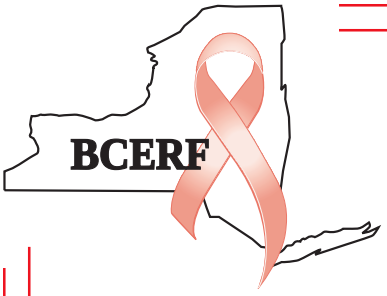

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Critical Evaluation of Mancozeb's Breast Cancer Risk

by

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Author's Note: The reader is encouraged to read the attached document, Appendix B, which includes an explanation of the BCERF Breast Cancer Risk Classification System, before reading this Critical Evaluation.

I. Introduction

Mancozeb is a polymeric complex of manganese and zinc ethylenebis-(dithiocarbamate) (Hayes et al., 1991). It is used as a protective fungicide (to prevent fungal growth) and in foliar and seed treatments to control a wide range of pathogens in field crops, fruits, ornamentals and vegetables (Worthing, 1991). It has been chosen for this evaluation because it is the most used fungicide in New York State (NYS). It is widely used on apples, grapes, potatoes, onions and tomatoes grown in this state (Gianessi and Anderson, 1995b). It is also used for control of fungal diseases in conifer and fir trees (FS/USDA, 1994). Mancozeb exposure has been implicated in causing prostate and thyroid gland dysfunction and reproductive impairment in humans and experimental animals (Clement and Colborn, 1992). Mancozeb belongs to the class of ethylene bisdithiocarbamate (EBDC) fungicides. All EBDC fungicides share a common metabolite, degradation product and manufacturing process contaminant, ethylenethiourea (ETU) (Vettorazzi et al., 1995). The major toxicological concern from exposure to mancozeb is considered to be the presence of ETU (USEPA, 1987b). ETU has been classified as possibly carcinogenic to humans, based on sufficient evidence for its carcinogenicity in experimental animals by IARC (IARC, 1987). However, mancozeb has not undergone a complete review for its carcinogenic potential by either the Environmental Protection Agency (EPA) or the International Agency for Research on Cancer (IARC).

A. History of Use and Nomenclature:

Mancozeb belongs to the structurally related group of EBDC fungicides. Mancozeb's fungicidal properties were reported in 1961. It was commercially introduced by Rohm and Haas Co. and E.I. duPont de Nemours and Co. (Worthing, 1991). Mancozeb is a fungicide with protective action (Worthing, 1991). It can thus be used for prevention as well as in treatments to control fungal growth. It is effective in controlling fungi causing anthracnose, leaf blights, downy mildew, rusts, seed decay, seedling blights, turf diseases, and other fungal diseases (USEPA, 1987a). In the Pacific Northwest, mancozeb has been used to control pear psylla nymphs (USEPA, 1987a). Mancozeb also protects harvested products from deterioration caused by fungal attack (USEPA, 1987a).

B. Usage:

Mancozeb is used as a fungicide for field crops, fruits, vegetables, nuts and turf (Meister, 1999). It is used as a dust, flowable suspension, liquid suspension, water dispersible granules or wettable powder (Meister, 1999). It may be applied to foliage using aerial or ground equipment. Mancozeb seed treatments may be applied using dip tanks or dusting equipment (USEPA, 1987a). Mancozeb is registered for use in many countries on horticultural and agricultural food crops as well as on ornamentals, tobacco and in forestry (FAO/WHO, 1993).

1. Agricultural Use:

Mancozeb was the fourth most used fungicide in agriculture in the United States (US) during 1990-93. The estimated agricultural usage of this fungicide was eight million lbs of active ingredient (AI) per year during this time period (Gianessi and Anderson, 1995a). EPA's estimates indicate that seven to ten million lbs of AI of mancozeb was used for agricultural purposes on cropland in the year 1997 (Aspelin and Grube, 1999). Mancozeb was the highest used fungicide in NYS, with 724,111 lbs of AI used annually on croplands during 1990-93 (Gianessi and Anderson, 1995b). According to the estimates published by the US Department of Agriculture (USDA) in 1997, 321 thousand lbs of AI mancozeb was applied on apple orchards in NYS (USDA, 1999).

Mancozeb is used as a fungicide for potatoes, tomatoes, apples, wheat, corn, watermelons, safflower, sorghum, peanuts, flax, cereal grains, grapes and onions (Meister, 1999; USGS, 1992). It is also used for seed treatment for cotton.

