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

009045

JAN 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Maneb - Developmental Toxicity Studies

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

FROM: Linda L. Taylor, Ph.D. Linda Taylor 1/6/92
Toxicology Branch II, Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 1/9/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 1/14/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant: Maneb Registration Group (ATOCHEM North America)
Chemical: manganese ethylene-1,2-bisdithiocarbamate; ethylene thiourea
Synonym: Maneb; ETU
Project No.: 1-2102
Caswell No.: 539
Record No.: none; Case: 818618; Submission: S401318
Identifying No.: 014505
MRID No.: 41965801
Action Requested: Please review Maneb - ETU data for GLN 83-3A.

Comment: In response to the Maneb Comprehensive Data Call-In of April 1, 1987, the Registrant has submitted a report: "Prenatal Toxicity Study of Maneb in Rats", which contains the data from three studies on Maneb alone and with varying levels of added ETU. These studies were combined and reformatted into a single report and include "detailed statistical and technical evaluation of the entire data base to permit risk analysis."

The original studies were entitled: "Study to Determine the Prenatal Toxicity of Manganese ethylene-1,2-bis-dithiocarbamate in Rats: (# 88/0522; H.Th. Hofmann & J. Peh, dated 3/4/77); "Study to Determine the Prenatal Toxicity of Manganese ethylene-1,2-bis-dithiocarbamate Containing 0.75% ETU in Rats" (# 88/0523; H.Th.

Hofmann & J. Merkle, dated 6/12/78); and "Study to Determine the Prenatal Toxicity of Manganese ethylene-1,2-bis-dithiocarbamate Containing 2% ETU in Rats" (# 88/0524; H.Th. Hofmann & J. Merkle, dated 7/26/78).

These three studies and the combined study report have been reviewed, and the DER is attached. Under the conditions of the study, administration of Maneb at dose levels of 20, 100, 133.33, 187.5, and 500 mg/kg to pregnant rats from Day 6 through Day 15 of gestation resulted in decreased body weight and body-weight gain in the maternal animals, an increase in the number of dead fetuses and dead implantations/dam, decreased fetal body weight and body length, decreased placental weight, and an increase in anomalies/variations/retardations at the 500 mg/kg dose level. Decreased maternal body weight and placental weight were noted at the 187.5 mg/kg dose level also, although this group displayed a significant decrease in body weight compared to the control prior to dosing. A significant increase in dead implantations/dam was observed at this latter dose also.

These three studies are classified Core supplementary, pending submission of (1) information on the number of fetuses with multiple anomalies at the 500 mg/kg dose level in Study # 522; (2) the identity (and number of fetuses with each) of the anomalies observed in the 172 fetuses at the 500 mg/kg dose level in Study # 524; (3) individual data for each fetus from all 3 studies; (4) individual data for each dam with respect to the number of corpora lutea and implantations; (5) clarification of the discrepancies/errors enumerated in DER and in Appendix A, appended to the DER; (6) clarification of the amount of exogenous/endogenous ETU in the various groups in Studies # 523 & 524 (listed as mg of ETU in the combined study report); (7) Batch numbers for the Maneb test material used in each study; (8) a description of the test material; (9) information on dose preparation, concentrations attained, and analytical data; and (10) a statement on how the testing practices of the testing facility compare to the GLP's. These 3 studies do not satisfy the guideline requirement (83-3) for a developmental toxicity study in rodents.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 1/6/92
Section II, Tox. Branch II (H7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 1/9/92
Section II Head, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

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

MRID NO.: 419658-01

TEST MATERIAL: Maneb with < 0.01, 0.75, and 2.0% ETU

SYNONYMS: Manganese ethylene-1,2-bisdithiocarbamate/ethylene thiourea

STUDY NUMBER: 88/0522, 88/0523, 88/0524

SPONSOR: BASF Corporation, Ludwigshaven, Germany

TESTING FACILITY: BASF Gewerbehygiene und Toxikologie, Ludwigshaven, Germany

TITLE OF REPORT: Prenatal Toxicity Study of Maneb in Rats

AUTHORS: RW Kapp, Jr., LJ Schellhaas, VJ Piccirillo

REPORT ISSUED: May 6, 1991 (original study dates: 3/4/77, 6/12/78, and 7/26/78)

QUALITY ASSURANCE: No quality assurance statement was provided; the Good Laboratory Practice Statement indicates that the studies were conducted prior to the effective data of the EPA GLP Standards. There is a statement indicating that the studies were conducted in compliance with the FDA Guidelines for Reproductive studies for safety evaluation of drugs for human use [1966]. Although it is stated that several areas (not identified) are not in compliance with current standards, the Sponsor's position is that the studies were conducted in an exemplary manner. There is no statement on how the practices of the testing facility compare to the GLP's, whether any periodic quality assurance-type inspections were performed, or whether any audits of the final reports were performed by a Quality Assurance Specialist prior to submission to the Agency. The numerous errors/discrepancies noted during this review (see DISCUSSION) suggest none was performed.

CONCLUSIONS: Under the conditions of the study, administration of Maneb at dose levels of 20, 100, 133.33, 187.5, and 500 mg/kg to pregnant rats from Day 6 through Day 15 of gestation resulted in decreased body weight and body-weight gain in the maternal animals, an increase in the number of dead fetuses and dead implantations/dam, decreased fetal body weight and body length,

decreased placental weight, and an increase in anomalies/variations/retardations at the 500 mg/kg dose level. Decreased maternal body weight and placental weight were noted at the 187.5 mg/kg dose level also, although this group displayed a significant decrease in body weight compared to the control prior to dosing. A significant increase in dead implantations/dam was observed at this latter dose also.

These three studies are classified Core supplementary, pending submission of (1) information on the number of fetuses with multiple anomalies at the 500 mg/kg dose level in Study # 522; (2) the identity (and number of fetuses with each) of the anomalies observed in the 172 fetuses at the 500 mg/kg dose level in Study # 524; (3) individual data for each fetus from all 3 studies; (4) individual data for each dam with respect to the number of corpora lutea and implantations; (5) clarification of the discrepancies/ errors enumerated in DER and in Appendix A, appended to the DER; (6) clarification of the amount of exogenous/endogenous ETU in the various groups in Studies # 523 & 524 (listed as mg of ETU in the combined study report); (7) Batch numbers for the Maneb test material used in each study; (8) a description of the test material used in each study; (9) information on dose preparation, concentrations attained, and analytical data; and (10) a statement on how the practices of the testing facility compare to the GLP's. These 3 studies do not satisfy the guideline requirement (83-3) for a developmental toxicity study in rodents.

A. MATERIALS

1. Test Compound: Maneb, with varying concentrations of ETU; Description: none provided; Batch #: none provided, but indicates that this information is in the BASF Archives; Purity: 99.99% technical grade Maneb, not provided for ETU; Source: not provided. NOTE: Purity information was not in the original final reports.

2. Test Animals: Species: rat; Strain: Sprague-Dawley; Age: adult; Weight: females: 201-233 g; Source: WIGA, Sultzfeld, Germany.

