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

Office of Prevention, Pesticides and Toxic Substances

HED DOC. NO. 013837

DATE: November 15, 1999

MEMORANDUM

SUBJECT: **MANEB**: Report of the Hazard Identification Assessment Review Committee on Maneb

FROM: Linda L. Taylor, Ph.D.
Reregistration Branch I
Health Effects Division (7509C)

THRU: Jess Rowland, Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Christina Swartz
Reregistration Branch I
Health Effects Division (7509C)
and
Anne Overstreet
Chemical Review Manager
Special Review and Re-registration Division (7508W)

PC Code: 014505

On May 4, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee [HIARC] evaluated the toxicology data on Maneb and selected toxicological endpoints and dose levels of concern appropriate for use in risk assessments for different exposure routes and durations based on the available data, for acute dietary, chronic dietary, and occupational/residential exposure. The Committee also assessed both the acute and chronic reference dose [RfD] for Maneb and addressed the potential enhanced sensitivity of infants and children from exposure to Maneb, as

required by the Food Quality Protection Act [FQPA] of 1996. The conclusions reached at the meeting are presented in this report.

With respect to the Toxicology Endpoint Selection, the HIARC determined that (1) the acute neurotoxicity study should be used for deriving the acute reference dose for acute dietary risk assessment for **the General Population [including Infants and Children]**; (2) the rat developmental toxicity study should be used for deriving the acute reference dose for acute dietary risk assessment for **Females 13+**; (3) the mouse carcinogenicity study should be used for deriving the chronic reference dose for chronic dietary risk assessment; (4) the 21-day dermal toxicity study should be used for deriving the short-term, the intermediate-term, and long-term dermal exposure risk assessments; and (5) the subchronic oral toxicity study in rats should be used for deriving the inhalation [any time period] exposure risk assessment. **NOTE:** Maneb was not evaluated previously by the Health Effects Division's Toxicology Endpoint Selection Committee [TES].

Committee Members in Attendance

Members present were: Robert Fricke, Karen Hamernik, Susan Makris, Nancy McCarroll, Mike Ioannou, PV Shah, Virginia Dobozy, Pam Hurley, David Anderson, Nicole Paquette, Jess Rowland [Co-Chaiman], Brenda Tarplee [Executive Secretary]. Member *in absentia* Kathleen Raffaele, Pauline Wagner, Tina Levine. Data were presented by Linda Taylor of Reregistration Branch I.

Also in attendance were Whang Phang, Christina Swartz, Michael Metzger, Jeff Dawson, Randy Perfetti.

Data Presentation and Report Presentation: _____

Linda L. Taylor, Ph.D.
Toxicologist

Report Concurrence: _____

Brenda Tarplee
Executive Secretary

Chemical Name: MANEB

I. INTRODUCTION

On May 4, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee evaluated the toxicology data base of Maneb, established a Reference Dose (RfD) and selected the toxicological endpoints for acute and chronic dietary, as well as occupational, exposure risk assessments. The HIARC also addressed the potential increased susceptibility of infants and children from exposure to Maneb as required by the Food Quality Protection Act (FQPA) of 1996.

II. HAZARD IDENTIFICATION

A. ACUTE DIETARY (Acute Reference Dose)

(1) Acute Reference Dose (RfD) - General Population [including Infants and Children]

Study Selected: acute neurotoxicity - rat **Guideline #:** 870.6200; §81-1

MRID No.: 43947601

Executive Summary: The administration of a single dose of Maneb Technical [91.2%] via gavage to Sprague-Dawley Crl:CD®BR rats [12/sex/group] at dose levels of 0, 500, 1000, and 2000 mg/kg resulted in a slight decrease in body-weight gain [♂♂ 89%/♀♀ 84% of control overall] in both sexes at the high dose, which may have resulted in the slight impairment of forelimb grip strength in females at the high-dose level. However, since decreased grip strength, as well as limb weakness, impaired mobility, dragging of hindlimbs, unsteady gait, and paralysis of hindlimb, have been observed in several studies following repeat exposure to Maneb, this finding cannot be discounted. NOTE: No cholinesterase or NTE measurements were obtained.

The NOAEL for systemic toxicity is 1000 mg/kg, and the LOAEL for systemic toxicity is 2000 mg/kg, based on decreased body-weight gains. The NOAEL for acute neurotoxicity is 1000 mg/kg, and the LOAEL is 2000 mg/kg, based on the slight impairment in forelimb grip strength in females.

Dose and Endpoint for Risk Assessment: NOAEL of 1000 mg/kg; slight impairment of forelimb grip strength at 2000 mg/kg.

Comments about Study/Endpoint: The endpoint is appropriate for acute dietary exposure because the effects were observed after one exposure. Although decreased body weight may have resulted in the slight impairment of forelimb grip strength in females at the high-dose level, due to the fact that decreased grip strength, as well as limb weakness, impaired mobility, dragging of hindlimbs, unsteady gait, and paralysis of hindlimb, have been observed in several studies following repeat exposure to Maneb, this finding cannot be discounted. It is to be noted that the oral LD_{50} for Maneb is greater than 5 grams. Additionally, dams in the rat developmental toxicity study displayed neurobehavioral clinical

signs by day 11 [following 6 days of dosing at 500 mg/kg/day; incidence increased with time of exposure and persisted]. NOAEL for this effect was 100 mg/kg/day.

Uncertainty Factor (UF): 100 (10X for interspecies, 10X for intraspecies)

$$\textbf{Acute RfD} = \frac{\text{1000 mg/kg [NOAEL]}}{\text{100 [UF]}} = \textbf{10 mg/kg}$$

(2) Acute Reference Dose (RfD) - Females 13+

Study Selected: rat developmental toxicity **Guideline #:** 870.3700; §83-3(a)

MRID No.: 42520001

Executive Summary: In a developmental toxicity study [MRID 42520001], 25 Sprague-Dawley Crl:CD®BR female rats/group were administered Maneb [90.4% a.i.] *via* gavage at dose levels of 0, 20, 100, and 500 mg/kg/day from gestation days 6 through 15.

There were no apparent treatment-related deaths, although one high-dose dam was euthanized on gestation day 16 for "humane reasons". At the 500 mg/kg/day dose level, neurobehavioral clinical signs [impaired mobility (all dams), dragging of the hindlimbs (80%), hunched posture (76%), unkempt appearance (72%), excessive chewing (60%), and prostration (16%)] were observed by day 11 of gestation, which increased in incidence with time during dosing, and persisted throughout the study in about half of the dams. Other clinical signs observed at the 500 mg/kg/day dose level were soft stool (96%), decreased defecation (72%), red material on the forelimbs, eyes, and nose, red/yellow staining on the urogenital area, and paleness/body cool to the touch (2 dams). Soft stool was also observed at the mid-dose level.

