of both body weight and organ weight) was used if significant (1% level) heterogeneity of variance was revealed in the organ weight (body weight) data by Bartlett's test, and if the transformed data showed less heterogeneity of variance than the untransformed data. Intergroup comparisons were carried out using Williams' test for a dose-related response.

B. STUDY DESIGN

1. Methodology: On arrival, 148 males and 145 females (up to 4/sex from the same litter were used) were examined for abnormalities/signs of ill health; 6/sex were designated for health check purposes and were sacrificed within 24 hours of arrival and subjected to routine macroscopic examination. Lungs, liver, kidneys, spleen, and heart were preserved. The remaining animals were weighed and, following a 7-day quarantine period, were weighed again and assigned to four groups by computerized stratified randomization to give approximately equal initial mean body weights (avoiding inclusion of litter mates within the same study group. Treatment began following a second 7-day acclimation period. The animals were housed in one room, 4/cage (sexes separately) during the pre-mating period, with male cages interspersed among the female cages to promote development of regular estrous cycles. During the mating period (20 days), one male and one female were housed together in plastic breeding cages. At the end of the mating period, the males were returned to their former cages and cagemates, and the females were housed in individual breeding cages for the birth and rearing of their young. Suitable nesting material was provided. All animals had free access to

diet (Labsure Laboratory Diet No. 2) and tap water.

Exposure of the F0 animals to the test material via the diet began when they were 6 weeks of age and continued for 70 days, at which time they were mated (16 weeks of age). Exposure continued until all litters were weaned. The F0 dams were allowed to rear their young to Day 21 post partum, at which time 24 pups/sex/group were selected to form the basis of the F1 generation. Direct exposure of the F1 animals actually began when the pups started to eat the treated diets; however, the filial generation were considered to start at a synchronized point when selected offspring were approximately 4 weeks of age; following 84 days of exposure (16 weeks of age), the animals were mated; exposure continued until all litters were weaned.

There were 28 rats/sex/group in the F0 generation and 24 rats/sex/group in the F1 generation. The test material was incorporated into the diet at fixed concentrations of 0, 75, 300, and 1200 ppm and fed to both sexes throughout two consecutive generations.

2. Dose preparation: The test material as supplied was weighed out and added to a weighed amount of sieved diet and stirred to give a pre-mix of suitable strength. The dietary concentrations required were obtained from this pre-mix by direct dilution with additional diet and further mixing. The diets were prepared freshly at weekly intervals. It was stated that the dietary inclusion levels remained constant throughout the study. The diets were analyzed for homogeneity and stability, and the achieved concentrations were measured at various (6) intervals during the study.

RESULTS

The mean concentrations of Maneb in the dose formulations analyzed during the study were all within 7% of the nominal concentrations. The diet was shown to be homogeneously mixed and stable for up to 14 days.

Because the dietary levels of Maneb were maintained at constant levels throughout the study, the achieved intake of the test material (mg/kg/day) decreased in the parental animals (both generations) as the animals grew. Also, the F1 animals had higher intakes at the start of treatment than the F0 animals due to their younger age.

Achieved Intakes of Maneb (mg/kg/day)

Week	MALES			FEMALES		
Group*	75	300	1200	75	300	1200
F0 generation						
1	7.9	31.1	121	7.9	31.1	121
2	6.8	27.3	103	7.3	28.6	112
3	6.1	24.2	93	6.8	26.4	104
4	5.4	22.0	83	6.2	24.8	98
5	5.0	20.3	81	6.1	23.9	102
6	4.7	19.1	77	5.8	23.4	105
7	4.4	17.8	71	5.4	21.8	95
8	4.3	17.1	69	5.1	21.4	92
9	4.1	16.5	66	4.9	20.6	86
10	4.0	16.3	66	4.8	19.2	85
Overall	5.3	21.2	83	6.0	24.1	100
F1 generation						
5	10.9	42.2	163	10.5	41.2	157
6	9.2	38.3	149	9.4	36.7	145
7	7.6	30.0	119	7.9	30.4	118
8	6.5	26.0	101	7.0	26.9	109
9	5.6	22.5	87	6.3	25.1	103
10	5.2	20.6	82	6.0	23.3	98
11	4.6	18.5	76	5.6	21.9	98
12	4.3	17.5	72	5.2	20.7	95
13	4.1	16.8	70	5.1	20.2	94
14	3.9	15.6	64	4.8	18.5	88
15	3.8	14.9	63	4.6	18.6	84
16	3.6	14.6	59	4.4	18.0	78
Overall	5.8	23.1	92	6.4	25.1	106

* ppm

3. Parental Investigations

(a) Clinical Observations: All animals were handled regularly and examined for obvious changes or signs of reaction to treatment, with twice-daily checks for morbidity and mortality, once-a-day check for signs of toxicity, and a detailed physical examination at each weighing interval throughout the study. Animals showing marked signs of ill health were sacrificed to prevent cannibalism or autolytic degeneration. All animals dying on test or sacrificed were weighed and subjected to post mortem examination to establish cause of death. A full spectrum of tissues was preserved for possible histopathological examination.