3. Statistics: Statistical analyses performed are discussed on pages 25-28 of the report (pages appended). The original statistical analyses are described in the appended pages from the original reports.

B. STUDY DESIGN

NOTE: The original studies were entitled: Study to Determine the Prenatal Toxicity of Manganese ethylene-1,2-bis-dithiocarbamate in Rats (# 88/0522; H.Th. Hofmann & J. Peh, dated 3/4/77); Study to Determine the Prenatal Toxicity of Manganese ethylene-1,2-bis-dithiocarbamate Containing 0.75% ETU in Rats (# 88/0523; H.Th. Hofmann & J. Merkle, dated 6/12/78); Study to Determine the Prenatal Toxicity of Manganese ethylene-1,2-bis-dithiocarbamate Containing 2% ETU in Rats (# 88/0524; H.Th. Hofmann & J. Merkle, dated 7/26/78).

1. Methodology: This study consists of three studies, one performed in 1975 [# 38/0522] and two performed in 1978 [#'s 88/0523 and 88/0524]. The latter two studies were performed concurrently using the same control groups. A combined total of 314 females were utilized as follows:

Study	# Females Dosed	Dose of Maneb []*
88/0522 GROUP		
1	24	untreated control
2	24	CMC control
3	23	20 mg/kg
4	24	100 mg/kg
5	25	500 mg/kg

Study	# Females Dosed	Dose of Maneb []*
88/0523 GROUP		
1	25	untreated control
2	24	CMC control
3	23	133.33 mg/kg [0.75]
4	25	500.00 mg/kg [0.75]
88/0524 GROUP		
1	25	untreated control
2	24	CMC control
3	24	187.5 mg/kg [2.0]
4	24	500.0 mg/kg [2.0]

* [%] ETU added; technical Maneb (<0.01% ETU; as indicated in combined report, not in the original report)

In the combined final report, it is stated that the females were assigned to the test groups via a "limit equalization" program, which was stated to be a BASF in-house statistical program (not further defined) documented in BASF files. In the original final report for the 1975 study, there is no mention of how the animals were assigned to the study. The two 1978 final reports indicate the "limit equalization" program was used and it is defined in the appended pages referred to under Statistics, above.

The test material was prepared (combined report indicates daily preparation, but only the 1975 study final report indicates daily; time-frame not provided in either 1978 report) in 0.5% CMC and administered by gavage once daily on gestation days 6 through 15. The concentration of the suspensions was adjusted so that the test dose was 10 mL/kg. Only the 1978 studies indicated that the dose was based on the Day 0 body weights. There were a vehicle control (0.5% CMC, 10 mL/kg) and an untreated control. There were no mixing, concentration, or analytical data provided, but these were said to be in the BASF Archives. The feed in # 88/0522 was standardized feed pellets Altromin R from ALTROMIN, Lage/L., Germany; that in #'s 88/0523 and 88/0524 was standardized feed pellets, Herilan MRH from Eggersmann KC, Rintein/Weser, Germany. It is not stated in the combined report whether the feed was available ad libitum, but the individual reports indicate it was; tap water was provided ad libitum also.

The 1975 study (# 88/0522) was performed with technical grade Maneb and the 1978 studies (#'s 88/0523 & 88/0524) were performed with technical Maneb in combination with ETU (0.75% & 2.0% ETU, respectively). It is to be noted that technical

Maneb is not defined in any of the reports. With regard to endogenous ETU (discussed in the combined report only), it is stated that the in vivo bioconversion rate of Maneb to ETU is generally accepted as approximately 7.5%. A table was provided showing the various dose levels of all 3 studies and the increasing levels of the total amount of ETU (endogenous plus exogenous):

Maneb Dose Level [% exogenous ETU]	Endogenous ETU (mg)*	Exogenous ETU (mg)*	Total ETU (mg)*
20 mg/kg [<0.01]	1.5	0.02	1.52
100 mg/kg [<0.01]	7.5	0.10	7.60
133.33 mg/kg[0.75]	10.0	1.00	11.00
187.5 mg/kg [2.0]	14.0	3.75	17.75
500 mg/kg [<0.01]	37.5	0.50	38.00
500 mg/kg [0.75]	37.5	3.75	41.25
500 mg/kg [2.0]	37.5	10.00	47.50

* It would appear that the units used for ETU are inaccurate; these should read mg/kg ETU. It was noted in the combined report that the teratogenic NOEL for ETU is 5 mg/kg, and severe teratogenic effects are observed at 10 mg/kg. NOTE: Only the 20 mg/kg dose of Maneb is below a no-effect dose for ETU (if total ETU is considered and the units are mg/kg), and one would expect all doses above the 100 mg/kg dose level of Maneb to produce severe teratogenic effects. If one considers exogenous ETU alone and, using the Registrant's units (mg), then all doses above 133.33 mg/kg Maneb + 1 mg ETU should display severe teratogenic effects. If mg/kg are used and only exogenous ETU considered, only the 500 mg/kg Maneb + 10 mg/kg ETU dose should display severe teratogenic effects.

There were a total of 11 individual groups in these 3 studies; the final report submitted combines all 3 studies into one study. The two untreated and the two CMC controls were combined so that there were two control groups in the combined study. The groups in the combined study were identified as shown below.

GROUP	Maneb Dose Level	Total ETU (mg)
1	Untreated control	0.00
2	CMC control	0.00
3	20 mg/kg	1.52
4	100 mg/kg	7.60
5	500 mg/kg	38.00
6	133.33 mg/kg	11.00
7	500.00 mg/kg	41.25
8	187.5 mg/kg	17.75
9	500.0 mg/kg	47.50

For # 88/0522, females were housed with untreated males at the end of each day. There is no mention of the number of females housed with a male. For the two other studies, it is stated that four (4) untreated females were mated with one untreated male of tested fertility. Each morning a vaginal smear was taken and examined for the presence of sperm, and the day on which this occurred was designated as Day 0 of gestation. Dosing was from Day 6 through Day 15 of gestation. There is no information on how the pregnant dams were housed after mating; all three original reports state that the animals were accommodated in pairs, but it is not evident that this means after mating also, or whether each dam had a separate nesting box.

Clinical Observations

Each dam was examined for clinical signs and mortality each study day, and body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. Animals dying on test were necropsied, and the uterus was examined for implantations. On Day 20, all surviving dams were sacrificed via Cesarean section, examined grossly, and the uterus was removed in toto for detailed examination. The following were recorded: # of implantation and resorption sites, live and dead fetuses, and corpora lutea. The sex, weight, and lengths of the live fetuses were determined.

The following parameters (as defined in the report) were used for assessment:

1. Conception rate: # of pregnant animals related to the # of animals that were sperm positive;
2. Implantations: total # of implantations and the mean # per pregnant animal were calculated;
3. Live fetuses: total # of live fetuses and their sex were determined;
4. Dead implantations: differentiated as follows:
   a) Early resorptions-Salewski Method: uterus placed into a 10% ammonium sulfide solution, which stains the early resorption a brown color.
   b) Early resorptions: detectable with the naked eye as yellowish brown spots on the uterus.
   c) Intermediate resorptions: embryos that have died and started to be resorbed, with no parts of the body recognizable to the naked eye.
   d) Late resorptions: embryos that have died and started to be resorbed, with individual parts of the body being recognizable to the naked eye.
   e) Dead fetuses: fetuses that showed no spontaneous breathing and were hypoxemic at Cesarean section.
5. Percentage of dead implantations: calculated from a-e described under 4. above.