Decreased body weight was observed in the high-dose dams from day 7 on, and the magnitude of the difference increased with time [76%-97% of control]. The mid-dose dams displayed a slight [94-96% of control] decrease in body weight compared to the control dams also. Body-weight gain [overall; days 0-20] was decreased at the mid- [86% of control] and high-dose [41% of control] levels compared to the control, and during the exposure phase, the mid-dose dams displayed a decrease [71% of control] and the high-dose dams displayed a negative body-weight gain compared to the controls. Terminal body weights were also decreased at the mid- [94% of control] and high- [78% of control] dose levels, net body weight [77% of control] was decreased at the high-dose level, and the net body-weight change at the high-dose level was minus 2 grams compared with a 67-gram gain for the control dams. Food consumption at the mid- [mainly during dosing period] and high- [throughout study] dose levels was decreased compared to the control values.

The numbers of pregnant females with offspring at termination, corpora lutea, implantation sites, and live fetuses were comparable among the groups, and there were no dead fetuses. There was a dose-related increase in total resorptions and in resorption/dam at the mid- and high-dose levels, with the mid-dose displaying the highest percent of early resorptions and the high-dose displaying the highest percent of

late resorptions, the latter exceeding the concurrent and historical control. With respect to late resorptions, the crown-rump length of the one late resorption in the control measured 3.1 cm, while the greatest length observed in the 13 late resorptions in the high-dose group was 2.3 cm [average 1.8 cm]. 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]. Numerous skeletal variations were observed in the high-dose group only. The majority of the skeletal variants at the high-dose level are indicative of a developmental delay, as evidenced by reduced ossification of various skeletal structures, and many of the findings at the high dose exceed the maximum values of the historical control.

The maternal NOAEL is 20 mg/kg/day, and the maternal LOAEL is 100 mg/kg/day, based on increased clinical signs [soft stool], decreased body-weight gain and food consumption. The developmental NOAEL is 20 mg/kg/day, and the developmental LOAEL is 100 mg/kg/day, based on increased post-implantation loss, increased resorption [total and resorption per dam], and decreased fetal viability. At the high-dose level, in addition to the maternal effects at the LOAEL, loss of body weight, neurobehavioral signs, and the death of one dam were observed. Developmental effects observed at the high-dose level included decreased fetal body weight and an increased incidence of malformations [bent limb bones] and developmental variations [retarded skeletal ossification and bent ribs].

Dose and Endpoint for Establishing Acute RfD: NOAEL of 20 mg/kg/day; decreased fetal viability.

Comments about Study/Endpoint: In order to be protective of females 13+, this endpoint was selected. At the high-dose level [500 mg/kg/day], fetal malformations [bent limb bones], maternal neurobehavioral signs, and one death were observed.

Uncertainty Factor (UF): 100 (10X for interspecies, 10X for intraspecies)

$$\textbf{Acute RfD} = \frac{\text{20 mg/kg [NOAEL]}}{\text{100 [UF]}} = \textbf{0.2 mg/kg}$$

This Risk Assessment is Required.

B. CHRONIC DIETARY [Reference Dose (RfD)]

Study Selected: mouse carcinogenicity **Guideline #:** 870.4200/§83-2

MRID No.: 42642401

EXECUTIVE SUMMARY: In a carcinogenicity study [MRID 42642401], 75 Crl: CD-1®(ICR)BR mice/group were administered Maneb [89.5% a.i.] *via* the diet at dose levels of 0, 60 [males 8.6/females 10.8 mg/kg/day], 240 [males 34.8/females 45.0 mg/kg/day], and 2400 [males 354.7/females 439.3 mg/kg/day] ppm [interim sacrifice of 20 mice/sex/group at weeks 52-53] for at least 78 weeks [≈18 months].

There were no apparent treatment-related deaths, although twice as many males at the mid- and high-dose levels died as died in the control and low-dose groups. Clinical signs were comparable among the groups [both sexes]. Slight decreases in body weight were observed in the mid-dose females [96% of control] at various times during the study and in both sexes at the high-dose level throughout the study [males 94%-97%/females 92%-97% of control]. Body-weight gain [overall] was decreased at the high-dose level [males 90%/females 84% of control], with the main decreases occurring initially. During the week 1-13 interval, body-weight gain was 91% of control for the mid-dose females, 85% of control for the high-dose females, and 76% of control for the high-dose males. Terminal body weights were comparable among the males, but the high-dose females displayed a slight decrease [93% of control] compared to the controls.

In both sexes, there was an apparent treatment-related decrease in erythrocyte counts, hemoglobin, and hematocrit values at the high-dose level at the interim and terminal sacrifices, although statistical significance was not always attained. Mean thyroxine [T_4] values were decreased in both sexes at the high-dose level at both the interim and terminal sacrifices compared to the control values, and the females displayed a dose-related decrease at the terminal sacrifice with all dose levels attaining statistical significance. There were no apparent treatment-related differences in either triiodothyronine [T_3] or thyroid stimulating hormone [TSH] at either sacrifice.

Increased thyroid weights [absolute and relative] were observed at the high-dose level in both sexes at the terminal sacrifice. There was a statistically significant, dose-related, decrease in absolute brain weight at termination in the mid- and high-dose females, but the increased relative brain weight was not statistically significant. There was no treatment-related increase in any microscopic lesion in the thyroid of either sex, although the high-dose females displayed an increased incidence of amyloid deposition compared to the control females. There was a treatment-related increase in the incidence of hepatocellular adenomas in both sexes at the high-dose level at the terminal sacrifice and when all mice are considered. In males, there was an apparent increase [doubling] in the incidence of alveologenic adenomas in the high-dose males at termination and when all mice are considered, although statistical significance was not attained. The dose levels are considered adequate for an assessment of the carcinogenicity potential of Maneb in mice, based on decreased body-weight gains during the first 13-week period.

No NOAEL was determined in this study. There was a dose-related decrease in the mean thyroxine [T_4] values in females at study termination [LOAEL 60 ppm (10.8 mg/kg/day)], and a NOAEL for this effect in females was not attained. At the high-dose level [2400 ppm (males 354.7/females 439.3 mg/kg/day)], both sexes displayed an increased incidence of hepatocellular adenomas, and the high-dose males displayed an apparent increase in alveologenic adenomas in the lungs.

Dose and Endpoint for Establishing Chronic RfD: LOAEL = 8.6 mg/kg/day, based on thyroid [target organ] effects [decreased T4]

Uncertainty Factor (UF): 300 (10X for interspecies, 10X for intraspecies, 3X for use of LOAEL)

$$\textbf{Chronic RfD} = \frac{8.6 \text{ mg/kg/day}}{300} = \textbf{0.029 mg/kg/day}$$

Chronic Reference Dose RfD: 0.029 mg/kg/day

Comments about Study/Endpoint: The NOAELs [≈5 mg/kg/day] in studies in the dog, monkey, and rat are supportive of this dose. The thyroid is a target organ for Maneb, and the EBDCs in general. The NOAEL for thyroid effects in the (1) **chronic dog study** is 200 ppm [males 6.36/females 7.18 mg/kg/day], and the LOAEL is 1000 ppm [males 33.84/females 35.25 mg/kg/day], based on clinical chemistry parameters indicative of thyroid toxicity, increased thyroid weight, and follicular (thyroid) hyperplasia; (2) **6-month monkey study** is 100 ppm [5.2-5.7 mg/kg/day; 7.3 mg/kg/day from JMPR, 1993], based o increased thyroid weight in males at the LOAEL of 300 ppm [15.5-16.8 mg/kg/day; (3) **90-day rat study** is 80 ppm [males 5/females 6 mg/kg/day], based on increased thyroid weights and follicular cell hyperplasia in males and decreased T_4 at 400 ppm [males 24/females 30 mg/kg/day]; (4) **2-generation reproduction study** is 75 ppm (F0 5.3/F1 5.8 mg/kg/day), based on an increased incidence of diffuse follicular epithelial hypertrophy/hyperplasia [F1] in males at the paternal LOAEL of 300 ppm (F0 21.2/F1 23.1 mg/kg/day).

