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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: **MANEB** - Developmental Toxicity Study in Rats

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

FROM: Linda L. Taylor, Ph.D Linda Taylor 1/28/93
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 2/1/93
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 2/2/93
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: ELF ATOCHEM North America, Inc.
Chemical: manganese ethylene-1,2-bisdithiocarbamate
Synonym: Maneb
Caswell No.: 539
Case No.: 818618
Submission No.: S428318
Identifying No.: 014505
DP Barcode: D184070
MRID No.: 425200-01

Action Requested: Please review the maneb terato. -rat data for GLN 83-3A (MRID 42520001). Please send a copy of the review and an updated **status** report for the tox. requirements for maneb.

Comment: A new rat developmental toxicity study has been performed on Maneb, since the Registrant was unable to obtain supporting information to upgrade the previous three studies [MRID # 419658-01 (TB II cover memo dated 1/15/92; DER dated 1/6/92). The new study has been reviewed and the DER is appended.

Under the conditions of the study, oral administration (via gavage) of Maneb at dose levels of 0, 20, 100, and 500 mg/kg to pregnant rats from Day 6 through Day 15 of gestation resulted in a decrease in body weight, body-weight gain, food consumption and an increase in the occurrence of clinical signs (soft stool) in the maternal

animals. Developmental toxicity was observed at the mid- and high-dose levels, as evidenced by the dose-related increase in postimplantation loss and a decrease in the numbers of viable fetuses. At the high-dose level, there was a decrease in fetal weight that exceeded both the concurrent and historical control values, and one skeletal malformation and several skeletal variations occurred that also exceeded both the concurrent and historical control values. The NOEL for maternal toxicity can be set at 20 mg/kg, the LEL at 100 mg/kg, based on decreased body weight/gain and food consumption. The NOEL for developmental toxicity can be set at 20 mg/kg, the LEL at 100 mg/kg, based on increased postimplantation losses and decreased fetal viability.

This study is classified Core Minimum, and it satisfies the guideline requirement (83-3) for a developmental toxicity study in rodents.

With regard to an update of the toxicology requirements for Maneb, TB II has provided you with several updates (TB II memos dated 2/13/92, 3/2/92, 5/29/92, 11/25/92); please refer to the TB II review memos sent to you on each of the studies that have been submitted to the Agency, transmitted by you to TB II for review. Each review has been forwarded to you with the classification of each study. Additionally, the TB II memo dated 11/25/92 informed you of a new data requirement, which was discussed with you and the Registrant in a meeting held 11/18/92.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 1/28/93
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 2/1/93
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity rat TOX. CHEM. NO.: 539

MRID NO.: 425200-01 Shaughnessy #: 014505

TEST MATERIAL: Maneb Technical

SYNONYMS: Manganese ethylene-1,2-bisdithiocarbamate

STUDY NUMBER: WIL-134011

SPONSOR: Maneb Registration Group, c/o NPC, Inc., Sterling, VA

TESTING FACILITY: WIL Research Laboratories, Inc., Ashland, OH

TITLE OF REPORT: A Developmental Toxicity Study of Maneb Technical in Rats

AUTHORS: M.D. Nemec

REPORT ISSUED: June 26, 1992

QUALITY ASSURANCE: A quality assurance statement was provided.

CONCLUSIONS: Under the conditions of the study, administration of Maneb at dose levels of 0, 20, 100, and 500 mg/kg to pregnant rats from Day 6 through Day 15 of gestation resulted in a decrease in body weight, body-weight gain, food consumption and an increase in the occurrence of clinical signs (soft stool) in the maternal animals. Developmental toxicity was observed at the mid- and high-dose levels, as evidenced by the dose-related increase in postimplantation loss and a decrease in the numbers of viable fetuses. At the high-dose level, there was a decrease in fetal weight that exceeded both the concurrent and historical control values, and one skeletal malformation and several skeletal variations occurred that also exceeded both the concurrent and historical control values. The NOEL for maternal toxicity can be set at 20 mg/kg, the LEL at 100 mg/kg, based on decreased body weight/gain and food consumption. The NOEL for developmental toxicity can be set at 20 mg/kg the LEL at 100 mg/kg, based on increased postimplantation loss and decreased fetal viability.