Data collected on fetuses are described below.

1. The fetuses were subjected to gross pathological examination; only changes in live fetuses were assessed.
2. Body weight of live fetuses.
3. Body length of live fetuses.
4. Weight of placentae of live fetuses.
5. Examination of fetal skeletons: 2/3 of the live fetuses of each dam (selected at random) were treated by a modification of the Dawson Method; fetuses were initially fixed in 96% alcohol and then clarified with potassium hydroxide solution and stained with Alizarin Red S. The fetuses were stored in 100% glycerol and examined for malformations, variations, and retardations.
6. Examination of fetal organs: the remaining 1/3 of the fetuses of each dam were fixed in Bouin's Solution for 14 days and then about 15 traverse sections of each fetus were prepared and examined via the Wilson Method.

The following criteria were provided.

MALFORMATIONS - morphological changes going beyond retardation and variation;
VARIATIONS - changes that occur regularly but do not affect the functioning of the individual;
RETARDATIONS - delays in development compared with normal at the time of the investigation.

C. RESULTS

Clinical Observations and Survival - Maternal

There was one death in the CMC (Study # 88/0522) control group, which was considered incidental. Three other deaths occurred and all were dams in the high-dose (500 mg/kg) groups; one occurred on Day 13 and one on Day 19 in Group 7 (0.75 % ETU); one death occurred on Day 19 in Group 9 (2.5% ETU). Based on severe clinical signs and deaths only at the high dose level of test material, these were considered to be treatment-related by the authors.

There were no signs of toxicity observed at the 20, 100, 133.33, and 187.5 mg/kg dose levels. At the 500 mg/kg dose level (Groups 5, 7, and 9), an unsteady gait, dragging of the rear limbs, diminished sensitivity to pain in the affected limbs, and paresis of the rear limbs were observed, and these were listed as severe. In Group 5 (no added ETU), 14 of 15 dams displayed paresis by Day 20 (first observed at Day 15/16), which persisted to sacrifice. It was stated that this group was slow to respond and slow to recover, although it is not apparent to this reviewer what the dams were slow to respond to/recover from. In Group 7, rear limb paresis was

noted earlier (in 1 dam at both Days 10 and 11, in 2 at Day 13, in 3 Day 14, and in 8 at Day 15). Two animals showed no clinical signs. By Day 19, most animals showed significant improvement, although several displayed an unsteady gait on Day 19. The same type of rear limb paresis was observed in Group 9 dams at Day 13, which steadily increased (not further defined-? # with or severity) throughout the dosing period. At Day 20, 15 of 24 dams continued to display significant paresis of the rear limbs.

Maternal Body Weight and Body-weight Gain

There were no differences among the groups with respect to body weight or body-weight gain until Day 15 of gestation. On Day 15, all three high-dose groups (500 mg/kg) displayed a significant reduction in body weight compared to both combined untreated (UT) and CMC control groups, and these deficits continued to Day 20. Additionally, body-weight gains for Days 0-15, 0-20, 5-15, and 15-20 intervals were significantly decreased in these 3 groups compared to controls. No data were provided for gravid uterus weights in the combined report. Data in the individual reports indicate a statistically significant decrease in uterine weight at the 500 mg/kg dose level in Study # 522 compared to both controls; at 500 mg/kg dose level compared to the untreated control in Study # 523; and at the 187.5 mg/kg dose level compared to the untreated control and at the 500 mg/kg dose level compared to both controls in Study # 524.

The authors of the combined report state that there appears to be an inverse relationship between the amount of exogenous ETU and body weight/body-weight gain at 500 mg/kg (see below).

Day	Body Weight (g) Group 5 [0.5 mg]	Group 7 [3.75 mg]	Group 9 [10.0 mg]
15	246*	233**	217**
20	283**	266**	245**
Interval (days)	Body Weight Gain (g) Group 5 [0.5 mg]	Group 7 [3.75 mg]	Group 9 [10.0 mg]
0-15	31**	21**	3**
0-20	68**	55**	29**
5-15	7**	-7**	-23**
15-20	37*	32**	25**

*p<0.05, **p<0.001 compared to untreated control;
[ ] exogenous ETU; each dose has 37.5 mg endogenous ETU

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The maternal body weight and body-weight gain were decreased (statistically significant) compared to the combined UT and CMC control value(s) only at the 500 mg/kg dose level in the combined report (see below).

Maternal Body Weight (% of Controls)

Dose* /Day	20	100	500	133	500	187	500
0	97	98	98	106	97	92	98
6	96	99	99	106	99	98	99
11	95	97	94	103	95	93	94
15	95	97	88♥♦	102	84♣♠	92	78♣♠
20	98	100	85♣♠	100	80♣♠	92	73♣♠

* mg/kg

p <0.05 UT ♥ CMC ♦ p <0.01 UT ♣ CMC ♠

Body-weight Gains (grams)

Dose (mg/kg) Time Interval	UT 0	CMC 0	20	100	500	133	500	187	500
0-15	60	59	53	55	31♣♠	50	21♣♠	56	3♣♠
0-20	116	115	115	118	68♣♠	101	55♣♠	106	29♣♠
6-15	37	35	33	31	7♣♠	27	-7♣♠	27	-23♣♠
15-20	56	56	62	64	37♥♦	51	32♣♠	50	25♣♠
0-6*	23	24	21	23	24	23	28	28	26

p <0.05 UT ♥ CMC ♦ p <0.01 UT ♣ CMC ♠

* no statistics performed for this interval

Body-weight gain on a "percent of control" basis is shown below.

Body-weight Gains (% of control)

Dose (mg/kg) Time Interval	20	100	500	133	500	187	500
0-15	88	92	52	83	35	93	5
0-20	100	102	59	87	47	91	25
6-15	89	84	19	75	-	73	-
15-20	111	114	56	91	57	89	45
0-6	91	100	104	100	122	122	113

The individual reports show a different picture with respect to body weights/body-weight gain. In Study # 522, the 500 mg/kg dose group displayed a significant decrease compared to both control groups at Days 15 and 20; in Study # 523, this dose level showed a significant decrease compared to both controls at Days 11, 15, and 20; in Study # 524, both the 187.5 mg/kg and 500 mg/kg dose groups displayed a significant decrease at Days 0, 6, 11, 15, and 20 compared to both controls. NOTE: The 187.5 mg/kg and 500 mg/kg dose groups both displayed a significant decrease in body weight prior to dosing. By combining the control groups from these studies, one loses sight of this fact. It is to be noted that of the 6 groups in the #523-524 studies, the 3 highest dose levels (187.5, 500, & 500 mg/kg) were made up of the lowest weight animals (see table below). A comparison of the % body-weight gain among the 500 mg/kg groups showed a lower gain with increasing levels of exogenous ETU levels.