RESULTS

Survival and Clinical Observations: There were 5 deaths during the study: one high-dose F0 female (Week 3) and one high-dose F1 female (Week 9), each considered to be related to treatment. Each was noted to be thin and showing locomotor difficulty, with the F1 animal having paralysis of the right hind limb. Necropsy revealed no other findings. One F0 control dam and one F0 dam of the low-dose group were sacrificed (for humane reasons) after having successfully reared their young to weaning, and one F1 male of the mid-dose group was found dead in Week 15. These latter 3 deaths were not considered to be related to treatment.

Four of the 27 surviving F0 females of the high-dose group showed an emaciated appearance between Weeks 5 and 8, which was considered to be a reflection of the retarded weight gain observed in this group. None of the mid- and low-dose F0 or any F1 parental animals displayed any treatment-related signs.

(b) Food and Water Consumption: Food intake was recorded weekly throughout the pre-mating phases, and resumed on Day 1 post partum for females, continuing to Day 21 post partum. Additionally, during the pre-mating phases, food conversion ratios were calculated. Water consumption was measured on a daily basis during the initial two and final two weeks of the pre-mating treatment periods for each generation.

RESULTS

Pre-mating period: Food consumption was decreased at the high-dose level in both sexes of both generations mostly during the first [F0 4-5; F1 6-7] weeks of the pre-mating periods, and the decrease in overall food consumption values was statistically significant [F0 ♂ 92%/♀ 93% of control (weeks 1-10); F1 ♂ 90%/♀ 89% of control (weeks 5-16)]. An increase in the food conversion ratio was observed in the high-dose females of both generations during the early part of the pre-mating period. There was a dose-related increase in water consumption at the mid- and high-dose levels in both sexes in each generation during the final two weeks prior to mating. Lactation period: For dams that littered, there was a consistent reduction in intake, compared to the controls, at the high-dose level during mid to late lactation (days 7 to 20) in both generations. For F1 females, slight reductions in food intake were observed in the low- and mid-dose dams also.

Food Consumption During Lactation

DAYS/ GROUP•	F0 Food Consumption (g/rat/day)				F1 Food Consumption (g/rat/day)			
	0	75	300	1200	0	75	300	1200
1-6	37	37	38	38	47	46	43	41
7-13	52	52	52	48	59	56	53	51
14-20	64	65	64	60	72	67	65	58

• PPM;

(c) Body Weight: All animals were weighed at the start of each generation (at 6 weeks of age for F0 generation ; at ≈ 4 weeks of age for F1 generation) and at weekly intervals thereafter. During the mating period, all females were weighed daily, and each was weighed daily until parturition. Weights are reported for Days 0 (day of occurrence of a positive indication of mating; i.e., sperm or plug), 7, 14, 17, and 20 of gestation. Weights of pregnant animals without a positive indication of mating are reported for appropriate days taken retrospectively from birth. Dams that littered were weighed on Days 0, 7, 14,

and 21 post partum.

RESULTS

F0 Generation: There was a decrease in body weight observed at the highest dose level in both sexes compared to the control values, with females displaying a larger decrease than the males. The overall body-weight gain was 88% of the control value in the high-dose males and 77% of the control value in the high-dose females.

F0 Generation Group Mean Body Weight

Week	Males (% of Control) Low	Mid	High	Females (% of Control) Low	Mid	High
0	102	101	100	101	103	103
1♦	101	99	96	101	99	94
2♦	99	97	92	100	100	93
3♦	99	99	91	94	93	85
4♦	98	97	90	100	98	86
5♦	98	96	90	100	98	84
6♦	98	97	90	99	97	84
7♦	100	97	90	99	97	86
8♦	100	97	90	100	97	87
9♦	100	97	90	100	98	88
10♦	102	98	91	100	98	87
11♦	100	97	90	99	97	86
12♦	99	97	89	99	96	86
13♦	99	97	89	97	97	88
14♥	101	97	90	100	98	90
15♥	101	97	91	98	97	91
16♥	101	98	92	97	96	91
17♣	101	97	92	99	97	91
18♣	101	97	92	99	97	91
19♣	101	98	92	100	96	90
BWG♦	101	95	84**	99	91	70**
BWG♥♦♣	100	96	88**	99	91*	77**

* p<0.05; ** p<0.01; ♦ pre-mating period; ♦mating period; ♥gestation; ♣lactation
BWG - body-weight gain: BWG♦ for weeks 0-10; BWG♥♦♣ for weeks 0-19

F1 Generation: The high-dose animals displayed a decrease in body weight compared to the control values at the start of the prescribed (84 days) exposure period, which persisted throughout the study. The females showed a larger decrease than the males after the first four weeks of treatment. The overall body-weight gain

was 90% of the control value in the high-dose males and 87% of the control value in the high-dose females.