Classification: Core Minimum. This study satisfies the guideline requirement (83-3) for a developmental toxicity study in rodents.

A. MATERIALS

1. Test Compound: Maneb, technical; Description: buff colored powder; Batch #: SR 798-19; Purity: 90.4%; Source: Atochem, North America, Bryan, TX. Vehicle: carboxymethylcellulose; Description: white powder, medium viscosity; Batch #: Lot # 116F-0231; Source: Sigma Chemical Co., St. Louis, MO.

2. Test Animals: Species: rat; Strain: Sprague-Dawley Crl:CD®BR; Age: sexually mature, ♀♀ ≈ 9 weeks old on receipt, ♂♂ ≈ 13 weeks old (for breeding only); Weight: virgin females: 175-224 g; Source: Charles River Breeding Laboratories, Inc., Portage, MI.

3. Statistics: All analyses were conducted using 2-tailed tests (minimum significance level of $p<0.05$), comparing each treated group to the vehicle control group. Statistical tests were performed by a Digital® MicroVAX 3400 computer, with appropriate programming. Fetal sex ratios: Chi-square test with Yates' correction factor; Malformations and variations: Fisher's Exact test; Early and late resorptions, dead fetuses, postimplantation losses: Mann-Whitney U-test; Corpora lutea, total implantations, viable fetuses, fetal body weights, maternal body weight and weight changes, maternal net body weight changes, gravid uterine weights, maternal food consumption: One-way ANOVA with Dunnett's test; Litter proportions of intrauterine data (considering the litter, rather than the fetus, as the experimental unit): Kruskal-Wallis test.

B. STUDY DESIGN

1. Methodology: Following a 12-day acclimation period, each female considered appropriate [in good health and within acceptable body-weight requirements (≥ 220 grams)] was placed with a resident male (length of time not stated; 1 ♂:1 ♀) from the same strain and source as the female. Following positive evidence of mating (confirmed by the presence of a copulatory plug in the vagina or the presence of sperm in a vaginal smear; day designated as Day 0 of gestation), the pair was separated. The mated females were consecutively assigned in a block design to one of four groups containing 25 rats each by the following randomization procedure: the first mated female was assigned to group 1, the second to group 2, the third to group 3, etc. This process was continued daily until 25 females were placed into each group.

Each female was housed individually until paired with a male and then after confirmation of mating; nesting material was not provided because the dams were sacrificed prior to expected delivery. Throughout the study, the females had free

access to feed (Purina® Certified Rodent Chow® #5002) and water (automatic watering system) ad libitum.

The dams (25/group) were administered either the test material or vehicle once daily via gavage for ten consecutive days beginning on gestation Day 6 and continuing through Day 15 (see Table 1, below). The dosing volume was 10 mL/kg for all groups, and the individual dosages were based on the most recent body weight.

Table 1. Group/Dose Levels

Group #	Dose level (mg/kg/day)	Dose concentration (mg/mL)
1	0	0
2	20	2
3	100	10
4	500	50

Dose preparation: An appropriate amount of test material was weighed out and triturated with a small amount of vehicle (0.5% aqueous carboxymethylcellulose) until a slurry was obtained. Additional vehicle was added to achieve the appropriate concentration for each group. The preparations were made daily, homogenized to reduce particle size, and stirred during the sampling and dosing procedures. Samples of the test material preparations were analyzed for homogeneity, concentration, and stability pre-test, and for concentration at ≈ the beginning, middle, and end of the study.

RESULTS

Because the dose preparations were not stable for the 10-day storage period examined, the preparations were made daily, ≈ one hour prior to dosing. The suspensions were said to have met the criteria for homogeneity. The mean concentrations and percent of the target dose levels attained are listed below. The low Day 0 values could not be explained. Several preparations were analyzed for ethylene thiourea (ETU) at various times after preparation, and the concentrations increased significantly over time, with levels detected after only one hour.