Body Weight (grams)

Group/ Day	UT #522	CMC #522	20	100	500	UT # 523/524	CMC # 523/524	133.33	500	187.5	500
0	214.6	216.7	212.2	215.3	214.8	223.6	221.4	232.7	212.2	200.5*	214.1*
6	235.2	238.8	232.7	238.7	238.7	248.7	247.7	256.1	239.8	228.8*	240.1*
% 0-20**	57	54	54	55	32	49	50	43	26	53	15

* significantly different from CMC at p<0.05 & from UT at p<0.01
* significantly different from both controls at p<0.05
** % gained between days 0 and 20

Food Consumption

No data were provided on food consumption.

Gross Pathological Observations

Maternal Observations: No gross pathology was observed in the untreated controls or in Groups 3, 4, 5, or 8 [20, 100, 500 (no exogenous ETU), or 187.5 mg/kg, respectively].

In one CMC control dam, there was gross dilation of the left renal pelvis, such that only the cortical layer was still present. One dam of this group that died prior to Cesarean section showed numerous yellowish white foci on the surface of the left kidney which penetrated into the cortex; the renal pelvis was dilated, the cortex was pale in color, and several yellowish white foci were observed on the surface of the right kidney.

In Group 6 [133.33 mg/kg plus 1 mg (per kg) ETU], one dam displayed hemopericardium, and in another the urinary bladder was filled to distention and both kidneys were greatly enlarged with substantial loss of the perirenal adipose

tissue.

In the 500 mg/kg group (Group 7) with 3.75 mg (per kg) ETU added, eight dams displayed substantial loss of the perirenal adipose tissue, which was also observed in the two dams that died. In the 500 mg/kg group with 10 mg ETU added, all sacrificed dams displayed a substantial loss of perirenal adipose tissue, which was observed in the dam that died also.

The author stated that the loss of perirenal adipose tissue at the 500 mg/kg dose levels that had ETU added (Groups 7 and 9) but not at this level without added ETU (Group 5) appears to be associated with exogenous ETU. However, this lesion was noted only in the 1978 study, which had only Maneb doses to which exogenous ETU was added. Since the 1978 study did not include a 500 mg/kg dose level without exogenous ETU, a definitive statement cannot be made regarding the occurrence of this lesion at the 500 mg/kg dose level and exogenous ETU. Additionally, TB II notes that the loss of perirenal adipose tissue was observed at the 133.33 mg/kg dose level (Group 6), which had 1 mg ETU added, but not at the 187.5 mg/kg dose level (Group 8), which had 3.75 mg ETU added. Groups 7 and 8 had the same amount of exogenous ETU (3.75 mg), but Group 7 did not display a loss of perirenal adipose tissue.

Maneb Dose Level [% exogenous ETU]	Endogenous ETU (mg)*	Exogenous ETU (mg)*	Total ETU (mg)*	Loss of PRAT-
20 mg/kg [<0.01]	1.5	0.02	1.52	0
100 mg/kg [<0.01]	7.5	0.10	7.60	0
133.33mg/kg[0.75]	10.0	1.00	11.00	1
187.5 mg/kg [2.0]	14.0	3.75	17.75	0
500 mg/kg [<0.01]	37.5	0.50	38.00	0
500 mg/kg [0.75]	37.5	3.75	41.25	10
500 mg/kg [2.0]	37.5	10.00	47.50	21

- = dams displaying loss of perirenal adipose tissue (PRAT)

Cesarean Section Observations

With the exception of pre-implantation loss, the data listed in the following table, as well as the statistical significance are as presented in the combined study report. In many instances, the data could not be confirmed due to illegible numbers in the study reports. Additionally, individual data for the number of corpora lutea and implantations were not provided.

Table III: Cesarean Section observations▼

	Study # 522					Study #'s 523 & 524						combined	
GROUP:	UT	CMC	20	100	500	UT	CMC	133.3	187.5	500	500	UT	CMC
#Animals Assigned	24	24	23	24	25	25	24	23	24	25	24	49	48
#Animals Mated	23	23	22	24	25	21	21	17	20	23	21	44	44
Pregnancy Rate (%)	96	96	96	100	100	84	88	74	83	92	88	90	92
Maternal Wastage													
#Died	0	1	0	0	0	0	0	0	0	2	1	0	1
#Died/pregnant	0	1	0	0	0	0	0	0	0	2	1	0	1
#Non pregnant	1	1	1	0	0	0	0	6	4	2	3	5	4
#Aborted	0	0	0	0	0	0	0	0	0	0	0	0	0
#Premature Delivery	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Corpora Lutea	301	308	282	332	344	312	295	258	273	305	294	613	603
Corpora Lutea/dam	13.09	14.0	12.8	14.0	13.8	14.9	14.1	15.2	13.7	14.5	14.7	13.9	14.0
Total Implantation	299	288	267	310	335	221	233	200	244	239	202	520	521
Implantations/Dam	13.0	13.09	12.1	12.9	13.4	10.5	11.1	11.8	12.2	11.4	10.1♥	11.8	12.1
Total Live Fetuses	291	278	255	292	304	212	211	189	221	222	177	503	489
Live Fetuses/Dam	12.7	12.6	11.6	12.2	12.2	10.1	10.1	11.1	11.1	10.6	8.9	11.4	11.4
Mean Litter %	97.4	94.6	95.8	95.0	90.3	95.4	88.4	94.8	90.6	93.6	85.0	97.0	92.6
Total Resorptions	8	10	12	18	16	9	22	11	23	17	19	17	32
Early (Salewski)	0	0	0	0	0	0	0	0	0	0	0	0	0
Early	6	8	10	18	11	6	17	7	17	10	9	12	25
Intermediate	2	2	2	0	5	3	5	4	6	7	8	5	7
Late	0	0	0	0	0	0	0	0	0	0	2	0	0
Resorptions/Dam	0.4	0.4	0.5	0.8	0.6	0.4	1.1	0.6	1.2	0.8	1.0	0.4	0.7
Litters w/ resorptions/ total # litters (%)	26.1	39.1	36.4	45.8	36.0	28.6	76.2	41.2	75.0♣	52.4♥	70.0♣	27.3	58.1♣
Total Dead Fetuses	0	0	0	0	15	0	0	0	0	0	6	0	0
Dead Fetuses/Dam	0	0	0	0	0.6	0	0	0	0	0	0.3	0	0
Litters w/ NLI*	26.1	39.1	36.4	45.8	48.0	28.6	76.2	41.2	75.0♣	52.4♥	70.0♣	27.3	58.9♣
Total Dead Implants	8	10	12	18	31	9	22	11	23	17	25	17	32
Dead Implants/Dam	0.35	0.45	0.55	0.75	1.24	0.43	1.05	0.65	1.15	0.81	1.25	0.39	0.74
Mean Placental Weight (g)	0.60	0.60	0.64	0.61	0.54	0.63	0.57	0.57	0.54	0.53	0.48	NA	NA
Mean Fetal Weight (gm)@	3.67	3.62	3.76	3.70	3.14♥	3.56	3.61	3.43	3.79	3.27	2.30♣	3.62	3.61
Mean Fetal Length@	3.62	3.60	3.64	3.64	3.40♣	3.65	3.61	3.60	3.65	3.53	2.98♣	3.63	3.60
# of Runts	1	0	1	2	1	1	3	2	1	1	1	2	3
Postimplantation Loss(%)	2.56	3.36	4.2	5.3	9.7	4.6	11.56	5.2	9.4	6.4	15.0	3.5	7.4
Sex Ratio (% Male)	51.2	50.7	54.9	48.3	54.2	49.5	49.3	53.4	52.5	58.6	51.4	50.5	50.1
Preimplantation Loss(%)^	0.69 0.66	6.5 6.5	5.5 5.3	7.9 7.7	2.9 2.6	29.2 29.2	21.0 20.7	22.4 22.5	10.9 10.6	21.4 21.6	31.3 31.3	15.1 15.2	13.6 13.6