This Risk Assessment is Required.

C. OCCUPATIONAL / RESIDENTIAL EXPOSURE–DERMAL

Percentage (%) Dermal Absorption: 1%

1. DERMAL ABSORPTION

Study Selected: dermal absorption **Guideline #:** §85-2; 870.7600

MRID No.: 41669301

Executive Summary: In a dermal absorption study [MRID 41669301], radiolabeled Maneb [91% a.i.] was applied dermally to male Charles River rats [4/dose/exposure period] on the anterior dorsal region [application site 10 cm^2] at dose levels of 0.1, 1.0, 10.0 mg/rat [suspensions in "Inert Ingredients for MANEB PLUS ZINC F4"] and 0.1 mg/rat [deionized water] for 0.5, 1, 2, 4, 10, and 24 hours. Following the exposure period, the rats were sacrificed and the application site was washed four times. There were three groups of rats that were exposed for 24 hours, with one group being sacrificed after 24, 48, and 72 hours. Those in the 48- and 72-hour groups had their application sites washed as above after the 24-hour exposure period, and these rats were placed into metabolism cages and urine and feces were collected for 24-hour durations prior to their sacrifice time.

Dermal absorption of Maneb is relatively small. Following exposure to Maneb using a formulation vehicle suspended in water, dermal absorption [as a % of dose] was below the limit of detection for the

first 10 hours. In order to produce measurable absorption, a total exposure period of 72 hours, which included 24 hours of Maneb exposure and a wash, was required. Four to five times as much Maneb [applied as an aqueous solution] remains on/in skin after washing as found following exposure to the formulation-vehicle suspension. Time-related dermal absorption was greater with the aqueous Maneb solution than with the formulation vehicle suspension. The amount absorbed increased with time of exposure, ranging from <0.1% following a half-hour exposure to 5.1% following a 24-hour exposure period, a wash, and sacrifice 48 hours later. Measurable quantities of the dose remain on the skin following a wash and, when applied as an aqueous solution, Maneb appears to remain on the skin for continued absorption. In general, whole blood/plasma concentrations were below the limit of detection. For risk assessment, a dermal absorption value of 1% is adequate.

---

2. SHORT-TERM DERMAL (1 - 7 days)

Study Selected: 21-day dermal toxicity study **Guideline #:** §82-2/870.3200

MRID No.: 40876101

Executive Summary: In a 21-day dermal toxicity study [MRID 40876101], 5 Hra:(NZW)SPF rabbits/sex/group were administered Maneb [86% a.i.] *via* dermal application [once a day for 6 hours, 5 days/week] for 15 days at concentrations of 0, 100, 300, or 1000 mg/kg/day.

There were no deaths, and clinical signs, body-weight, food consumption, hematology, serum chemistry, and organ weights [except the thyroid] were comparable among the groups [both sexes]. The high-dose males displayed a decrease in body-weight gain overall [61% of control; not statistically significant], but the females displayed comparable body-weight gains among the groups. Increased thyroid weight [absolute (130% of control) and relative (132% of control)] was observed in males at the high-dose level. Comparable thyroid weights were observed among the female groups. There was a dose-related increase in testes weight in males. Thyroid follicular cell hypertrophy was observed in all high-dose males and in two high-dose females. Increased colloid material was observed in four high-dose, two mid-dose, and one in both the low-dose and control females. Slight dermal irritation was observed in both sexes at all dose levels but not in the controls, and the females displayed a greater effect than the males. T_3, T_4, and TSH were not assessed in this study.

The NOAEL for systemic effects is 100 mg/kg/day, and the LOAEL is 300 mg/kg/day, based on microscopic thyroid changes [both sexes]. Slight dermal irritation was observed at all dose levels at the site of application.

Dose and Endpoint for Risk Assessment: NOAEL of 100 mg/kg/day; microscopic thyroid changes [increased colloid material] in both sexes at LOAEL of 300 mg/kg/day. At the high dose level [1000 mg/kg/day], follicular cell hypertrophy was observed in all males and in 2 of 5 females, and increased thyroid weights [absolute and relative] were observed in males.

Comments about Study/Endpoint: Although T_3, T_4, and TSH were not assessed in this study, the study is considered adequate based on the fact that microscopic lesions in the thyroid and thyroid weight are

observed in other [oral] studies of varying duration at or below dose levels where changes in clinical chemistry parameters indicative of thyroid effects [T_3, T_4, ^{131}I, and/or TSH] are observed. Additionally, the lowest dose where thyroid effects have been observed is≈10 mg/kg/day [monkey, **based on increased thyroid weight**. NOTE: ^{131}I absorption decreased/microscopic lesions in thyroid were observed only at HDT (300 ppm) 145/171 mg/kg/day]. The no-effect dose range for thyroid effects in the available studies is ≈5 mg/kg/day -≈20 mg/kg/day [NOAEL in 90-day rat study (80 ppm) 5 mg/kg/day (males)/6 mg/kg/day (females); increased thyroid weights/follicular cell hyperplasia in males; decreased T_4 both sexes at LOAEL of 400 ppm [males 24/females 30 mg/kg/day]; in chronic rat (300 ppm) 20.4 (males)/21.8 (females) mg/kg/day; thyroid effects: increased half-life for ^{131}I-retention, decreased mean T_4, increased thyroid weight in both sexes only at HDT 1000 ppm (68/75 mg/kg/day); in 2-generation reproduction (5.3 mg/kg/day/75 ppm), thyroid hyperplasia observed in males at 300 ppm (21 mg/kg/day); T_3, T_4, ^{131}I, and/or TSH not measured]; in the mouse carcinogenicity study, no NOAEL for decreased T_4 in females was attained [LOAEL 60 ppm/10.8 mg/kg/day (females). Using a dermal absorption factor of 2% for Maneb, the high dose in the 21-day dermal study equates to 20 mg/kg/day, and the low dose equates to 2 mg/kg/day. *NOTE: Dams in the rat developmental toxicity study displayed neurobehavioral signs [impaired mobility, dragging of hindlimbs] following oral exposure during gestation days 6-15 at a dose level of 500 mg/kg/day [NOAEL for this effect 100 mg/kg/day]. But as noted above, dermal absorption is low, and the 21-day dermal study provides a more conservative value for this risk assessment.*

A known target organ was affected following dermal exposure, and the endpoint and route of exposure are appropriate. Thyroid effects are observed following exposure to Maneb *via* the oral route of exposure in all species examined and following both short and long-term exposure. Maneb is also a dermal sensitizer.