Table 2. Mean Concentrations [mg/mL] and (% Target Dose Recovered)

Study Phase Analyzed	Low Dose	Mid Dose	High Dose
pre-test	1.69 (84.3)	8.83 (88.3)	48.6 (97.3)
Day 0	1.31 (65.5)	7.01 (70.1)	40.8 (81.6)
mid-study	1.57 (78.5)	8.97 (89.7)	46.2 (92.3)
study end	1.93 (96.5)	8.00 (80.0)	50.0 (100)

Clinical Observations

Each dam was examined for moribundity and mortality twice daily, and detailed clinical signs were recorded for each dam (prior to test material administration during the dosing period) from Day 0 through 20 of gestation. Additionally, each dam was observed for signs of toxicity ≈ 1, 2, and 4 hours after each dosing. Individual maternal body weights were recorded on Days 0, 6-16, and 20 of gestation. Gravid uterine weight, net body weight (Day 20 body weight - weight of uterus and contents), and net body-weight change (Day 0-20 body-weight change - the weight of the uterus and contents) were recorded. Individual food consumption was recorded on gestation Days 0, 6-16, and 20. Animals dying on test were necropsied, but fetal findings for these dams were not recorded. **Cesarean Section:** On Day 20, all surviving dams were sacrificed, and the thoracic, abdominal, and pelvic cavities were opened and examined. The uterus and ovaries were removed, and the number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. Only those maternal tissues displaying gross findings were preserved for possible histopathological examination. Uteri with no macroscopic evidence of nidation were excised, opened and placed in 10% ammonium sulfide solution for the detection of early implantation loss (Sallewski, 1964). Uterine data were summarized using two methods of calculation: (1) group mean litter basis and (2) proportional litter basis. Examples:

$$(1)\ \text{postimplantation loss/litter} = \frac{\#\ \text{dead fetuses, resorptions (early/late)/group}}{\#\ \text{gravid females/group}}$$

$$(2)\ \text{summation per group (5)} = \frac{\text{postimplantation loss/litter (5)}^{*}}{\#\ \text{of litters/group}}$$

$$^{*} = \frac{\#\ \text{dead fetuses, resorptions (early/late)/litter}}{\#\ \text{implantation sites/litter}} \times 100$$

Fetal Morphological Examination: Each fetus was sexed, weighed, tagged for identification (dam # and fetus #), and a detailed external examination of each was conducted of at least the eyes, palate, and external orifices. Crown-rump measurements were recorded for late resorptions and the tissues were discarded. Each fetus was examined viscerally (modification of Stuckhardt and Poppe fresh dissection technique, 1984), which included the heart and major vessels. The sex of each fetus was verified by an internal examination. Fetal kidneys were examined and graded for renal papillae development (Woo and Hoar, 1972). Heads from ≈ one-half of the fetuses from each dam were placed in Bouin's fixative for subsequent soft-tissue examination (Wilson sectioning technique, 1965). The heads of the remaining one-half of the fetuses were examined by a mid-coronal slice. All carcasses

were eviscerated and fixed in 95% ethyl alcohol. Following fixation, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S (Dawson, 1926). The skeletal examination was conducted utilizing low power magnification via a stereomicroscope. External, visceral, and skeletal findings were recorded as developmental variations or malformations. The fetal developmental findings were summarized by (1) presenting the incidence of a given finding both as a % of the # of fetuses and the # of litters available for examination in the group and (2) by considering the litter as the basic unit for comparison and calculating the # of affected fetuses in a litter on a proportional basis as follows:

$$\text{summation per group (\%)} = \frac{\text{viable fetuses affected/litter (\%)}^{*}}{\text{\# of litters/group}}$$

$$^{*} = \frac{\text{\# viable fetuses affected/litter}}{\text{\# viable fetuses/litter}} \times 100$$

C. RESULTS

Clinical Observations and Survival - Maternal: No deaths occurred, but one high-dose dam was euthanized on Day 16 of gestation for humane reasons. Only dams in the high-dose (500 mg/kg/day) group displayed neurobehavioral clinical signs, the primary findings being impaired mobility (all dams), dragging of the hindlimbs (80%), hunched posture (76%), unkempt appearance (72%), excessive chewing (60%), and prostration (16%). The first signs were observed on Day 11 of gestation and increased in incidence with time during dosing. In nearly half of the dams, these persisted throughout the study. Other clinical signs noted in the high-dose dams were soft stool (96%) and decreased defecation (72%). Two high-dose dams displayed paleness and body cool to the touch, and red material on the forelimbs, eyes, and nose, and red and yellow staining on the urogenital area were slightly increased at the high dose compared to the control and are considered signs of maternal toxicity. At the mid-dose level, soft stool was the only clinical sign observed. The low-dose and control dams displayed clinical findings of a similar kind and at a similar frequency.