@statistical significance compared to combined UT ($p<0.05$ ♥; $p<0.01$ ♣); CMC ($p<0.05$ ♦; $p<0.01$ ♠); both ($p<0.05$ ♪; $p<0.01$ ♫)

units not provided in any of the reports; * NLI non-live implants

@ # for the combined controls could not be verified by this reviewer because much of the data in the original reports could not be deciphered !

^ calculated by this reviewer; first row is calculated using mean #'s/dam; second row is calculated using total #'s/group; preimplantation loss for the individual studies is as follows: Study #522 UT = 0.7; CMC = 6.5; Study #523-4 UT = 29.2; CMC = 21.0

NA = could not be calculated because individual data not available

NOTE: Regarding the decreases in mean fetal body weight, it should be pointed out that significant differences were observed between the 500 mg/kg dose and respective control groups in each of the three studies; in Study #'s 522 and 524, a statistically significant decrease was attained at the $p<0.01$ level when compared to either (UT or CMC) control group; in # 523, statistical significance at the $p<0.05$ level was attained when compared to the CMC control group.

Fetal Body Weight (grams)/Body Length (cm)

Group/	UT #522	CMC #522	20	100	500	UT # 523/524	CMC # 523/524	133.33	500	187.5	500
Body weight											
♀	3.78	3.70	3.85	3.81	3.164♣	3.52	3.71	3.52	3.33♣	3.86	2.33♣♣
♂	3.56	3.55	3.66	3.59	3.09♣♣	3.47	3.50	3.32	3.19♣	3.68	2.27♣♣
♀ & ♂	3.67	3.62	3.76	3.70	3.14♣♣	3.56♣♣	3.61	3.43	3.27♣	3.79	2.30♣♣
Body length											
♀	3.66	3.62	3.67	3.68	3.41♣♣	3.64	3.63	3.63	3.56	3.67	2.98♣♣
♂	3.57	3.57	3.61	3.61	3.39♣♣	3.62	3.58	3.53	3.49♥	3.62	2.97♣♣
♀ & ♂	3.62	3.60	3.64	3.64	3.40♣♣	3.65♣	3.61	3.59	3.53♥	3.65	2.98♣♣

♣ # in final report; not recalculated by LLT
♦ significantly different from CMC control at $p<0.01$; ♦ at $p<0.05$
♣ significantly different from UT control at $p<0.01$; ♥ at $p<0.05$

With regard to the significant decrease in the # of implants/litter in the 500 mg/kg group (2% ETU), significance is attained only when compared to the combined UT controls, not to the concurrent control groups of Studies # 523-524. The number of live fetuses/dam was reported as comparable among the groups (combined study), although the 500 mg/kg fetuses (Group 9) in Study # 524 were statistically significantly fewer than the concurrent UT at $p<0.01$. The number of live fetuses at 137.5 mg/kg (Study # 524) was greater than the concurrent UT at $p<0.05$. The total number of dead implants/litter was increased (dose-related) at the 187.5 and 500 mg/kg dose levels in Study # 524 compared to the concurrent UT control group ($p<0.05$ and $p<0.01$, respectively), although not on a mean litter % basis, and there was no increase at the other 500 mg/kg dose level (Group 7). An increase in the total number of dead implants was observed in Study # 522 at 500 mg/kg on a mean litter % basis only, compared to the concurrent UT ($p<0.05$). With regard to the % litters with nonlive implants, the concurrent CMC control for Studies #523/524 has a value of 76%.

There was a higher percentage (58.6%) of males in Group 7 (500 mg/kg - 0.75 ETU) compared to control [concurrent CMC and combined UT (as stated on page 44); this reviewer notes that the combined CMC value is below the combined UT value, suggesting significance was attained compared to this control also. TB II points out that all groups (except Group 4) had fewer female pups than male pups. Since neither of the other 500 mg/kg dose groups displayed a

similar increase in the number of males to females, the increase is considered incidental.

Fetal Observations: With the exception of one fetus (Study # 523/524) with anasarca (generalized edema), all UT control fetuses were unremarkable. The CMC control (Study # 523/524) had 1 fetus with anasarca and one with malpositioning of the extremities (both from same litter); all other fetuses were unremarkable. No remarkable findings were observed at the 20, 100, 133.33, and 187.5 mg/kg dose levels.

In the 500 mg/kg dose groups, numerous changes were observed in each group, but Group 7 with 0.75% added ETU displayed considerably fewer changes than the other two (500 mg/kg) groups. Of the 177 fetuses in Group 9 (2% added ETU), 172 were listed with multiple anomalies (not further defined). Based on the data provided, it appears that these 172 did not display any of the anomalies observed (and listed) in the other two groups, since the table of malformations (Table 3, page 69-70) shows very few entries for Group 9. If the multiple anomalies are different than those listed for the other animals, these should have been identified in the report. If they are the same anomalies, the summary table needs to be revised to reflect the correct number of each in order to compare the findings with those of Group 5. As summarized in the combined study report, the changes observed in Group 5 included short tail, meningocele, accessory toe, shortened toes, macroglossia, syndactyly, kyphosis, oligodactyly, and shortened splayed toes in numerous fetuses. Group 7 had one fetus with malpositioning of the extremities, one with curled tail, and 27 with kinked tails. Group 9 showed 172 fetuses with multiple anomalies, and of the 5 fetuses for which data on anomalies were presented, malpositioning of extremities, short tail, and syndactyly were each found in 2 fetuses; macroglossia, meningocele, and a very short tail were each found in one fetus. The following table demonstrates the lack of consistency in anomalies observed at the 500 mg/kg dose levels among the three studies.