This Risk Assessment is Required

---

3. INTERMEDIATE-TERM DERMAL (1-Week to Several Months)

Study Selected: 21-day dermal toxicity study **Guideline #:** §82-2/870.3200

Dose and Endpoint for Risk Assessment: NOAEL of 100 mg/kg/day; microscopic thyroid changes [increased colloid material] in both sexes at LOAEL of 300 mg/kg/day.

Comments about Study/Endpoint: see Short-Term

This Risk Assessment is Required

---

4. LONG-TERM DERMAL (Several Months to Lifetime)

Study Selected: 21-day dermal toxicity study **Guideline #:** §82-2/870.3200

Dose and Endpoint for Risk Assessment: NOAEL of 100 mg/kg/day; microscopic thyroid changes [increased colloid material] in both sexes at LOAEL of 300 mg/kg/day.

Comments about Study/Endpoint: see Short-Term

This Risk Assessment is Required.

---

5. OCCUPATIONAL / RESIDENTIAL EXPOSURE—INHALATION (ANY-TIME PERIOD)

Study Selected: subchronic oral - rat **Guideline #:** §82-1(a); OPPTS 870.3100

MRID No.: 40982601

Executive Summary: In a subchronic oral toxicity study [MRID 40982601], 15 Sprague-Dawley Crl:CD®BR rats/sex/group were administered Maneb [77.9% a.i.; reported as 86% by Sponsor] *via* the diet for 13 consecutive weeks at concentrations of 0, 80 ppm [males 5/females 6 mg/kg/day], 400 ppm [males 24/females 30 mg/kg/day], and 1300 ppm [males 77/females 103 mg/kg/day]. Five rats/sex/group were used in the 4-week recovery phase of the study, and the other 10 rats/sex/group were sacrificed at 14 weeks.

There were no deaths, and clinical signs in the males were comparable among the groups. Tremors were observed in 2 mid-dose and 2 high-dose females during weeks 5, 8, 9, and 12. Decreased body weight [84% of control] was observed in the high-dose females at week 13, and decreased body-weight gains [males 89% /females 66% of control] were observed in the high-dose rats of both sexes overall [weeks 0-13]. Decreased body-weight gain was observed only initially in males [weeks 0-4: 83% of control] but throughout the study in females [weeks 0-4: 61%/weeks 4-8: 67%/weeks 8-13: 76% of control]. During the recovery period, both sexes of the high-dose group gained significantly more weight than the control group [males 175%/females 228% of control]. A slightly lower food consumption was observed for both sexes at the high dose. No treatment-related effects were observed on the ophthmological or hematology parameters monitored. Following 13 weeks of dosing, there was a dose-related decrease in T_4 and a dose-related increase in TSH in both sexes, although statistical significance was attained only for T_4 at the high-dose level in females. Following the recovery period, T_4 and TSH values were comparable among the groups for both sexes. The high-dose males displayed an increase in T_3 following exposure, and the high-dose females displayed a slight increase also, although neither increase attained statistical significance. There were no other treatment-related effects observed in either sex in the clinical chemistry parameters monitored. Thyroid weight [absolute] was increased in males at the mid-dose level and in both sexes at the high-dose level, although statistical significance was not attained for the female value. Relative thyroid weights were also increased at the high-dose level in both sexes, but only the male value attained statistical significance. Kidney weight [absolute] was increased at all dose levels in both sexes compared to the control [no dose response], and relative kidney weight was statistically significantly increased in the high-dose females. Treatment-related follicular cell hyperplasia was

observed in 1 mid-dose male, all 10 high-dose males, and in 2 high-dose females following 13 weeks of exposure. Four high-dose males also displayed increased colloid in the thyroids. Following the recovery period, 1 of the 5 high-dose males displayed a follicular cell adenoma. In the kidneys, all of the mid- and high-dose rats of both sexes and 2 of the 10 low-dose males displayed a tubular pigment. Following the recovery period, 3 of the 5 males and 4 of the 5 females at both the mid- and high-dose levels displayed this same pigment. None of the 5 recovery phase rats/sex at the low dose displayed this renal pigment.

The NOAEL is 80 ppm [males 5/females 6 mg/kg/day], and the LOAEL is 400 ppm [males 24/females 30 mg/kg/day], based on thyroid effects [increased thyroid weights and follicular cell hyperplasia in males and decreased T4.

Dose and Endpoint for Risk Assessment: 5 mg/kg, based on thyroid effects [increased thyroid weights and follicular cell hyperplasia in males and decreased T_4].

Comments about Study/Endpoint: The selected dose should be used for short-term, intermediate-term, and long-term inhalation risk assessments. An oral study was selected because the subchronic inhalation toxicity study is classified Unacceptable due mainly to the fact that the dose levels used were too low. Additionally, it was determined that the dose levels tested were too low, and it is noted that the TSH values were going in the wrong direction. The 13-week **oral** toxicity study was selected for use in this risk assessment, pending the submission of an adequate 90-day inhalation toxicity study. Since the selected dose was an oral dose, 100% absorption factor should be used for route-to-route extrapolation.

This Risk Assessment is Required.

D. Margins of Exposure

A margin of exposure (MOE) of 100 is considered to be adequate for occupational exposure risk assessments. The MOE for residential risk assessment will be determined by the FQPA Safety Factor Committee.

E. Recommendation for Aggregate Exposure Risk Assessment

For **acute exposure**, add the food and water using the high end exposure combined with the acute RfD. NOTE: There are two acute RfDs [females 13+ and the general population, including infants and children].

For **short-, intermediate-, and long-term exposures**, the aggregate systemic [oral], dermal, and inhalation exposure risk assessments are appropriate due to the common toxicological endpoint [thyroid effects] observed *via* the three routes of exposure.

$$\text{Total MOE} = \frac{1}{\frac{1}{} + \frac{1}{} + \frac{1}{}}$$

12

$MOE_{(oral)}$ $MOE_{(dermal)}$ $MOE_{(inhalation\text{-}oral\ equivalents)}$

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. In a combined chronic toxicity/carcinogenicity study in rats study [Accession No. 259628; MRID 00129979], precancerous dysplasia was observed in 7 control males and 11 high-dose males, papilloma in 3 high-dose males and in none of the control males, carcinoma of the squamous epithelium in 2 high-dose males and none of the control males, and pavement carcinoma in one high-dose male and none of the controls. One control male displayed a urinary bladder carcinoma and another a cysto-papillary tumor. A urinary bladder tumor [cysto-papillary tumor] was observed in one control female only. Pituitary adenomas were observed in 18 control females and 26 high-dose females. Pituitary carcinoma was observed in 2 control females and none of the high-dose females. There was no apparent increase in thyroid tumors in either sex. However, due to the lack of any significant effect on survival or body-weight gain during the first 90-day interval, it appears that the rats could have tolerated higher dose levels. It is concluded that the dose levels were not adequate for the assessment of carcinogenic potential. There was a significant increase in thyroid weight [main target organ] in both sexes at study termination [≈150% of control] at the high-dose level, although there were no histopathological findings in the thyroid. Effects on thyroid function [decreased T_4, increased ^{131}I retention] were evident at various times during the study, although statistical significance was not always attained. There were no adverse effects on survival, body weight/gain, food consumption, auditory, ophthalmological, hematological, clinical chemistry, and urinalysis parameters in either sex. **The *ad hoc* Committee [HED Document No. 013554] concluded that the rat study was not acceptable but did not recommend that the study be repeated at this time. However, the requirement to repeat the rat study is RESERVED.** Future requests for a re-evaluation of the classification of Maneb, based on a mechanistic approach to risk assessment would warrant/necessitate submission of adequate long-term studies to assess the full carcinogenic potential for Maneb.