F1 Generation Group Mean Body Weight (% of Control)

Week	Males (% of Control) Low	Mid	High	Females (% of Control) Low	Mid	High
4♦	100	98	89	97	97	89
5♦	99	97	88	98	97	89
6♦	99	97	89	97	97	89
7♦	99	97	89	98	97	87
8♦	100	97	39	97	97	84
9♦	100	97	88	98	96	81
10♦	101	97	87	99	97	78
11♦	102	98	88	99	96	78
12♦	102	98	88	100	97	80
13♦	102	98	88	99	97	82
14♦	103	99	89	100	97	83
15♦	104	100	90	100	97	84
16♦	104	99	90	100	97	84
17♦	104	99	90	100	98	86
18♦	104	98	89	100	98	85
19♦	104	98	89	100	98	85
20♥	106	99	90	95	95	85
21♥	105	99	90	97	93	84
22♥	105	99	90	100	98	91
23♦	105	99	90	101	98	92
24♦	105	98	90	100	98	91
25♦	106	98	90	100	99	91
26	106	98	90	99	97	88
BWG♦	106	99	91**	102	96	82**
BWG♥♦♦♦	108	99	90*	100	97	87**

* p<0.05; ** p<0.01; ♦ pre-mating period; ♦mating period; ♥gestation; ♦lactation
BWG - body-weight gain: BWG♦ for weeks 4-16; BWG♥♦♦♦ for weeks 4-26

Body weight was decreased throughout gestation at the high-dose level in both generations compared to the control values, with the F1 dams displaying a larger decrease than the F0 dams. Body-weight gain was comparable among the F0 dams during gestation, but was slightly lower in the high-dose F1 dams compared to controls (88%) over the entire gestation period.

Day	F0 Body Weight [gestation] (% of Control)			F1 Body Weight [gestation] (% of Control)		
0	99	97	87	99	95	82
7	98	97	87	100	96	83
14	99	97	87	99	95	84
17	99	97	88	99	96	85
20 BWG 0-20	98 98	97 98	88 92	98 97	95 94	84 88*
Day	**F0 Body Weight [lactation] (% of Control)**			**F1 Body Weight [lactation] (% of Control)**		
0	97	96	87	97	95	82
7	98	98	90	98	94	87
14	98	97	92	96	94	87
21	98	98	94	97	94	90

Throughout the entire lactation period, the high-dose dams of both generations displayed statistically significant increases in body-weight gain compared to their respective controls, but with no comparable increase in food consumption (based on limited food consumption data available).

Body-Weight Change During Lactation

DAY/ GROUP*	F0 BODY-WEIGHT CHANGE (g)				F1 BODY-WEIGHT CHANGE (g)			
	0	75	300	1200	0	75	300	1200
7	8.1	10.5	14.3	19.4**	9.0	12.6	5.5	24.2*
14	16.0	17.4	18.9	31.1**	18.7	16.6	14.6	31.5*
21	-3.7	-1.0	2.4	19.5**	2.9	5.4	-0.3	28.4**

* PPM; *P<0.05; **P<0.01

(d) Pregnancy Rate, Mating Performance, and Gestational Period

The pregnancy rate was determined as the percent of surviving paired females that became pregnant. With regard to mating performance, vaginal smears were taken daily during the 20-day mating period to enable the number of animals that mated on a specific day to be determined in order to (1) detect whether pregnancy was interrupted after mating; (2) detect marked anomalies of the estrus cycle; and (3) determine the median pre-coital time for the group. The gestation period for females that littered was taken as the time between the day of successful mating and the day on which pups were first seen.

RESULTS

F0 Generation: Four of the 28 low-dose females did not become pregnant; 85.7% of the low-dose females and all other females in the other groups became pregnant. The mean pre-coital time

was comparable among the groups (see below). With the exception of one control dam (total resorption), all dams reared their young to weaning. Gestation times were comparable among the groups. F1 Generation: There was a dose-related increase in the number of non-pregnant females (1, 2, 2, 3), although there was an adequate pregnancy rate (95.8, 91.7, 91.7, and 87.0%) in all groups. The mean pre-coital time was slightly longer at the high-dose level compared to the control and all other groups of this generation and all groups of the F0 generation (see below). With the exceptions of one low-dose dam with total resorption and one control dam with total litter loss, all dams reared their young to weaning. Gestation times were comparable among the groups.