Maternal Body Weight and Body-Weight Gain: From Day 7 on, the high-dose dams displayed a statistically significant decrease in body weight compared to the control value, with the magnitude of the decrease increasing with each subsequent dose (Table 3). The mid-dose dams also displayed decreased body weight (statistically significant from Day 11 on), but the magnitude of the decrease was small (94-99% of the control value). The high-dose dams also displayed decreased body-weight gains throughout the study compared to the control values, and the mid-dose dams displayed lower gains during the dosing period and overall (Table 4).

Table 3. Maternal Body Weights (% of control)

Day/Dose	20 mg/kg	100 mg/kg	500 mg/kg
0	101	99	100
6	100	98	99
7	100	97	97*
8	99	97	95**
9	99	97	94**
10	100	97	94**
11	99	96*	92**
12	99	96*	91**
13	99	95*	88**
14	99	95*	85**
15	99	95**	80**
16	98	94**	76**
20	98	94*	78**

Table 4. Maternal Body-weight Gains (grams)

Interval/dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
0-6	34	30	31	31
6-7	1	1	-1	-5**
7-8	6	6	5	0**
8-9	5	4	3	3
9-10	3	5	3	4
10-11	7	5	5	1**
11-12	4	5	4	-1**
12-13	4	3	2	-5**
13-14	4	6	4	-5**
14-15	9	7	6	-7**
15-16	8	8	6	-6**
16-20	61	60	59	51
6-9	11	10	8	-3**
9-12	15	14	11	4**
12-16	25	23	18	-23**
6-16	51	47	36*	-23**
0-20	147	138	127*	61**

Terminal body weight was significantly decreased at the mid- and high-dose levels (dose-related). The high-dose dams displayed a mean net body-weight loss, decreased mean gravid uterine weight, and decreased net body weight. The mid- and low-dose levels dams also displayed slightly lower values for these parameters, but statistical significance was not attained (Table 5).

Table 5. Mean Net Body Weight/Gains and Gravid Uterine Weight (g)

Parameter/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Initial body weight	235	237	233	236
terminal body weight	382	375	360*	297**
% body weight change+	63	58	55	26
gravid uterine weight	80	77	72	63**
net body weight	302	298	288	234**
net body weight change	67	61	55	-2.0**

+ statistics not performed; * $p<0.05$; ** $p<0.01$

Food Consumption: Food consumption was decreased throughout the study at the high-dose level (on both a grams/animal and grams/kg basis) and throughout most of the study at the mid-dose level (on a grams/animal basis), compared to the control values. At the mid-dose level, a significant decrease in food consumption on a grams/kg basis was displayed during the first 3-day interval of dosing and during the entire dosing interval. The low-dose values were comparable to those of the control.

Table 6. Food Consumption During Gestation

Interval/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
grams/dam/day				
0-6	22	21(95)•	22(100)	22(100)
6-7	22	22(100)	20*(91)	17**(77)
7-8	23	22(96)	20*(87)	15**(65)
8-9	23	22(96)	21(91)	17**(74)
9-10	22	23(105)	20*(91)	18**(82)
10-11	24	22(92)	21*(88)	16**(67)
11-12	24	23(96)	22(92)	16**(67)
12-13	24	23(96)	21*(88)	13**(54)
13-14	24	23(96)	21*(88)	13**(54)
14-15	23	22(96)	21(91)	10**(43)
15-16	25	23(92)	22*(88)	9**(36)
16-20	26	25(96)	25(96)	18**(69)
6-9	23	22(96)	20**(87)	16**(70)
9-12	23	23(100)	21*(91)	16**(70)
12-16	24	23(96)	21*(88)	11**(46)
6-16	23	23(100	21*(91)	14**(61)
0-20	23	23(100)	22*(96)	17**(74)
grams/kg/day				
0-6	88	84(95)	87(99)	86(98)
6-7	82	81(99)	76(93)	63**(77)
7-8	83	82(99)	76(92)	58**(70)
8-9	84	81(96)	76(90)	64**(76)
9-10	79	82(104)	74(94)	66**(84)
10-11	82	78(95)	75(91)	59**(72)
11-12	81	79(98)	76(94)	59**(73)
12-13	79	77(97)	72(91)	50**(63)
13-14	79	78(99)	74(94)	47**(59)
14-15	76	73(96)	71(93)	37**(49)
15-16	78	74(95)	72(92)	33**(42)
16-20	74	73(99)	76(103)	65**(88)
6-9	83	81(98)	75*(90)	61**(73)
9-12	81	80(99)	75(93)	61**(75)
12-16	78	76(97)	73(94)	43**(55)
6-16	80	78(98)	75*(94)	54**(68)
0-20	80	78(98)	78(98)	66**(83)