2. In a carcinogenicity study in mice [MRID 42642401], there was a treatment-related increase in the incidence of hepatocellular adenomas in both sexes at the high-dose level at the terminal sacrifice and when all mice are considered. In males, there was an apparent increase [doubling] in the incidence of alveologenic adenomas in the high-dose males at termination and when all mice are considered, although statistical significance was not attained. The dose levels are considered adequate for an assessment of the carcinogenicity potential of Maneb in mice, based on decreased body-weight gains during the first 13-week period. During the week 1-13 interval, body-weight gain was 91% of control for the mid-dose females, 85% of control for the high-dose females, and 76% of control for the high-dose males. Body-weight gain [overall] was decreased at the high-dose level [males 90%/females 84% of control], with the main decreases occurring initially. The *ad hoc* Committee [HED Document No. 013554] concluded that the mouse study was acceptable.

3. Maneb has been tested in a series of *in vitro* and *in vivo* genotoxicity assays [MRIDs 40091302, 40091303, 259018,40091301, 259073, 265753, 259071, 40163901], and it is concluded that Maneb is capable of inducing genotoxic effects [review memo dated September 25, 1989, K. Dearfield to A.

Kocialski]. Additionally, as discussed above, Maneb and the other EBDCs are known to be converted to ETU. In a review of mutagenicity studies on ETU [Dearfield, K.L., Mutation Research 317 (1994), 111-132], it was concluded that ETU exhibits weak genotoxic potential.

4. Classification: The *ad hoc* HED Cancer Assessment Review Committee [CARC] concluded that because the EBDCs are known to be converted to ETU, each EBDC should be classified as **Group B2**, and after applying the metabolic conversion factor for EBDC to ETU of 0.075, the Q_1* of ETU will be used. It was also agreed that there was no need for an assessment of Maneb by the full CARC at this time. Based on the weight of evidence, the Committee is confident that the risk would not be underestimated with respect to cancer potential for all of the EBDCs using the ETU data. [HED Document No. 013554, dated July 7, 1999].

IV. FQPA CONSIDERATIONS

1. Neurotoxicity

In an **acute neurotoxicity study** [MRID 43947601], there was a slight impairment of forelimb grip strength in females at the high-dose level. Although this was accompanied by a slight decrease in body-weight gain, decreased grip strength, as well as limb weakness, impaired mobility, dragging of hindlimbs, unsteady gait, and paralysis of hindlimb, have been observed in several studies following repeat exposure to Maneb. Therefore, this finding cannot be discounted. NOTE: Cholinesterase and NTE were not measured in this study. NTE was affected in the subchronic neurotoxicity study in rats.

In a **subchronic neurotoxicity study** [MRID 43947602], high carriage, impaired mobility, and decreased fore- and hind-limb grip strength were observed in the high-dose females, there was a dose-related decrease in NTE in the males, and digestion chambers in the tibial nerve, sciatic nerve, and/or peroneal nerve was observed only in treated rats [both sexes].

EVIDENCE OF NEUROTOXICITY FROM OTHER ORAL TOXICITY STUDIES

In a **rat developmental toxicity study** [MRID 41965801], treatment-related clinical signs were observed at the 500 mg/kg/day dose levels, which consisted of unsteady gait, dragging of the rear limbs, diminished sensitivity to pain in the affected limbs, and paresis of the rear limbs.

In the definitive **rat developmental toxicity study** [MRID 42520001], neurobehavioral clinical signs [impaired mobility (all dams), dragging of the hindlimbs (80%), hunched posture (76%), unkempt appearance (72%), excessive chewing (60%), and prostration (16%)] were observed by day 11 of gestation at the 500 mg/kg/day dose level. These signs increased in incidence with time during dosing and persisted throughout the study in about half of the dams. Other clinical signs observed at the 500 mg/kg/day dose level were soft stool (96%), decreased defecation (72%), red material on the forelimbs, eyes, and nose, red/yellow staining on the urogenital area, and paleness/body cool to the touch (2 dams).

In the **mouse carcinogenicity study** [MRID 42642401], there was a statistically significant, dose-related, decrease in absolute brain weight at termination in the mid- [240 ppm; 45 mg/kg/day] and high-dose 2400 ppm; 439.3 mg/kg/day] females, and the increased relative brain weight was not statistically significant.

In a **subchronic oral toxicity study in rats** [MRID 40982601], tremors were observed in 2 mid-dose [400 ppm (30 mg/kg/day)] and 2 high-dose [1300 ppm (103 mg/kg/day)] females during weeks 5, 8, 9, and 12.

In the **chronic toxicity/carcinogenicity study in rats** [MRID 00129979], there was an increased incidence of microscopic lesions of the skeletal muscle in the high-dose [1000 ppm; 74.5 mg/kg/day] females compared to the controls [females 41 *vs* 20].

OTHER DATA FROM THE LITERATURE

There are published data/articles regarding human exposure to Maneb and its possible role in pathogenesis of idiopathic Parkinson's disease. Additionally, chronic manganese intoxication induces Parkinsonism among miners and some categories of manufacturers exposed to manganese ores. Disulfiram poisoning is associated with permanent extrapyramidal syndromes, and ethylene-bis-dithiocarbamate [EBDTC] and carbon disulfide are the main metabolites of Disulfiram. Carbon disulfide is a well-known neurotoxic compound that causes parkinsonism, and it can react with endogeneous amino acids and monoamines to produce dithiocarbamates. [Meco, G.; Bonifati, V.; Vanacore, N.; Fabrizio, E. (1994). Parkinsonism After Chronic Exposure to the Fungicide Maneb (manganese ethylene-bis-dithiocarbamate). Scand. J. Work Environ. Health 20, 301-305]

2. Developmental & Reproductive Toxicity

(I) Developmental Toxicity: In a developmental toxicity study [MRID 42520001], 25 Sprague-Dawley Crl:CD®BR female rats/group were administered Maneb [90.4% a.i.] *via* gavage at dose levels of 0, 20, 100, and 500 mg/kg/day from gestation days 6 through 15.