Median Pre-coital Time (days)*

Generation/Group	Control	Low	Mid	High
F0	3.0	3.0	2.0	3.0
F1	2.0	3.0	3.0	3.5

*day by which half of the females successfully paired had conceived

(e) Sacrifice and Pathology

Shortly after the pups were weaned, the parental animals were sacrificed and examined macroscopically. The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, thyroid, ovaries, prostate (with seminal vesicles, coagulating gland), testes with epididymides, and thymus (if present). Additionally, a full range of tissues [CHECKED (X)] was preserved for histopathological examination. The uteri of apparently non-pregnant females were examined by the Salewski technique, and the reproductive tract off all apparently infertile animals (both sexes) was examined histologically.

Digestive system		Cardiovasc./Hemat.		Neurologic	
X	Tongue	X	Aorta	X	Brain
X	Salivary glands	X	Heart	X	Periph. nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal column
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		X	Adrenal gland
X	Cecum	X	Kidneys	X	Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	X	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate	Other	
X	Pancreas	X	Seminal vesicle	X	Bone
Respiratory		X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung	X	Cervix	X	All gross lesions
X	Nasal turbinates	X	Vagina		and masses
X	Pharynx				
X	Larynx				

Reproductive tract-associated tissues* (ovaries, pituitary, prostate, seminal vesicles with coagulating gland, testes with epididymides, uterus with cervix, and vagina) and possible target organs** (brain, kidneys, liver, and thyroid) were subjected to microscopic examination as follows:

> * all F0 and F1 adults of the control and high-dose groups and any apparently infertile animals from the other two dose levels; **thyroid and liver from all F0 and F1 adults/all groups and kidneys from F0 adults of the control and high-dose groups. NOTE: no indication of which animals had the brain examined.

RESULTS

Gross Pathology: Enlarged cervical lymph nodes were observed in greater numbers in the mid- and high-dose F0 males and in the high-dose F1 males compared to the control groups. In females, fluid distension of the uterus was increased with increasing dose in both generations. NOTE: Table 21a incorrectly lists the incidence of enlarged cervical lymph nodes for all three treated male groups (incidence reported for low, mid, and high doses: 13, 7, 11, respectively).

Macroscopic Finding/ Generation/Dose group*	F0 Generation				F1 Generation			
	0	75	300	1200	0	75	300	1200
Cervical lymph node - males enlarged	3/28♦	6/28	13/28	12/28	4/24	2/24	4/23	7/24
Uterus - females fluid distension	3/28	3/28	4/28	5/27	3/24	3/24	5/24	6/23

* ppm; ♦ # rats with finding/# examined

Organ Weights: Adults: There was an increase in thyroid weight observed at the highest dose level in both sexes in both generations, which is related to Maneb exposure. In males, there was a dose-related increase in lung weight at the mid- and high-dose levels in both generations, increased liver and kidney weights at the highest dose level in the F0 generation and at the mid- and high-dose levels in the F1 generation, and an increase in testes weight at all dose levels in the F0 generation only. Thymus weight was decreased in the males in both generations, but only the mid- and high-dose F1 males showed statistical significance. In females, a similar increase in thyroid weight was observed at the highest dose level in both generations compared to the control values. Other differences noted from control values were increased liver and kidney weights at the high-dose level in the F0 generation, an increase in lung weight in the high-dose F1 group, and a decrease in brain weight at the high dose level of the F1 generation. Thymus weight (absolute) was decreased (dose-related)

in both generations, but statistical significance was not attained.

MALES (Adults) Organ Weight Data (% Control)

Dose (PPM)	F0 Generation			F1 Generation		
	75 ppm	300 ppm	1200 ppm	75 ppm	300 ppm	1200 ppm
Organ Weight♦						
BRAIN	100 (100)	100 (100)	99 (99)	100 (98)	100 (100)	99 (100)
THYROIDS	108 (108)	113 (114)	147 (151)**	95	99	124**
KIDNEY	103 (102)	103 (105)	101 (106)*	110 (103)	108 (107)*	100 (107)*
LIVER	95 (94)	99 (101)	108 (116)**	111 (101)	109 (108)*	108 (116)**
LUNGS	104 (104)	108 (109)**	110 (115)**	110 (105)	113 (112)**	100 (114)**
THYMUS	106 (105)	90 (92)	86 (92)	98 (92)	83 (82)*	78 (83)*
TESTES	105 (105)*	104 (105)*	103 (105)*	105 (102)	97 (97)	95 (97)
SEM. VES.+ PROST.	95 (94)	96 (96)	93 (95)	96 (90)	95 (94)	86 (90)

♦ ( ) value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p<0.01$; analysis of covariance using final body weight as covariate, followed by Williams' test

FEMALES (Adults) Organ Weight Data (% Control)