Anomaly	Study # 522 fetuses litters	Study # 523 fetuses litters	Study # 524 fetuses litters
Malpositioning of Extremities	95/304 10/24	1/222 1/21	2/177 2/20 1/211 1/21 CMC
Meningocele	99/304 10/24	none observed	1/177 1/20
Short Tail	214/304 21/24	1/222 1/21	2/177 2/20
Short Toes	60/304 6/24	none observed	none reported
Micrognathia	20/304 4/24	none observed	none reported
Macroglossia	30/304 3/24	none observed	1/177 1/20
Syndactyly	17/304 3/24	none observed	1/177 1/20
Kyphosis	17/304 3/24	none observed	none reported
Oligodactyly	14/304 3/24	none observed	none reported
Curled Tail	7/304 7/24	1/222 1/21	none reported
Toe short/splayed	12/304 1/24	none observed	none reported
Cleft Thoracic Ventricle	UT 6/291 6/23 CMC 2/278 2/22 20 7/255 7/22 100 6/292 5/24 38/304 16/24	UT 3/212 3/21 CMC 1/211 1/21 133 1/189 1/17 11/222 6/21	UT 3/212 3/21 CMC 1/211 1/21 187 5/221 4/21 none reported
Hydrocephaly	84/304 23/24	38/222 5/21	5/177 2/20
Dilatation of 4th ventricle & Sylvian Aqueduct	72/304 21/24	none observed	none reported
Brachygnathia	81/304 8/24	none observed	none reported
Cleft Palate	UT 1/291 1/23 97/304 10/24	133 1/189 1/17 6/222 1/21	1/177 1/20
Aplasia of Thoracic Ventricle	42/304 6/24	none observed	none reported
Wavy Ribs (bilateral)	UT 1/291 1/23 CMC 2/278 1/22 20 3/255 3/22 100 4/292 1/24 12/304 7/24	UT 2/212 2/21 CMC 2/211 2/21 133 4/189 3/17 15/222 5/21	UT 2/212 2/21 CMC 2/211 2/21 187 4/221 3/20 none reported

NOTE: Unless otherwise indicated, the data in the table are for the 500 mg/kg dose groups; UT=untreated control, CMC=carboxymethylcellulose control, 20/133/187 are the other dose groups; the #'s under fetuses and litters are: # fetuses affected/total # of fetuses and # litters affected/total # of litters.

With regard to Study # 524, the data are misleading, since data are reported for only 5 fetuses from 3 litters (of the 177 total live fetuses/20 litters from 20 litters born in this 500 mg/kg dose group). In Study # 524, the general term "Multiple Anomalies" is listed for the high-dose group fetuses (172/177), but no explanation is provided. In the Summary table on page 69, the highest number of fetuses displaying any malformation is 5 (177 -

172 = 5). It is not apparent whether all other fetuses had all of the defect types listed or only defects not listed. It is therefore difficult to compare Group 9 with the other 500 mg/kg groups (Groups 5 and 7). In general, two of the three 500 mg/kg groups (Groups 5 and 9) show numerous malformations, which are increased compared to control values both on a per litter basis and on the number of fetuses displaying the defects. From the data, one cannot determine the number of fetuses in Group 5 that had multiple anomalies.

The following two tables summarize the anomalies (TABLE A)/ variations/retardations (TABLE B) observed in the three studies reviewed. Shown are the number (and percent) of fetuses with the observation, the number (and percent) of litters with fetuses with the observation, and the mean litter percent. For the last 500 group in TABLE A, the numbers may be misleading since the fetuses with several anomalies were not identified as to which (or how many) had a particular anomaly; the mean litter % was calculated assuming those with multiple anomalies did not have the particular anomaly listed.

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TABLE A

OBSERVATION	DOSE GROUP* AND STUDY #										
Anomalies	UT #522	CMC #522	20 #522	100 #522	500 #522	UT #523-24	CMC #523-24	133.3 #523	187.5 #524	500 #523	500 #524
# fetuses	291	278	255	292	304	212	211	189	221	222	177
# litters	23	22	22	24	24	21	21	17	20	21	20
C-Section											
Malpos. Ext.											
# F w/ (%)	0	0	0	0	95(31)	0	1(0.5)	0	0	1(0.45)	2
# L w/ (%)	0	0	0	0	10(42)	0	1(4.8)	0	0	1(4.8)	2
litter %	0	0	0	0	31±43	0	0.4±1.8	0	0	0.5±2.2	63±23
Short tail											
# F w/ (%)	0	0	0	0	214(70)	0	0	0	0	1(0.45)	2
# L w/ (%)	0	0	0	0	21(88)	0	0	0	0	1(4.8)	2
litter %	0	0	0	0	70±35	0	0	0	0	1.6±7.3	6.3±23
Meningocele											
# F w/ (%)	0	0	0	0	99(33)	0	0	0	0	0	1
# L w/ (%)	0	0	0	0	10(42)	0	0	0	0	0	1
litter %	0	0	0	0	32±43	0	0	0	0	0	5±22
Short toes											
# F w/ (%)	0	0	0	0	60(20)	0	0	0	0	0	0
# L w/ (%)	0	0	0	0	6(25)	0	0	0	0	0	0
litter %	0	0	0	0	UTC	0	0	0	0	0	0
Skull											
Brachygnathia											
# F w/ (%)	0	0	0	0	81(27)	0	0	0	0	0	0
# L w/ (%)	0	0	0	0	8(33)	0	0	0	0	0	0
litter %	0	0	0	0	26±40	0	0	0	0	0	0
Cleft palate											
# F w/ (%)	1(0.3)	0	0	0	97(32)	0	0	1(0.5)	0	6(2.7)	1
# L w/ (%)	1(4)	0	0	0	10(42)	0	0	1(5.9)	0	1(4.8)	1
litter %	0.4±1.7	0	0	0	31±42	0	0	0.7±3.0	0	2.9±13	5±22
Dilat.4 vent.											
# F w/ (%)	0	0	0	0	72(24)	0	0	0	0	0	0
# L w/ (%)	0	0	0	0	21(88)	0	0	0	0	0	0
litter %	0	0	0	0	24±14	0	0	0	0	0	0
Hydroce. int.											
# F w/ (%)	0	0	0	0	84(28)	0	0	0	0	38(17)	5
# L w/ (%)	0	0	0	0	23(96)	0	0	0	0	5(24)	2
litter %	0	0	0	0	28±9.9	0	0	0	0	19±39	10±31
Spine											
Cleft vert.											
# F w/ (%)	6(2.1)	2(0.7)	7(3)	6(2)	38(13)	3(1)	1(0.5)	1(0.5)	5(2.3)	11(5)	0
# L w/ (%)	6(26)	2(9.1)	7(32)	5(21)	16(67)	3(14)	1(4.8)	1(5.9)	4(20)	6(28.6)	0
litter %	2.3±4.1	0.8±2.5	2.9±4.3	2.1±4.6	12.8±13	1.2±3.1	0.4±1.8	0.7±3.0	2.8±5.9	5.7±10.5	0
Ribs											
Wavy ribs bi											
# F w/ (%)	1(0.3)	2(0.7)	3(1)	4(1)	12(4)	2(0.9)	2(0.9)	4(2.1)	4(1.8)	15(7)	0
# L w/ (%)	1(4)	1(5)	3(14)	1(4)	7(29)	2(10)	2(10)	3(17.6)	3(15)	5(24)	0
litter %	0.4±1.7	0.8±3.6	1.3±3.3	1.3±6.3	4.1±8.4	1.0±3.4	0.8±2.5	2.3±5.4	1.9±4.6	7.0±13	0
Wavy ribs uni											
# F w/ (%)	2(0.7)	3(1)	5(2)	4(1)	0	2(0.9)	0	3(1.6)	1(0.45)	5(2)	0
# L w/ (%)	2(8.7)	3(14)	4(18)	2(8)	0	1(4.8)	0	2(11.8)	1(5)	5(24)	0
litter %	0.7±2.3	1.0±2.6	1.9±4.2	1.3±4.9	0	0.8±3.6	0	1.4±4.1	0.4±1.7	3.4±7.9	0
Kidneys											
DPCB	0	0	0	0	12(4)	0	0	0	0	0	0
# F w/ (%)	0	0	0	0	6(25)	0	0	0	0	0	0
# L w/ (%)	0	0	0	0	3.9±8.1	0	0	0	0	0	0
litter %											
DCPU											
# F w/ (%)	1(0.3)	0	0	0	5(1.6)	0	0	0	0	0	0
# L w/ (%)	1(4)	0	0	0	5(21)	0	0	0	0	0	0
litter %	0.4±1.9	0	0	0	1.8±3.7	0	0	0	0	0	0
Hydroureter											
# F w/ (%)	1(0.3)	0	0	0	16(16)	0	0	0	0	0	0
# L w/ (%)	1(4)	0	0	0	6(25)	0	0	0	0	0	0
litter %	0.4±1.9	0	0	0	4.7±9.4	0	0	0	0	0	0