There were no apparent treatment-related deaths, although one high-dose dam was euthanized on gestation day 16 for "humane reasons". At the 500 mg/kg/day dose level, neurobehavioral clinical signs [impaired mobility (all dams), dragging of the hindlimbs (80%), hunched posture (76%), unkempt appearance (72%), excessive chewing (60%), and prostration (16%)] were observed by day 11 of gestation, which increased in incidence with time during dosing, and persisted throughout the study in about half of the dams. Other clinical signs observed at the 500 mg/kg/day dose level were soft stool (96%), decreased defecation (72%), red material on the forelimbs, eyes, and nose, red/yellow staining on the urogenital area, and paleness/body cool to the touch (2 dams). Soft stool was also observed at the mid-dose level.

Decreased body weight was observed in the high-dose dams from day 7 on, and the magnitude of the difference increased with time [76%-97% of control]. The mid-dose dams displayed a slight [94-96% of control] decrease in body weight compared to the control dams also. Body-weight gain [overall; days 0-

20] was decreased at the mid- [86% of control] and high-dose [41% of control] levels compared to the control, and during the exposure phase, the mid-dose dams displayed a decrease [71% of control] and the high-dose dams displayed a negative body-weight gain compared to the controls. Terminal body weights were also decreased at the mid- [94% of control] and high- [78% of control] dose levels, net body weight [77% of control] was decreased at the high-dose level, and the net body-weight change at the high-dose level was minus 2 grams compared with a 67-gram gain for the control dams. Food consumption at the mid- [mainly during dosing period] and high- [throughout study] dose levels was decreased compared to the control values.

The numbers of pregnant females with offspring at termination, corpora lutea, implantation sites, and live fetuses were comparable among the groups, and there were no dead fetuses. There was a dose-related increase in total resorptions and in resorption/dam at the mid- and high-dose levels, with the mid-dose displaying the highest percent of early resorptions and the high-dose displaying the highest percent of late resorptions, the latter exceeding the concurrent and historical control. With respect to late resorptions, the crown-rump length of the one late resorption in the control measured 3.1 cm, while the greatest length observed in the 13 late resorptions in the high-dose group was 2.3 cm [average 1.8 cm]. 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]. Numerous skeletal variations were observed in the high-dose group only. The majority of the skeletal variants at the high-dose level are indicative of a developmental delay, as evidenced by reduced ossification of various skeletal structures, and many of the findings at the high dose exceed the maximum values of the historical control.

The maternal NOAEL is 20 mg/kg/day, and the maternal LOAEL is 100 mg/kg/day, based on increased clinical signs [soft stool], decreased body-weight gain and food consumption. The developmental NOAEL is 20 mg/kg/day, and the developmental LOAEL is 100 mg/kg/day, based on increased post-implantation loss, increased resorption [total and resorption per dam], and decreased fetal viability. At the high-dose level, in addition to the maternal effects at the LOAEL, loss of body weight, neurobehavioral signs, and the death of one dam were observed. Developmental effects observed at the high-dose level included decreased viability and fetal body weight and an increased incidence of malformations [bent limb bones] and developmental variations [retarded skeletal ossification and bent ribs].

(ii) Rabbit Developmental Toxicity: In a developmental toxicity study [MRID 40982401], female Himalayan Chbb:HM rabbits [15/group] were administered Maneb [90.6% a.i. with 2% ETU] at dose levels of 0 [CMC (carboxymethylcellulose control)], 5 mg/kg/day, 20 mg/kg/day, and 80 mg/kg/day from gestation day 6 through gestation day 18.

One high-dose doe [not pregnant] died on day 8, and three high-dose does were sacrificed [1 aborted (day 21), 1 delivered early (day 28), and one was sacrificed moribund on day 23 (pregnant) following a considerable loss of body weight]. One mid-dose female [not pregnant] was sacrificed on day 16 because

of a dosing error. There were no treatment-related clinical signs.

In general, body weight was comparable among the groups throughout the study, although slight decreases [94%-95% of control] noted in the high-dose does attained statistical significance on gestation days 11, 23, and 25. Prior to dosing, the high-dose group displayed a significantly lower [1.6% of control] body-weight gain than the controls, and during the post dosing period also displayed a significantly lower body-weight gain [49% of control] than the control. During the dosing period, there was a dose-related increase in body-weight gain. Overall [gestation days 0-29], the high-dose does displayed a decreased body-weight gain [59% of control] compared to the controls. Corrected body-weight gain was comparable among the groups. Food consumption was decreased during the dosing period [78% of control] and overall [90% of control] at the high-dose level compared to the control.

Pregnancy rates were comparable among the groups. Only the control group [1 doe] had dead fetuses [7]. The high-dose had the lowest number of corpora lutea and implantations [per doe also], but statistical significance was not attained. The number of fetuses [42] and the number of fetuses/doe [3.82] were decreased significantly at the high-dose level compared to the control [79 and 6.08, respectively], and the total number of resorptions [21] and the number of resorption/doe [1.91] were increased at the high-dose level compared to the control [5 and 0.36, respectively]. Additionally, at the high-dose level, there was an increase in the percent of litters with resorptions [72.7%] compared to the control [38.5%]. Postimplantation loss was increased at the high-dose level [30.6%] compared to the control [14.4%]. All seven fetuses [entire litter] were resorbed in one high-dose doe. Fetal body weights and body lengths were comparable among the groups for both sexes. Only one runt [low dose] was observed. Placental weight of the high-dose female fetuses was increased compared to the control. Uterine weight was decreased at the high-dose level [65% of control]. The sex ratio was comparable among the groups. There were no apparent, treatment-related, effects on external, visceral, or skeletal variation/anomalies/retardations/ malformations.

No NOAEL or LOAEL were established for maternal or developmental toxicity in the rabbit due to deficiencies in the study.

This nonguideline developmental toxicity study in rabbits is classified Unacceptable. The study is nonguideline because too few animals/group [15/group] were used. It is classified Unacceptable mainly because of questions regarding the dose administered during the study. Other deficiencies include the lack of information on (1) the examination of the fetal head and brain, (2) sperm used for insemination, (3) animal assignment for the three phases of the study, and the fetal x-rays employed in the skeletal examinations were never submitted to the Agency for review. A new rabbit developmental toxicity study is required to assess the skeletal delays observed in the rat developmental toxicity study.

(iii) Reproductive Toxicity: In a 2-generation reproduction study [MRID 42049401], 28 [F0]/24 [F1] Crl: CD®(SD)BR VAF/Plus rats/sex/group were administered Maneb [87.3%/89.6%] via the diet [from age 6 weeks (F0)/4 weeks (F1)] for 70 days [F0]/84 days [F1] during the pre-mating period and through gestation and lactation for two generations at dose levels of 0, 75 [F0 males 5.3 /F0 females 6.0 mg/kg/day; F1 males 5.8/F1 females 6.4 mg/kg/day], 300 [F0 males 21.2/F0 females 24.1 mg/kg/day; F1 males 23.1/F1 females 25.1 mg/kg/day], and 1200 ppm [F0 males 83/F0 females 100 mg/kg/day; F1

17

males 92/F1 females 106 mg/kg/day].