Dose (PPM)	F0 Generation			F1 Generation		
	75 ppm	300 ppm	1200 ppm	75 ppm	300 ppm	1200 ppm
Organ Weight♦						
BRAIN	99 (99)	100 (100)	98 (98)	99	98	96**
THYROIDS	96 (97)	100 (103)	142 (152)**	105 (105)	99 (98)	129 (133)**
KIDNEY	99 (101)	99 (104)	99 (113)**	100 (101)	99 (100)	97 (105)
LIVER	97 (98)	98 (103)	103 (117)**	97 (97)	97 (97)	101 (106)
LUNGS	101 (106)	104 (111)	99 (106)	102 (103)	106 (107)	105 (109)*
THYMUS	92 (93)	85 (88)	78 (91)	89 (91)	80 (82)	75 (86)
OVARIES	93 (94)	97 (101)	98 (108)	99 (99)	99 (99)	95 (100)

♦ ( ) value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p<0.01$; analysis of covariance using final body weight as covariate, followed by Williams' test

Histopathology: Adults - A target organ for Maneb is the thyroid. Minimal diffuse follicular epithelial hypertrophy/hyperplasia was displayed in both sexes of the high-dose groups in both generations, which was associated with significantly increased thyroid weights. Mid-dose F1 males also displayed this lesion. Minimal centrilobular hepatocyte enlargement was observed in males at the high-dose level in both generations and was associated with significantly increased liver weights. No comparable lesions were observed in the high-dose females, although liver weights were significantly increased. There was a reduction/absence of spermatozoa in the testes (usually with reduction/absence of spermatozoa in the associated epididymides) observed in 5/24 F1 males at the high-dose level compared with 1/24 in the control. In most cases, the affected testes was reported to be small/blue/flaccid macroscopically. Based on eleven comparable concurrent studies, the incidence is marginally greater than the highest historical control value of the testing laboratory (4/24 F1 males). The author noted that the change in 4 of the 5 affected Maneb males was unilateral and each of these males was found to be fertile at pairing (at about 16 weeks of age). Additionally, no change was observed in the accessory sex organs and no effect was detected in overall mating performance at this dose level.

Microscopic Lesions Observed in Adult Rats

Microscopic Finding/ Generation/Dose group*	F0 Generation				F1 Generation			
	0	75	300	1200	0	75	300	1200
Thyroid - males								
minimal diffuse follicular epithelial hypertrophy/ hyperplasia	0/28	0/28	0/28	16/28	0/24	0/24	2/23	15/24
cystic follicular hyperplasia	0/28	0/28	0/28	1/28	0/24	0/24	0/23	3/24
cystic follicles	0/28	0/28	0/28	1/28	0/24	0/24	0/23	1/24
follicular adenomata	0/28	0/28	0/28	0/28	0/24	0/24	0/23	2/24
Liver - males								
centrilobular hepatocyte enlargement	0/28	0/28	0/28	8/28	0/24	0/24	0/23	4/28
Testes								
reduction/absence spermatogenesis	0/28	0/28	0/28	0/28	1/24	0/24	0/23	5/24
Thyroid - females								
minimal diffuse follicular epithelial hypertrophy/ hyperplasia	0/28	0/28	0/28	13/28	0/24	0/24	0/24	8/23

* ppm; ♦ # rats with finding/# examined

4. Offspring Investigations

(a) All litters: As soon after parturition as possible, the pups were counted, individually identified within the litter by toe amputation, sexed, weighed, and examined for external abnormalities. With minimal nest disturbance, all litters were examined daily for dead and/or abnormal pups. F0 Generation - Litters were standardized [random basis, using a list of numbers (1 to 20) generated by computer in random order for each litter] where possible, to 4 pups/sex/litter at Day 4 post partum. Excess pups were sacrificed and examined macroscopically. For one pup/sex/litter (selected on the basis of median body weight), specified organs (see below) were weighed and tissues preserved for possible histopathological examination. Surviving pups were weighed on Days 4, 8, 12, and 21 post partum. At Day 21 post partum, 24 pups/sex/group were selected (no details) to form the basis of the F1 generation. On or shortly after Day 21, excess pups were sacrificed and examined macroscopically, with any abnormal tissue being preserved. Sex of each pup was confirmed by gonadal inspection. F1 Generation - Same as above, except at Day 21 post partum, one pup/sex in each of the 24 litters per group were retained for further study. On or shortly after Day 21, all other pups were sacrificed and examined macroscopically and handled as above for excess pups. NOTE: It is not stated when the pups selected for further study (post weaning development) were sacrificed.