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

Gross Pathological Observations

Maternal Observations: On gestation Day 16, one high-dose dam was sacrificed in extremis. Several dark red areas in the stomach were noted, as were dark contents in the ileum and stomach and yellow contents in the cecum. Eighteen normally-developing implantations were observed in utero. In the ovaries, the number of corpora lutea was 24 (14 left/10 right. Two other high-dose dams had dark red fluid/contents in the uterus (both horns) at scheduled sacrifice; each had 6 late

resorptions observed in utero. There were no other treatment-related findings.

Cesarean Section Observations

A summary of the observations is provided in Table 7 below. The number of pregnant females with offspring at termination was not adversely affected by treatment. The numbers of corpora lutea, implantation sites, and live fetuses were comparable among the groups. There were no dead fetuses. The total number of resorptions was increased (dose-related) at the mid- and high-dose levels, and the number of resorptions per dam was also increased at these two dose levels compared to the control. Additionally, the number and percent of litters with resorptions were increased at the mid- and high-dose levels compared to the control, but the mid-dose group displayed the greater increase. On a proportional basis, the % of early resorptions was significantly increased at the mid-dose level compared to the control value and, although the high-dose was also increased compared to the control, the increase was less than that at the mid dose. The % of late resorptions at the high-dose level exceeds both the concurrent (0.3%) and historical (3.2%) control values. With regard to crown-rump length, the one late resorption in the control group measured 3.1 cm; the greatest length in the 13 late resorptions in the high-dose group was 2.3 cm (average 1.8 cm). Post-implantation losses were increased at the mid- and high-dose levels (dose-related), with concomitant decreases (dose-related) being displayed in the % of viable fetuses at these dose levels.

Mean fetal weight (both sexes together and separately) was decreased at the high-dose level compared to the concurrent and historical control data [only data on combined sexes was provided for the latter]. Fetal sex ratios were not affected by treatment.

Table 7: Cesarean Section observations*

GROUP:	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
#Animals Assigned	25	25	25	25
#Animals Mated	22	24	26	25
Pregnancy Rate (%)	88	96	96	100
Maternal Wastage				
#Died	0	0	0	1
#Died/pregnant	0	0	1	1
#Non pregnant	3	1	1	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	372	402	388	417
Corpora Lutea/dam	16.9	16.8	16.2	17.4
Total Implantation	340	360	356	372
Implantations/Dam	15.5	15.0	14.8	15.5
Total Live Fetuses	324	345	324	331
Live Fetuses/Dam	14.7	14.4	13.5	13.8
Mean Litter %	95.4	96.1	90.9*	98.7*
% Viable				
Total Resorptions	16	15	32	41
Early (%)	15 (4.3)	15 (3.9)	32 (9.1)*	28 (7.7)
Late (%)	1 (0.3)	0	0	13 (3.6)
Resorptions/Dam	0.73	0.63	1.33	1.71
Total Resorptions (%)	4.6	3.9	9.1*	11.3*
Litters w/ resorptions/ total # litters (%)	11/22 (50)	9/24 (38)	19/24 (79)	17/24 (71)
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Total Dead Implants	16	15	32	41
Dead Implants/Dam	0.73	0.63	1.33	1.71
Mean Fetal Weight (gm)	3.5	3.5	3.5	2.9**
Mean ♂♂ Fetal Weight (gm)	3.6	3.5	3.6	3.0**
Mean ♀♀ Fetal Weight (gm)	3.4	3.4	3.3	2.8**
Mean Fetal Length#				
# of Runts	4.6	3.9	9.1*	11.3*
Postimplantation Loss(%)	54	53	59	51
Sex Ratio (% Male)	53.7	50.5	53.4	50.4
Preimplantation Loss(%)				