UT (untreated), CMC (carboxymethylcellulose) controls; #'s mg/kg Maneb

UTC = unable to calculate due to error in data presentation (31 out of 17 fetuses were listed as displaying short toes in one litter)

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TABLE 8

Variation/ Retardation	UT	CMC	20	100	500	UT	CMC	133.3	187.5	500	500
# fetuses	291	278	255	292	304	212	211	189	221	222	177
# litters	23	22	22	24	24	21	21	17	20	21	20
Tail kink											
# F w/ (%)	0	0	0	0	59(29)	0	0	0	0	27(18)	0
# L w/ (%)	0	0	0	0	17(71)	0	0	0	0	10(50)	0
litter %	0	0	0	0	19±24	0	0	0	0	14±18	0
Skull retard.											
# F w/ (%)	5(2.6)	5(2.7)	1(0.6)	28(14)	163(80)	4(2.8)	1(0.7)	18(26)	6(5.3)	29(20)	12(10)
# L w/ (%)	4(17)	2(9)	1(4.5)	9(38)	21(88)	2(9.5)	1(4.8)	7(41)	4(20)	8(38)	2(10)
litter %	1.7±3.9	1.8±6.7	0.4±1.6	9.2±19	55±23	2.0±8.2	0.8±3.6	9.5±14	2.6±6.3	13±22	6.6±20
Gen. retard.											
# F w/ (%)	3(1)	1(0.4)	2(0.8)	2(0.7)	36(12)	7(3)	2(0.9)	5(3)	1(0.5)	25(11)	59(33)
# L w/ (%)	3(13)	1(4.5)	2(9.1)	2(8.3)	8(33)	3(14)	2(10)	2(12)	1(5)	7(33)	11(55)
litter %	.98±2.5	0.4±1.7	0.8±2.6	0.7±2.5	10.7±22	2.7±7.2	1.2±6.0	2.5±7.8	0.4±1.7	12.5±23	37±36
Kidney DPB											
# F w/ (%)	0	0	0	0	14(14)	0	0	0	0	0	14(24)
# L w/ (%)	0	0	0	0	7(29)	0	0	0	0	0	9(45)
litter %	0	0	0	0	4.2±8.7	0	0	0	0	0	7±11
Kidney DPU											
# F w/ (%)	1(.03)	0	1(1.2)	1(1.0)	13(13)	0	2(2.9)	0	0	0	9(15)
# L w/ (%)	1(4.3)	0	1(4.5)	1(4.2)	10(42)	0	2(9.5)	0	0	0	6(30)
litter %	0.4±1.7	0	0.5±2.1	0.4±1.7	4.4±7.3	0	0.8±2.5	0	0	0	5.7±10
Testes DT											
# F w/ (%)	0	0	2(0.7)	0	16(5.3)	1(0.5)	0	0	0	0	5(2.8)
# L w/ (%)	0	0	2(9.1)	0	8(33.3)	1(4.8)	0	0	0	0	4(20)
litter %	0	0	0.9±2.8	0	5.1±8.9	0.6±2.7	0	0	0	0	4±10
Testes FDT											
# F w/ (%)	0	0	0	0	3(1.0)	0	0	0	0	0	20(11)
# L w/ (%)	0	0	0	0	1(4.2)	0	0	0	0	0	8(40)
litter %	0	0	0	0	.96±4.7	0	0	0	0	0	8.3±13

D. Discussion

In the three 500 mg/kg dose level groups, dams displayed an unsteady gait, dragging of the rear limbs, paresis of the rear limbs, and diminished sensitivity to pain in the affected limbs. Both body weight and body-weight gain were decreased in these 3 groups also when compared to their concurrent and the combined control groups. With regard to gross pathological changes, a loss of perirenal adipose tissue was observed in both 500 mg/kg groups that contained exogenous ETU and at the 133.33 mg/kg dose level with exogenous ETU. Although this lesion was observed in treated groups only, it occurred only in the 1978 studies and, due to the lack of any significant difference between the amount of ETU in the groups displaying 100% incidence (exogenous ETU-10 mg/kg; total ETU-47.5 mg/kg) and 48% incidence (exogenous ETU-3.75 mg/kg; total ETU-41.25 mg/kg) compared with 6% incidence (exogenous ETU-1 mg/kg; total ETU-10 mg/kg) and 0% incidence (exogenous ETU-0.5 mg/kg; total ETU-38 mg/kg and exogenous ETU 3.75 mg/kg; total ETU-14 mg/kg), no definitive statement can be made regarding exogenous ETU and the occurrence of this lesion.

There was no difference in pregnancy rate or in the number of corpora lutea/dam among the groups when compared to their concurrent control groups or to the concurrent control groups. When compared to the combined control groups, the 500 mg/kg dose level with 2% exogenous ETU displayed fewer implantations/dam than the UT control only. The percent of litters with non live-implants (NLI)

and the # litters with resorptions/# total litters were both increased at 187.5+2% ETU, 500+0.75% ETU, and 500+2% ETU compared to the DT control, but both 500 mg/kg groups showed a smaller increase than the 187.5 mg/kg dose level. Fetal body weight was decreased in two of the 500 mg/kg dose groups (0.5 and 2% exogenous ETU) compared to the DT control, and body length was decreased in these same groups compared to both combined control groups.

When these parameters were compared to their respective concurrent control groups, no differences were noted in the # implantations/dam or # resorptions/dam. There was a dose-related increase in the total # of dead implants/dam at the 187.5+0.75% ETU and 500+2% ETU dose levels but not at the 500+0.75% ETU dose level. Statistical significance was not attained in the dose-related increase observed in this parameter in the 1975 study, but there was a significant increase in the mean litter % of dead implantations at the 500 mg/kg dose level. The # live fetuses/dam was decreased at the 500+2% ETU dose level, and the mean litter % of live fetuses was decreased in a dose-related manner in the 1975 study (statistical significance was attained at the low dose only).