Formulae used in assessing litter parameters for each litter reared to weaning:

(1) pre-birth loss: $\frac{\text{(\# of implantations - total \# young at birth}}{\text{\# of implantations}} \times 100$

(2) pup loss at birth: $\frac{\text{total \# young at birth - \# live young}}{\text{total \# young at birth}} \times 100$

(3) pup loss at Day 4: $\frac{\text{total \# young at birth - \# live young at Day 4*}}{\text{total \# young at birth}} \times 100$

(4) cumulative pup loss: $\frac{\text{total \# young at Day 4♦ - \# live at Day x♥}}{\text{total \# young at Day 4O}} \times 100$

* pre-cull; ♦ post-cull; ♥ Day 8, 12, or 21

RESULTS

There were no apparent adverse effects of treatment on implantation rate, litter size, or pup mortality in either generation. NOTE: Although statistical significance was attained at the high-dose level for the decreased number of implants in the F1 generation, the mid-dose showed a lower number, which was not significant, and the number of implants at the high-dose was comparable to those observed in the F0 generation. Litter weight was comparable among the groups at birth and on Days 4 and 8 post partum in both generations. A significant decrease in both mean pup and litter weights was observed at the high-dose level in the F1 generation on Day 12 post partum and in both generations at the high-dose level on Day 21 post partum compared to control values.

F0 LITTER DATA

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
# implants	13.6	13.6	14.1	13.1
Pre-birth loss (%)	11.9	10.3	9.6	6.1
litter size	12.0	12.0	12.6	12.3
% pup loss	0.2	1.8	1.1	0.9
litter weight	73.7	74.0	80.6	77.2
pup weight	6.3	6.3	6.5	6.4
Day 4 litter size	11.7	12.0	12.5	12.2
cum. loss to Day 4	2.4	2.0	1.8	1.4
cum. loss Day 4-21	0	1.2	0	0
litter wt pre-cull	113.9	120.5	124.2	119.6
LITTER SIZE				
Day 4 pre-cull	7.5	7.9	8.0	7.9
Day 8	7.5	7.9	8.0	7.9
Day 12	7.5	7.8	8.0	7.9
Day 21	7.5	7.8	8.0	7.9
LITTER WEIGHT				
Day 4	74.8	81.4	80.9	77.9
Day 8	139.8	151.4	154.5	144.0
Day 12	214.9	230.5	233.0	218.3
Day 21	427.5	446.9	449.3	412.5* (96)♦
MEAN PUP WEIGHT				
Day 4	10.2	10.3	10.1	10.0 (98)
Day 8	18.8	19.3	19.3	18.5 (98)
Day 12	28.9	29.5	29.1	27.9 (96)
Day 21	57.4	57.2	56.2 (98)	52.8* (92)

F1 LITTER DATA

PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
# implants	14.8	15.1	13.5	13.7*
Pre-birth loss (%)	8.5	8.4	13.0	6.7
litter size	13.7	14.0	13.3	13.0
% pup loss	0.9	0.9	0	0.4
litter weight	81.4	80.6	81.5	77.9 (96)
pup weight	6.0	5.9	6.3	6.1
Day 4 litter size	13.5	13.5	13.1	12.4
cum. loss to Day 4	2.6	3.7	1.3	5.1
cum. loss Day 4-21	1.1	1.2	0	2.6
litter wt pre-cull	131.0	126.7 (97)	132.2	118.4 (90)
LITTER SIZE				
Day 4 pre-cull	8.0	7.9	7.7	7.9
Day 8	7.9	7.8	7.7	7.7
Day 12	7.9	7.8	7.7	7.7
Day 21	7.9	7.8	7.7	7.7
LITTER WEIGHT				
Day 4	78.7	75.3 (96)	79.5	76.1 (97)
Day 8	146.9	139.7 (95)	144.0	137.2 (93)
Day 12	228.4	218.9 (96)	224.6	205.2** (90)
Day 21	447.8	436.2 (97)	428.9 (96)	391.3** (87)
MEAN PUP WEIGHT				
Day 4	9.9	9.6	10.5	9.6 (97)
Day 8	18.7	17.8 (95)	18.9	17.6 (94)
Day 12	29.1	27.9 (96)	29.4	26.4** (91)
Day 21	57.1	55.6 (97)	56.0	50.7** (89)

♦ (% of control value); * $p<0.05$; ** $p<0.01$

(b) During the pre-weaning period, all offspring were examined to determine the age at which the following developmental stages were attained.

(1) Surface-righting reflex: from Day 1 post partum to 100% success. The reflex was tested by placing the pup on its back on a flat surface; reflex considered present if the pup righted itself within ≈ 2 seconds.
(2) Startle reflex: from Day 11 to 100% success. The reflex was tested by sounding a buzzer held 15 cm from the ears of the pup; reflex considered present if pup jerked its hind limbs at the sound.
(3) Air-righting reflex: from Day 14 to 100% success. The reflex was considered present if the pup was able to land on its feet when dropped onto a cushioned surface from a height of 31 cm.
(4) Pupil reflex: once, on Day 20. The reflex was considered present if pupil contraction occurred when a light was shone into the eyes.

RESULTS

In both generations, the startle response was delayed slightly in the mid- and high-dose pups. All other parameters were comparable among the groups in both generations.