2. Non-cropland Use:

Mancozeb formulations are available for use in homes and nurseries, for flowers, ornamental trees, shrubs, turf sod, and golf courses (Meister, 1999). In forestry, it is used to control fungal diseases in conifer and fir trees (FS/USDA, 1994). Estimates of amount of mancozeb used for non-agricultural uses were not available.

C. Chemical Information

Table 1. Chemical information on mancozeb

Common Names: Mancozeb; mancozebe; manzeb (Worthing, 1991)
Chemical Name: [[1,2-ethanediylbis[carbamodithioato]](2-)] manganese mixture with [[1,2-ethanediylbis[carbaodithioato]] (2-)]zinc (Worthing, 1991)
Chemical Formula: $[\text{-SCSNHCH}_2\text{CH}_2\text{NHCSSMn-}]_x(\text{Zn})_y$ (Worthing, 1991)
CAS Registry Number: 8018-01-7 (formerly 8065-65-6) (Montgomery, 1993)
Major Metabolites: ethylene thiourea (ETU), ethyleneuria, and ethelenebisisothyocyanate sulfide (IPCS, 1988)
Mode of Action: Mancozeb inhibits production of ATP in fungi by forming a complex with metal-containing enzymes (USEPA, 1987b)

Figure 1. Chemical structure of mancozeb

II. Current Regulatory Status

A. Regulatory Status:
Manozeb has been classified by EPA in the toxicity class IV (practically nontoxic) and is registered as a General Use Pesticide (GUP) (EXTOXNET, 1998).

The carcinogenic potential of ETU prompted EPA to conduct a Special Review of EBDC in 1987 (USEPA, 1989). In September, 1989, registrants for EBDC fungicides applied to EPA to amend their usage on food crops and removed 42 of the 55 registered food uses from the end use labels for mancozeb. This action left only 13 registered uses of mancozeb on food commodities (asparagus, bananas, cranberries, figs, grapes, onions, peanuts, potatoes, sugar beets, sweet corn, tomatoes, pome fruits and cereal grains). The registrants also petitioned EPA to reduce tolerances for the remaining 13 food uses (USEPA, 1989).

All EBDC labels were revised in 1992 to restrict rotation in the use of fungicides within this family for a growing season. In other words, if growers use mancozeb on a crop at the start of the season, no other EBDC fungicide may be used on the same crop in the same growing season (PMEP, 1992). EPA has set limits on the total amount of EBDC fungicide used on specific crops per season (USEPA, 1989). Further, there is a restricted entry interval (REI) of 24 hours (hrs) imposed for fields treated with EBDC fungicides such as mancozeb (PMEP, 1992).

A position document published by EPA in 1992 announced the cancellation of some types of residential uses of mancozeb on home garden turf and fruit trees. Other home garden uses were allowed to remain registered, subject to certain conditions (USEPA, 1996b).

B. Clean Water Act Requirements:
There has been no maximum contaminant level or health advisories set for levels of mancozeb in public drinking water supplies (USEPA, 1996a).

C. Workplace Regulations:
The Occupational Safety and Health Commission (OSHA) has set a time-weighted average (TWA) exposure limit of 5 mg/m^3 for mancozeb (EXTOXNET, 1998).

D. Food Tolerances:
The amount of pesticide permitted to occur on the edible portion of raw agricultural commodities and in processed foods, called tolerances, are set by EPA. Residues of dithiocarbamates, including mancozeb, are calculatedå9n terms of zinc ethylene bisdithiocarbamate, a common metabolite. Total zinc ethylene bisdithiocarbamate residues from all dithiocarbamates, including mancozeb, on any raw agricultural commodity should not exceed the highest tolerance established for any member of this class of chemicals (USEPA, 1998). Residue tolerances for mancozeb range between 4 to 7 parts per million (ppm) in fruits, and squashes; 2 ppm in or on sugar beets and carrots; 0.5 ppm on popcorn grain, fresh corn, cottonseed, onions, and peanuts; and 0.1 ppm (negligible residue) in asparagus and corn grain (except popcorn grain) (USEPA, 1998).

III. Summary of Evidence of Overall Carcinogenicity

A. Human Studies:
Epidemiological studies of cancer incidences in applicators and manufacturing workers known to have used or worked in areas treated with mancozeb were not found in the open scientific literature. Two cohort mortality studies have mentioned the possibility of exposure to mancozeb and are discussed below. However, these cohorts had a very small number of cases and data on potential for exposure to mancozeb were lacking, making these studies less useful for an evaluation of mancozeb's carcinogenic potential.