Fetal Observations: The numbers of fetuses and litters available for evaluation, as well as the numbers of fetuses and litters with malformations, are listed in Table 8, below.

Table 8. Number of Fetuses and Litters Examined/Number with Malformations

Parameter/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
# Available for Exam fetuses (litters)	324 (22)	345 (24)	324 (24)	331 (24)
# With Malformations fetuses (litters)	4 (4)	3 (3)	4 (4)	7 (5)

External Malformations/Variations - External malformations (microphthalmia, anophthalmia, bent tail, omphalocele, filamentous tail) were observed in 1(1), 0(0), 2(2), and 4(4) fetuses(litters) in the control, low-, mid-, and high-dose groups (Table 9). No external variations were observed.

Table 9. Numbers of Fetuses (Litters) with External Malformations/Variations

External Findings/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
MALFORMATIONS				
bent tail	0	0	0	1 (1)
omphalocele	0	0	0	1 (1)
microphthalmia &/or anophthalmia	0	0	2 (2)	1 (1)
filamentous tail	1 (1)	0	0	0
localized fetal edema	0	0	0	1 (1)

Although the percentage of litters with microphthalmia and/or anophthalmia at the mid dose (8.3%) slightly exceeded the maximum value in the historical control data (8.0%), the percentage of fetuses (0.6%) was the same as the maximum value of the historical control data (0.6%). Additionally, the incidence in the mid-dose group was greater than that in the high-dose level; therefore, the occurrence can be attributed to chance.

Visceral Malformations/Variations - Soft tissue malformations [hydrocephaly, retroesophageal aortic arch, retina(s) folded] were observed in 2 (2), 2(2), 1 (1), and 0 (0) fetuses (litters) in the control, low-, mid-, and high-dose groups, respectively. One control fetus displayed a kidney cyst. No treatment-related effects were observed.

Skeletal Malformations/Variations - Skeletal malformations (bent limb bone(s), vertebral agenesis, vertebral anomaly with or without associated rib anomaly) were observed in 2 (2), 1 (1), 1 (1), and 3 (1) fetuses (litters) in the control, low-, mid-, and high-dose groups, respectively (Table 10). With respect to bent limb bones, the low-dose fetus had a bent left femur, and at the high dose, the anomalies involved a bent radius, ulna, and/or scapula (bilateral and/or unilateral). The % of fetuses affected at the high-dose (0.9%) was slightly greater than the maximum value in the historical control

(0.6%) data, but the % of litters affected (4.2%) was less than the maximum (4.5%) observed in the historical control. It is also noted that the body weights of the three affected high-dose fetuses were low (1.7, 2.4, and 2.4 grams compared with a control mean of 3.5 grams). The vertebral anomalies consisted of thoracic arches, centra and ribs that were absent, smaller than normal, malpositioned, fused or malformed in the control and thoracic and lumbar arches and/or centra, a sacral centrum and ribs that were smaller than normal, fused, unossified, shorter than normal and/or reduced in ossification in the mid-dose fetus. The control fetus with vertebral agenesis also displayed a filamentous tail (external examination).

Table 10. Numbers of Fetuses (Litters) with Skeletal Malformations/Variations

Skeletal Finding/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
bent limb bone(s)	0	1(1)	0	3(1)
vertebral anomalies	1(1)	0	1(1)	0
vertebral agenesis	1(1)	0	0	0

Numerous skeletal variations were observed in the high-dose group only (Table 11). The majority of the skeletal variants observed at the high-dose level are indicative of a developmental delay, as evidenced by reduced ossification of various skeletal structures. Many of the findings at the high dose exceed the maximum values of the historical control data (Table 12).