Mean Age (days post partum) for Attaining Startle Response

Generation/Group	Control	Low	Mid	High
F0	34.1	34.1	34.5*	34.5**
F1	34.0	34.1	34.3*	34.5**

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

(c) Indices of Sexual Maturation: The onset of vaginal opening was monitored in all females from 28 day post partum, and the occurrence of the balanopreputial skinfold was monitored in all males from 35 days post partum. The prepuce of each male, which is initially fused to the glans penis, was examined daily until the prepuce could be fully retracted.

RESULTS

There was a slight delay in the mean age of occurrence of vaginal opening in the F1 high-dose females, which the authors considered likely to reflect delayed physical development due to the treatment-related body-weight gain effect rather than as indicative of Maneb specifically interfering with mechanisms controlling sexual maturation in females (see table below). The age of occurrence of vaginal opening in the mid- and low-dose females was comparable to the control. There was no clear effect of treatment on the mean age of occurrence for balanopreputial skinfold cleavage in F1 males.

Number of F1 Females* Showing Vaginal Opening on Day "X" Post Partum

Group (ppm)/ Day "X"	28	29	30	31	32	33	34	35	36	37	38	39	Mean age (days)
0	1	-	1	3	7	4	3	4	-	1	-	-	32.8
75	-	1	2	5	4	6	2	2	1	-	1	-	32.6
300	1	1	-	6	7	3	2	2	-	-	1	1	32.5
1200	-	-	-	-	6	-	6	1	3	3	4	1	35.0

* N=24 for each group

Number of F1 Males Showing Balanopreputial cleavage on Day "X" Post Partum

Dose (ppm)/ Day "X"	38	39	40	41	42	43	44	45	46	Mean Age (days)
0	-	2	3	6	3	6	4	-	-	41.8
75	1	2	4	8	3	4	-	1	1	41.4
300	-	1	2	8	6	2	4	1	-	41.9
1200	-	-	3	7	3	5	3	1	2	42.4

* N=24 for each group

(d) Terminal studies: The following organs were weighed: adrenals, brain, heart, kidneys, liver, lungs, thyroid, ovaries, prostate (with seminal vesicles, coagulating gland), testes with epididymides, and thymus (if present) and preserved. The carcass was preserved with all other tissues in situ.

RESULTS

There were no macroscopic lesions observed that could be attributed to exposure to Maneb. Although an increase was observed in thyroid weight in the high-dose animals (both sexes/both generations), statistical significance was not attained. There was a dose-related decrease in thymus weight in F0 males at the mid- and high-dose levels and a small increase in kidney weight in this group compared to the control value. F0 females displayed dose-related increases in liver and kidney weight, which were statistically significant at the mid- and high-dose levels, and increased ovarian weights at these dose levels. Although the increase in ovarian weight is not dose-related, it may be treatment-related. There was a dose-related increase in liver weight, which was statistically significant at all dose levels, in both sexes of the F1 generation. F1 males displayed a dose-related increase in testes weight (significant only at the high dose) and a decrease in heart weight at the high-dose level.

MALES (Offspring at Weaning) Organ Weight Data (% Control)

Dose (PPM)	F0 Generation			F1 Generation		
	75 ppm	300 ppm	1200 ppm	75 ppm	300 ppm	1200 ppm
Organ Weight•						
BRAIN	100 (100)	99 (100)	98)101)	99 (99)	99 (99)	99 (101)
THYROIDS	113 (113)	90 (90)	111 (116)	107 (94)	107 (103)	106 (111)
KIDNEY	97 (98)	101 (103)	95 (104)*	99 (102)	99 (100)	93 (102)
LIVER	100 (101)	101 (99)	98 (96)	102 (106)*	105 (107)*	104 (118)**
LUNGS	96 (97)	99 (101)	93 (101)	95 (97)	88 (88)	86 (95)
THYMUS	102 (98)	88 (86)*	97 (84)*	98 (100)	91 (92)	84 (92)
TESTES	99 (101)	101 (103)	92 (103)	96 (100)	101 (106)	102 (112)*
HEART	99 (101)	99 (101)	90 (97)	92 (96)	113 (99)	87 (86)**

• ( ) value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p<0.01$;

FEMALES (Offspring at Weaning) Organ Weight Data (% Control)

Dose (PPM)	F0 Generation			F1 Generation		
	75 ppm	300 ppm	1200 ppm	75 ppm	300 ppm	1200 ppm
Organ Weight•						
BRAIN	100 (101)	99 (101)	97 (100)	100 (100)	101 (101)	99 (101)
THYROIDS	88 (92)	78 (84)	104 (120)	93 (96)	95 (96)	95 (96)
KIDNEY	99 (101)	102 (105)**	96 (109)**	97 (101)	100 (102)	92 (103)
LIVER	102 (104)	104 (109)**	99 (113)**	101 (106)*	107 (110)**	108 (115)**
LUNGS	90 (91)	94 (97)	85 (95)	94 (96)	92 (94)	86 (89)
THYMUS	96 (97)	91 (94)	85 (95)	106 (92)	97 (99)	89 (98)
OVARIES	107 (109)	121 (125)*	111 (122)*	101	109	92
HEART	99 (100)	99 (102)	90 (99)	96 (99)	102 (104)	91 (100)