1. Cohort Study:
A retrospective mortality study of a cohort of 32,600 past and present employees (male and female) of a lawn care service company in Ohio evaluated any associations between exposure to different pesticides and cause of death. The cohort was young, with 70% white, non-Hispanic men; 2% African-American men; 1% Hispanic men; 0.4% other non-white men; 25% white, non-Hispanic women; 1% African-American women; 0.3% Hispanic women; and 0.2% other, non-white women. The total number of deaths recorded for the cohort (n = 307) during the years 1966 to 1990 indicated a decreased mortality from all causes in comparison to the mortality rate of the general US population (Zahm, 1997). There was an increase in incidence of non-Hodgkin's lymphoma (NHL) in male lawn applicators who had been employed for three or more years [standardized mortality ratio (SMR) = 7.11, 95% confidence interval (CI) 1.78-28.42], but it was based on only two deaths. Company records indicated moderate use of mancozeb. However, mancozeb was not used at the specific branch where the two cases of NHL had been employed (Zahm, 1997). Hence, although an increased mortality rate from NHL was recorded for this cohort, the increase was not associated with exposure to mancozeb.

2. Ecological Study:
An ecological study has compared the cancer mortality rates of four different geographic regions of Minnesota, using data collected by the National Center for Health Statistics. These four regions of the state have very different agricultural profiles (Schreinemachers et al., 1999). Cancer mortality rates between 1980 to 1989 among residents of the mostly urban and forested region of the northeast region of the State were compared to the cancer mortality rates from the more rural and farm-based region in the northwest. A survey on pesticide usage conducted by the Minnesota Department of Agriculture in 1990 indicated that the largest amount of fungicides, including mancozeb, was applied aerially to potatoes, sugar beets and wheat crops grown in the northwestern region. The mortality rate from thyroid cancer among white males in the northwestern region was found to be significantly higher [age-standardized mortality rate ratio (SRR) = 2.95, 95% CI 1.35-6.44] than the rates at the mostly urban and forested region, based on nine deaths. Other significant increases in mortality rates in white men in this region were from prostate cancer (SRR = 1.12, 95% CI 1.00-1.26) based on 352 deaths, and bone cancer (SRR = 2.09, 95% CI 1.00-4.34), based on nine deaths. Mortality rates from lung cancer were significantly decreased in both white men and women in this agricultural region (Schreinemachers et al., 1999).

While different pesticide use patterns were used to predict exposure in this study, data on specific exposures were lacking. Many different pesticides, including mancozeb were used in the northwestern region of the state, and the role of mancozeb in causing these cancers cannot be determined from this study. However, results of this study indicate that populations exposed to mancozeb should be followed for cancer incidences.

3. Summary, Human Studies:
The mortality rate from NHL was found to be higher in a cohort of lawn care service employees exposed to many pesticides, including mancozeb. However, the increase in mortality from NHL was unlikely to be due to exposure to mancozeb, since the two cases of NHL had worked at a branch where mancozeb was not used (Zahm, 1997). In another study, a correlation was found between higher use of pesticides, including mancozeb, in an agricultural region of Minnesota and increases in mortality rates from cancers of thyroid, bone and prostate in white men (Schreinemachers et al., 1999). In both these studies, many different pesticides including mancozeb were involved, and exposure data were lacking, making it difficult to assess the specific role of this fungicide in having caused these cancers. However, this ecological study serves as an indicator for the kinds of cancers that should be followed in populations that have been exposed to mancozeb.

B. Experimental Animal Studies:
Most results of long-term exposure effects of mancozeb in experimental animals have been presented in unpublished reports. These studies were reviewed at the Joint Food and Agricultural Organization (FAO) and World Health Organization (WHO) Meeting, 1993. Experimental design and results of any unavailable studies have been abstracted from this meeting report (FAO/WHO, 1993).

1. Mice:
Two different laboratories have independently studied the effects of long-term exposure to mancozeb, using the same strain of mice and very similar doses. Results from these two laboratories were similar. In the first of these studies, conducted at Inveresk Research International, Scotland, for Elf Atochem, groups of Charles River CD-1 mice (60/sex/dose) were fed 0, 25, 100or 1000 ppm mancozeb technical (88.6% mancozeb) in diet for 78 weeks. There was no statistically significant difference in the incidence of tumors in the mancozeb-treated groups compared to controls [Everett et al., 1992, as reviewed in (FAO/WHO, 1993)]. In the second study, which was conducted at Tegeris Laboratories, US, for Rohm and Haas, groups of Charles River CD-1 mice (94/sex/dose) were fed 0, 30, 100, or 1000 ppm equivalent of active ingredient of mancozeb (using 83% mancozeb) for 78 weeks. There were no treatment-related increases in neoplastic incidences [Schellenberger, 1991, as reviewed in (FAO/WHO, 1993)].