Table 11. Numbers of Fetuses (Litters) with Skeletal Variations

Skeletal Variation/Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
sternebra(e) #5 &/or #6 unossified	54(16)	78(17)	34(18)	104(18)
bent rib(s)	5(3)	3(1)	3(2)	28(9)
reduced ossification of vertebral arches	0	0	0	23(5)
reduced ossification of skull	0	0	0	14(5)
reduced ossification of vertebral centra	0	0	0	6(2)
sternebra(e) #1,2,3 &/or 4 unossified	0	0	1(1)	11(6*)
hyoid unossified	1(1)	0	2(1)	8(5)
pubis unossified	0	0	0	14(3)
ischium unossified	0	0	0	7(1)
entire sternum unossified	0	0	0	10(2)
14th full rib(s)	0	0	0	2(1)
27 presacral vertebrae	0	0	0	4(2)

Table 12. Incidence of Skeletal Variants at High-Dose vs Maximum Historical Control Value

VARIANT	% Affected		Maximum Values in Historical Control	
	FETUSES	LITTERS	FETUSES	LITTERS
bent rib(s)	8.5*	37.5*	4.2	20.8
sternebra(e) #5 &/or #6 unossified	31.4	75.0	36.6	100.0
reduced ossification of vertebral arches	6.9*	20.8*	1.9	10.0
reduced ossification of skull	4.2	20.8*	6.4	9.0
reduced ossification of vertebral centra	1.8	8.3	*	*
sternebra(e) #1,2,3 &/or 4 unossified	3.3*	25.0*	2.6	22.7
hyoid unossified	2.4	20.8	15.7	50.0
pubis unossified	4.2*	12.5*	1.2	3.3
ischium unossified	2.1*	4.2	0.3	4.2
entire sternum unossified	3.0*	8.3	0.7	3.3

* value exceeds maximum historical control data value; * variant not observed in historical control

D. Discussion

Maternal toxicity was evident at the high-dose level, with one dam euthanized on Day 16 of gestation. All other dams survived to study termination. Neurobehavioral signs were displayed at the high-dose level only. Other signs considered as signs of maternal toxicity include soft stool, decreased defecation, paleness, and body cool to the touch, red material on forelimbs, eyes, and nose, and staining on the urogenital area. There was an increased incidence of soft stool in the mid-dose dams, which was considered treatment related. No other treatment-related clinical signs were observed at the mid-dose level, and none were observed at the low-dose level.

Body weight was adversely affected at the high-dose level throughout the study. Body weight, body-weight gain, terminal body weight, net body weight, and net body-weight change were all reduced at the high-dose level. Body-weight gain during the dosing period and overall and terminal body weight were reduced at the mid-dose level also. Food consumption was also affected at these two dose levels.

There was a dose-related increase in total post-implantation loss, which was statistically significant at the mid- and high-dose levels, and there was a concomitant decrease in the percentages of viable fetuses. Mean fetal body weight was decreased at the high-dose level compared to the concurrent and historical control values. Associated with the lower body weights was an increased incidence of (1) bent limb bones (malformation) and (2) retarded skeletal ossification and bent ribs (developmental variations).

E. CONCLUSION

Under the conditions of the study, oral administration of Maneb at dose levels of 0, 20, 100, and 500 mg/kg for 10 days (gestation Days 6-15) resulted in maternal toxicity at the mid- (slight decrease in body-weight gain and food consumption, increased incidence of soft stool) and high-dose levels (sacrifice of one dam in extremis, body-weight losses, decreased food consumption, soft stool, neurobehavioral findings). Developmental toxicity was observed at the mid- (increased postimplantation loss and decreased fetal viability) and high-dose level (decreased fetal weight associated with skeletal malformation/variations, increased postimplantation loss, and decreased fetal viability). The NOEL for maternal toxicity can be set at 20 mg/kg, the LEL at 100 mg/kg, based on decreased body weight/gain and food consumption. The NOEL for developmental toxicity can be set at 20 mg/kg, the LEL at 100 mg/kg, based on increased postimplantation losses and decreased fetal viability. This study is classified Core Minimum, and it satisfies the guideline requirement (83-3) for a developmental toxicity study in rodents.