Two treatment-related deaths occurred at the high-dose level. One high-dose F0 female was sacrificed during week 3 and one high-dose F1 female was sacrificed during week 9. Each was noted to be thin and showing locomotor difficulty, and the F1 female had paralysis of the right hindlimb. Additional evidence of maternal toxicity at the high-dose level in both generations included decreased food consumption and body weight [78%-92% of control]. These latter effects were also observed in the high-dose males [males 88%-92%] of both generations. Body-weight gain was decreased during the 0-10 week interval [F0 males 84%/females 70% of control; F1 males 91%/females 82% of control] at the high dose in both generations (both sexes) and during the 0-19 week interval [F0 males 88%/females 77% of control; F1 males 90%/females 87% of control] at the high dose in both generations (both sexes) and in the mid-dose F0 females [91% of control]. Increased thyroid weight was observed at the high-dose level in both sexes in both generations, which correlated with the lesions observed [diffuse follicular epithelial hypertrophy/hyperplasia]. Other organ weight effects include increased liver weight of F0 adults at the high-dose level [both sexes] and in F1 adult males at the mid- and high-dose levels, increased kidney weights in the high-dose F0 males and females and in the mid- and high-dose F1 males, and increased lung weight in the mid- and high-dose males [both generations] and in the high-dose F1 females. Testes weight was significantly increased [comparable] at all dose levels in the F0 generation compared to the control. F1 females at the high-dose level displayed a significantly decreased brain weight. Centrilobular hepatocyte enlargement was observed in 8/28 F0 and 4/28 F1 males as compared to none in the male control groups. Reduced/absent spermatogenesis was observed in 5 of 24 high-dose F1 males compared to 1 of 24 control F1 males and none in any other group in either generation. There were no effects demonstrated on pregnancy rate, gestation times, implantation rate, litter size, pup mortality, or the ability to rear young to weaning in either generation, 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.

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 [statistically significant] in the startle response at the mid- and high-dose levels, and the onset of vaginal opening was delayed [not statistically significant] in the F1 high-dose female [35.0 days] offspring compared to the control [32.8 days]. There were various organ-weight effects, which were similar to those observed in the parental animals. Liver weight was increased in the mid- and high-dose F0 female offspring at weaning, and there were increases in ovarian weight in the mid- and high-dose F0 female offspring at weaning, and decreases in thymus weight in the mid- and high-dose F0 male offspring at weaning. Increased liver weight was observed at all dose levels in the F1 offspring [both sexes], but the increase observed at the low-dose level was of questionable significance.

The NOAEL for maternal toxicity is 75 ppm (F0 6.0/F1 6.4 mg/kg/day), and the LOAEL is 300 ppm (F0 24.1/F1 25.1 mg/kg/day), based on decreased body weight/body-weight gain and food consumption. At the high-dose level, 2 of the dams died, thyroid weights were increased, there was an increased incidence of diffuse follicular epithelial hypertrophy/hyperplasia, and a slightly longer pre-coital time for the F1 generation. The NOAEL for paternal toxicity is 75 ppm (F0 5.3/F1 5.8 mg/kg/day), based on a significant increase in lung [both generations] and liver [F1] weight and an increased incidence of diffuse follicular epithelial hypertrophy/hyperplasia [F1]. The paternal LOAEL is 300 ppm (F0 21.2/F1 23.1 mg/kg/day). At the high-dose [F0 83/F1 92 mg/kg/day] level

in males, decreased body weight/body-weight gain and food consumption were observed, and there were increases in lung [both generations] and thymus [F1 generation] weights and increases in the incidences of centrilobular hepatocyte enlargement [both generations] and diffuse follicular epithelial hypertrophy/hyperplasia of the thyroid [F1 generation]. The NOAEL for reproductive effects is 300 ppm [males F0 21.2/F1 23.2 mg/kg/day; females F0 24.1/F1 25.1 mg/kg/day], and the LOAEL for reproductive effects is 1200 ppm [males F0 83/F1 92 mg/kg/day; females F0 100/F1 106 mg/kg/day], based on delayed vaginal opening in the F1 female offspring. A slight delay in the startle response in the offspring was observed at 300 ppm [males F0 21.2/F1 23.2 mg/kg/day; females F0 24.1/F1 25.1 mg/kg/day] and 1200 ppm [males F0 83/F1 92 mg/kg/day; females F0 100/F1 106 mg/kg/day]. The NOAEL for fetal effects is 75 ppm (F0 6.0/F1 6.4 mg/kg/day), and the LOAEL is 300 ppm (F0 24.1/F1 25.1 mg/kg/day), based on a slight delay in the startle response in the offspring.

3. Determination of Susceptibility

There is qualitative evidence of susceptability in the rat developmental toxicity study in that severe fetal effects [deaths] were observed at a dose level that produced minimal maternal toxicity [decreased body-weight gain]. There is no evidence of increased susceptability in the rat 2-generation reproduction study. There is no adequate rabbit developmental toxicity study.

4. Determination of the Need for Developmental Neurotoxicity Study

There is **evidence of neurotoxicity** in the rat [tremors observed in females in a subchronic oral toxicity study; decreased NTE in males and decreased forelimb grip strength in females in a subchronic neurotoxicity study]. There is **evidence of developmental toxicity** in the rat [bent limb bones [malformation] and retarded skeletal ossification and bent ribs [developmental variations] at a dose level that produced neurobehavioral clinical signs [impaired mobility (all dams), dragging of the hindlimbs (80%), hunched posture (76%), unkempt appearance (72%), excessive chewing (60%), and prostration (16%)] in the dams by day 11 of gestation [after 5-6 doses]. There is no evidence of increased sensitivity in offspring in the rat 2-generation reproduction study, but there was a slight delay in the startle response and a delay in vaginal opening in the 2-generation reproduction study.

There is a **data gap** [rabbit developmental toxicity study]. A rabbit developmental toxicity study is required to assess the skeletal delays observed in the rat developmental toxicity study. ETU [metabolite, degradation product, contaminant] is associated with central nervous system anomalies in a rat developmental toxicity study. Manganese is associated with neuropathology, central nervous system effects, and idiopathic parkinsonism.

Based on the weight of evidence [clinical signs (unsteady gait, dragging of hindlimbs, diminished sensitivity to pain in affected limbs, paresis of hindlimbs, impaired mobility in dams in developmental toxicity studies; tremors in a subchronic toxicity study), decreased brain weight in the mouse carcinogenicity study, microscopic lesions of the skeletal muscle in the chronic rat study, impairment of forelimb grip strength in females in both the acute and subchronic neurotoxicity studies, and decreased NTE in males in the subchronic neurotoxicity study, along with an association of Maneb with Parkinsonism in the open literature], the HIARC determined that a developmental neurotoxicity study is required for Maneb.

5. Determination of the FQPA Factor:

The HIARC, based on hazard alone, recommends that the 10X FQPA safety factor should be retained due to data gaps for (i) a rabbit developmental toxicity study and (ii) a developmental neurotoxicity study in the rat. The application of an FQPA factor for the protection of infants and children from exposure to Maneb, as required by FQPA, will be determined during by the FQPA Safety Factor Committee.