• ( ) value adjusted for body weight; relates to organs where the within-group relationship to body weight was significant at $p<0.01$;

C. DISCUSSION

Evidence of maternal toxicity included two deaths in females at the high-dose level, decreased body weight/gain at the high dose and a decrease in overall body-weight gain at the mid dose, and an increase in thyroid weight at the high dose in both generations. Additionally, there were increases in thyroid, kidney, liver (both sexes of F0 and F1 males at the high dose), and lung (mid- and high-dose F0 and F1 males and high-dose F1 females) weights, decreases in thymus weight in the mid- and high-dose F1 males and in brain weight in the high-dose F1 females. The F0 males at all dose levels displayed significant (and comparable) increases in testes weight. Thyroid lesions were observed at the high-dose level in both sexes in both parental generations, which correlate with the increased thyroid weights. There were no effects observed on pregnancy rate, gestation time, implantation rate, litter size, pup mortality, or the ability to rear young to weaning in either generation. There was an apparent increase in the median pre-coital time at the high-dose level in the F1 generation. Litter and pup weight were unaffected until Day 12 post partum at the high-dose of the F1 generation and Day 21 post partum at the high-dose in both generations, at which time both were significantly below control values. In both generations, there was a slight delay in the startle response at the mid- and high-dose levels, and a delay in the onset of vaginal opening in the F1 high-dose female offspring. Similar organ-weight effects as observed in the parental animals were observed in the offspring in both generation, but there was no no-effect level for

liver weight increase for either sex in the F1 generation.

D. CONCLUSION

Under the conditions of the study, exposure to Maneb via the diet during pre-mating (10 weeks F0, 12 weeks F1) and through gestation and lactation, at dose levels of 0, 75, 300 and 1200 ppm, produced some maternal toxicity at the high-dose level. Food consumption and body weight were decreased at the high-dose level in both sexes of both generations. Overall body-weight gain was decreased at the high dose in both generations (both sexes) and in the mid-dose F0 females. There was no effect demonstrated on pregnancy rate, gestation times, or the ability to rear young to weaning, but the pre-coital time was slightly longer in the high-dose F1 dams compared to the concurrent control value and all other groups in both generations. Various organ weights (thyroid, liver, kidney, lung, testes) were affected by treatment, but a no-effect level was demonstrated for each except for the F0 testes weight. There was no effect of treatment on the implantation rate, litter size, or pup mortality in either generation. Litter and pup weights were comparable at birth among the groups in both generations, but there was a decrease observed in both in the F1 generation at Day 12 post partum and in both generations at Day 21 post partum. In both generations, there was a slight delay in the startle response of the offspring at the mid- and high-dose levels, and the onset of vaginal opening was delayed in the F1 high-dose female offspring. There were various organ-weight effects, which were similar to those observed in the parental animals, although no no-effect level was observed for the increase in liver weight for either sex in the F1 offspring.

The NOEL for maternal toxicity can be set at 75 ppm (≈ 6 mg/kg/day), the LEL at 300 ppm (≈ 25 mg/kg/day), based on decreased body weight/body-weight gain and food consumption. A NOEL for reproductive effects can be set at 75 ppm, the LEL at 300 ppm, based on a slight delay in the startle response in the offspring at the mid- and high-dose levels in both generations. The NOEL for the effect of Maneb on the testes weight of the adult males would be assessed more appropriately in a 90-day or chronic toxicity study. The NOEL for effects on the offspring can be set at 75 ppm, the LEL at 300 ppm, based on the delay of the startle response. Although there were increased liver weights at all dose levels in the F1 offspring of both sexes, the magnitude of the response between the dose levels was not comparable to the magnitude of the increase in the dose and is not viewed as biologically significant. This study satisfies the guideline requirements (83-4) for a 2-generation reproduction study.

Discrepancies noted in report: (1) On pages 78/79 (Tables 14a/14b), the mean age for attaining the parameter is listed as "days post coitum". This should read days post partum, according to the procedures listed on page 24 of the report. (2) On page 90 (Table 21a), the numbers of treated F0 adult males displaying enlarged cervical lymph nodes are incorrect (see above under Gross Pathology). (3) On page 33, under (f) Bodyweight change, it states that "a slight effect also being apparent for males receiving 300 ppm." This should read females, according to the data presented in Table 7a on page 82.