Due to the lack of information on (1) the number of fetuses with multiple anomalies at the 500 mg/kg dose level in Study # 522 and (2) the identity (and number of fetuses with each) of the anomalies observed in the 172/177 fetuses at the 500 mg/kg dose level in Study # 524, a comparison between Groups 5 (Maneb technical) and 9 (Maneb plus 2% ETU) with respect to anomalies cannot be made.

Because of the lack of consistency in the results from the studies run in 1975 and 1978, TB II suggests these studies should not be combined; i.e., the control groups should not be combined and compared to the results observed in the treated groups. The data from Study # 522 can be compared with that from Studies # 523 and 524 to determine an overall assessment of the developmental toxicity potential of Maneb itself and a limited assessment of the effect of added ETU. The latter assessment would involve comparing the two 500 mg/kg dose levels in Studies # 523 and 524, which were composed of 0.75% (3.75 mg/kg) and 2% (10 mg/kg) exogenous ETU, respectively.

E. CONCLUSION

There was no effect on the survival of the dams following dosing with Maneb on gestation days 6 through 15 at dose levels of 30, 100, 133.33, 187.5, and 500 mg/kg. The number of corpora lutea were comparable among the groups, as were the number of implantations. Maneb was maternally toxic at the 500 mg/kg dose levels, with and without exogenous ETU, as evidenced by unsteady gait, dragging of the rear limbs, diminished sensitivity to pain in the affected limbs, paresis of the rear limbs, and decreased body weight/body-weight gain. Additionally, a dose of 500 mg/kg resulted in a decrease in the number of live fetuses in the 500 mg/kg + 2% ETU

group only), an increase in the number of dead fetuses and dead implantations/dam, decreased fetal weight and body length, decreased placenta weight, and an increase in anomalies/variations/ retardations. At the next highest dose level (187.5 mg/kg), decreased maternal body weight and placenta weight were noted, although this group showed a significant decrease in body weight compared to the controls prior to dosing. Additionally, a significant increase in dead implantations/dam was observed at this dose level.

These three studies are classified Core supplementary, pending submission of (1) information on the number of fetuses with multiple anomalies at the 500 mg/kg dose level in Study # 522; (2) the identity (and number of fetuses with each) of the anomalies observed in the 172 fetuses at the 500 mg/kg dose level in Study # 524; (3) individual data for each fetus from all 3 studies; (4) individual data for each dam with respect to the number of corpora lutea and implantations; (5) clarification of the discrepancies/ errors enumerated in DER and in Appendix A, appended to the DER; (6) clarification of the amount of exogenous/endogenous ETU in the various groups in Studies # 523 & 524 (listed as mg of ETU in the combined study report); (7) Batch numbers for the Maneb test material used in each study; (8) a description of the test material used in each study; (9) information on dose preparation, concentrations attained, and analytical data; and (10) a statement on how the practices of the testing facility compare to the GLP's. These 3 studies do not satisfy the guideline requirement (83-3) for a developmental toxicity study in rodents.

APPENDIX A

DISCREPANCIES/ERRORS/PROBLEMS

1. page 9, at *, the statement is not correct. #522 & 523 did not share controls; # 523 & 524 had concurrent controls;
2. pages 29-44, results are presented on these pages, but all tables are labeled as Table 1 or Table 2 - for example, page 29 (Table 1), page 31 (table 1), page 35 (Table 2), page 36 (Table 1), pages 37-40 and 42-44 (each labeled Table 2);
3. pages 32 & 36, level of significance is stated to be 0.001 in several cases; this should probably be 0.01;
4. page 39, "of ETU" should be added to the last line;
5. page 40, for Group 7 under Litters with resorptions per total litters (%), the % is in error; i.e., 11/21 = 52.4, not 42.4.
6. page 45, no data listed for Group 9; Group 8 is listed twice (once as 187.5 mg/kg with no findings, then as 500 mg/kg);
7. page 46, ** stated to be <0.001, instead of 0.01;
8. page 47, fetal length has the units of grams;
9. page 65, # litters with resorptions for Group 5 should be 10, not 9 (p573); # for Group CMC should be 24, not 25 (p 570 & 775);
10. page 65, Summary Table-Group 5 listed with 25 litters; original report indicates 25 pregnant animals, but data are given for 24 litters (one litter contained all dead fetuses);
11. page 69, for Group 9, 3 fetuses are listed with short tail, but the original report lists 2;
12. page 592, Table 38 (summary table) lists 60 fetuses with ZV (short toes); Tables 74 & 77 list the individual litters. Ten dams had fetuses with short toes, and the number listed adds to 60; however, dam # 110 shows 17 fetuses, but 31 are listed with short toes (?);
13. pages 629-31, corpora lutea/dam and total implantations/dam are in error;
14. page 777, (Table 016 of original #524 study) Dam 87 of the 500 mg/kg group appears to have 4 dead, 2 aborted, and one each for early & intermediate resorptions, but 6 dead fetuses are indicated on page 773 for Group 500 mg/kg;
15. page 573, Table 19, the #'s appear to be listed under A (aborted) instead of D (dead), although none of the dams were reported to have aborted;
16. page 568. rows of #'s do not line up with parameters;
17. page 568, (Table 14 of original report # 522)-total dead implantations for untreated control is listed as 6 (0.35/dam), but 6 (early resorptions) & 2 (intermediate resorptions) are shown (23 dams, 8÷23=0.35);
18. pages 580-581 (Tables 26 & 27, resp.), both are labeled for Test Group 4; from dam #'s, Table 27 is Group 5.
19. pages 574-576 (Tables 20-22, resp.), no units are provided, and each table is "labeled" WEIGHT (apparently, Table 20 is body weight, Table 21 should read length, Table 22 is placenta weight);
20. pages 582-583 (Tables 28 & 29, resp.), data presented on these two pages are continuous; i.e., individual pup weights (fetus # 1-15 and 16-7) for the untreated control; other groups also have their data listed on single or two pages, with each page listed as

a different Table;
21. page 628 (Table 74), the data appear to be the # of fetuses in a given litter that displayed each anomaly; for Dam # 110, 17 live fetuses are listed, but for the anomaly ZV (short toes), 31 are listed????;
22. pages 692-3 (Tables 017 & 018 of original report of # 523), the fetal body weight/length for the ccmbined sexes is greater than the mean body weight for either sex alone;????? ; also, N=19 for males, 20 for females, and 21 combined and the # per litter was calculated using only those litters that had the sex indicated;
23. page 723 is a duplicate of page 722;
24. page 140, the heading "Multiple malformations" - the #'s do not line up correctly under the litter the # belongs to;
25. page 793, Table 032 lists 15 for Group 4 (Kidney-DPB), but Tables 047-048 (individual data) show 14;
26. Much of the data in the original reports could not be deciphered.

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Page _____ is not included in this copy.

Pages 25 through 31 are not included in this copy.

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___ Identity of product inert ingredients

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___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

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