V. DATA GAPS

The HIARC determined that the following studies are required:

1) Developmental Toxicity Study in the Rabbit
2) Developmental Neurotoxicity Study in the Rat
3) Subchronic Inhalation Study in the Rat

VI. HAZARD CHARACTERIZATION

The Maneb toxicology database is not complete; however, there are sufficient data for selecting acute and chronic dietary endpoints and short-term, intermediate-term, and chronic dermal and inhalation endpoints for risk assessment. A developmental toxicity study in the rabbit, a subchronic inhalation toxicity study in rats, and a developmental neurotoxicity study in the rat have been identified by the HED HIARC as data gaps.

Maneb is a fungicide in the class of dithiocarbamates, which includes Mancozeb and Metiram, and all of these compounds have a common metabolite/degradation product/contaminant; i.e., ethylenethiourea [ETU]. The findings in multiple studies demonstrate that the thyroid is a target organ for Maneb after single and multiple doses *via* the oral, dermal, and inhalation routes of exposure and across species [rat, dog, mouse, monkey]. Neurotoxicity is also a major toxic effect observed following both acute and subchronic exposures to Maneb.

Acute toxicity data show that Maneb is not acutely toxic to rats *via* the oral and inhalation routes of exposure or to rabbits *via* the dermal route of exposure. Maneb is not a skin or eye irritant, but it is a strong dermal sensitizer.

Thyroid effects have been observed following Maneb exposure to **dogs** [changes in clinical chemistry parameters indicative of thyroid toxicity, increased thyroid weight, and follicular (thyroid) hyperplasia]; **monkeys** [increased thyroid weight]; **rats** after 90-day and 2-year exposures [increased thyroid weights and follicular cell hyperplasia, decreased T_4], in a **2-generation reproduction study** [increased incidence of diffuse follicular epithelial hypertrophy/hyperplasia], and following 21-day dermal exposure [increased thyroid weight, thyroid follicular cell hypertrophy]; and **mice** [decreased T4, increased thyroid weights].

Neurotoxicity has been observed following exposure to Maneb. Following acute oral exposure, a slight impairment of forelimb grip strength was observed in female rats. Following oral exposure for 90 days [neurotoxicity study], impaired mobility, decreased fore- and hindlimb grip strength, and high carriage were observed in female rats, and a dose-related decrease in neurotoxin esterase [NTE] activity was observed in male rats. There was also a higher incidence of microscopic lesions in the nerves [both sexes]. Treatment-related clinical signs [unsteady gait, dragging of the rear limbs, diminished sensitivity to pain in affected limbs, paresis of rear limbs] were observed in a rat range-finding developmental

toxicity study, and in the definitive study, impaired mobility, dragging of hindlimbs, hunched posture, and prostration were observed. In the mouse carcinogenicity study, there was a dose-related decrease in absolute brain weight. Tremors were observed in several rats in a subchronic oral toxicity study. There was an increased incidence of microscopic lesions of the skeletal muscle of rats following long-term [2 year] exposure.

Supporting evidence for a concern regarding neurotoxicity and exposure to Maneb includes the following: Maneb contains manganese, a known neurotoxin, which has been associated with idiopathic Parkinson's disease; Disulfiram poisoning is associated with permanent extrapyramidal syndromes, and **ethylene-bis-dithiocarbamate and carbon disulfide** are the main metabolites of Disulfiram. Carbon disulfide is a known neurotoxin that causes parkinsonism, and it can react with endogeneous amino acids and monoamines to produce dithiocarbamates.

There is no evidence of prenatal developmental toxicity in the rat, but there is increased qualitative fetal susceptibility in rats. In the rat developmental toxicity study, fetal death occurred at a dose level where minimal maternal toxicity [decreased body-weight gains] occurred. There is no evidence of increased susceptibility in the rat 2-generation reproduction study. There is no acceptable rabbit developmental toxicity study, and a rabbit developmental toxicity study is required.

Because Maneb is known to be converted to ETU, it is classified as a Group B2, and after applying the metabolic conversion factor for EBDC to ETU [0.075], the Q_1* of ETU will be applied. Based on the weight of evidence, it is concluded that the risk would not be underestimated with respect to cancer potential for Maneb and all of the EBDCs using the ETU data.

VII. ACUTE TOXICITY ENDPOINTS

Acute Toxicity of Maneb

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral - rat	41975601	LD_{50} = >5000 mg/kg	IV
81-2	Acute Dermal - rabbit	41975602	LD_{50} = >2000 mg/kg	III
81-3	Acute Inhalation - rat	41975603	LC_{50} >1.3 mg/L	III
81-4	Primary Eye Irritation	41975604	not an eye irritant	III
81-5	Primary Skin Irritation	41975605	not a skin irritant	III
81-6	Dermal Sensitization	41975606	dermal sensitizer	N/A
81-8	Acute Neurotoxicity	43947601	systemic toxicity NOAEL 1000 mg/kg/ systemic toxicity LOAEL 2000 mg/kg, based on decreased body-weight gains. acute neurotoxicity NOAEL 1000 mg/kg/acute neurotoxicity LOAEL 2000 mg/kg, based on the slight impairment in forelimb grip strength.	N/A

VIII. SUMMARY OF TOXICOLOGICAL ENDPOINT SELECTION: MANEB

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary **Females 13+**	NOAEL 20 (UF = 100)	decreased fetal viability	rat developmental toxicity
		Acute RfD = 0.2 mg/kg	
Acute Dietary **general population**	NOAEL 1000 mg/kg (UF = 100)	slight impairment of forelimb grip strength	acute neurotoxicity
		Acute RfD = 10 mg/kg	
Chronic Dietary non-carcinogenic effects	LOAEL 8.6 (UF= 300)	mouse decreased T4	mouse carcinogenicity
		Chronic RfD = 0.029 mg/kg/day	
Chronic Dietary carcinogenic effects	$Q_1^* = 6.01 \times 10^{-2}$ $(mg/kg/day)^{-1}$	Maneb is classified as a **Group B2** carcinogen with a low-dose extrapolation approach for human risk assessment, based on ETU.	
Short-Term (Dermal)	NOAEL = 100	thyroid effects	21-day dermal
Intermediate-Term (Dermal)	NOAEL = 100	thyroid effects	21-day dermal
Chronic Dermal ✖/ Inhalation ✖ carcinogenic effects	NOAEL = 100	thyroid effects	21-day dermal
Chronic Dermal ✖/ Inhalation ✖ carcinogenic effects	$Q_1^* = 6.01 \times 10^{-2}$ $(mg/kg/day)^{-1}$	Maneb is classified as a **Group B2** carcinogen with a low-dose extrapolation approach for human risk assessment, based on ETU.	
Inhalation ✖ (Any Time Period)	NOAEL = 5	thyroid effects	13-week oral toxicity

✖appropriate route-to-route extrapolation should be performed for these risk assessments. Exposure values using a dermal absorption factor of 1% should be converted to equivalent oral doses and compared to the oral NOAEL. MOE for worker exposure risk assessments = 100.