Another study followed tumor incidences in mice dermally exposed to mancozeb. Two groups of female Swiss albino mice (20/group) were exposed to topical applications of 0 or 100 mg/kg mancozeb

in dimethyl sulfoxide (DMSO), while another two groups of mice were treated with 0 or 5 mg of the potent carcinogen benzo[*a*]pyrene (BaP) in acetone, three times a week, over sixty weeks (Shukla et al., 1990). Mancozeb-treated animals had significantly reduced body weight after the 30 weeks of exposure, indicating that the dose used was above the maximum tolerated dose (MTD). Five out of the 14 animals that survived 48 weeks of exposure to mancozeb had benign skin tumors. No tumors were reported for the group of 17 mice that did not receive either BaP or mancozeb. Survival rates were very low. Only six of the twenty mice in the mancozeb-treated group survived the 60-week period. The very small number of surviving animals and the use of only one dose of mancozeb (which was toxic) severely limits any conclusions that can be drawn from this study.

In a previously reported short-term study, the same group of investigators had treated female Swiss albino mice (20/group) with 0 or 100 mg/kg mancozeb in DMSO three times a week for three weeks, followed by 0, or 5 mg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in acetone two times a week for 12 weeks (Mehrotra et al., 1987). Mice treated with mancozeb or TPA alone had no tumors, but 9/20 (45%) mice treated with mancozeb and TPA had benign skin tumors. The authors presented the results of this short-term study as evidence for mancozeb's potential to initiate skin tumors. The absence of tumors in the group treated with mancozeb alone and the observation of tumors only after the start of TPA treatments in all groups indicate a possible synergistic action between TPA and mancozeb in promoting skin tumors. However, before a conclusion can be made on mancozeb's ability to initiate skin tumors, a long-term study of mancozeb exposure of mice through topical applications would be useful.

2. Rats:

In a long-term toxicity and carcinogenicity study conducted at Haskell Laboratories for Rohm and Haas, Charles River Crl:CDBR rats (72/sex/dose) were fed 0, 20, 60, 125 or 750 ppm mancozeb technical (83.8% pure) for two years. Male and female rats fed the highest dose had a significant decrease in body weight gain during the first year (p value not available), but body-weight gains in the second year were not significantly different from controls. Survival rates of treated and control groups were comparable. At the end of the first year, a significant increase in hypertrophy/hyperplasia of the thyroid gland was observed in males and female rats that had received the highest dose (p values not available). At the end of the second year, males treated with the highest dose of mancozeb had significantly increased incidence of thyroid follicular cell adenomas (20/61; 33%) and carcinomas (14/61; 23%); incidence in controls and p values not available. In females treated with the highest dose of mancozeb the incidence of thyroid follicular cell adenomas (6/61; 10%), and carcinomas (4/61; 7%) was increased, but not significantly (incidence level for controls, p values not available) [Stadler, 1990, as reviewed in (FAO/WHO, 1993)].

In another study conducted at the Hutington Research Center for Elf Atochem, Sprague-Dawley (CD) rats (70/sex/dose) were fed 0, 25, 100, or 400 ppm technical mancozeb (88.5% pure) for two years. During the initial six months, body weights of male and female rats fed the highest dose were significantly decreased (p value not available). Thyroid follicular cell adenomas incidences were found in 6/50 (12%), 2/50 (4%), 2/50 (4%) and 6/50 (12%) of the males in the respective treatment groups, while thyroid follicular cell adenocarcinomas incidences were 2/50 (4%), 0/50 (0%), 1/50 (2%) and 3/50 (6%), respectively. These incidences were not statistically different from controls (p values not stated). Thyroid follicular cell adenomas were observed in 0/50 (0%), 0/50 (0%), 2/50 (4%) and 2/50 (4%) females in the control and treated groups. Thyroid follicular cell adenocarcinoma was observed in only one female fed the highest dose. There was a significant increase in the height of the thyroid follicular epithelium at 400 ppm in males and females. Hence, non-neoplastic changes in the thyroid were significantly different in both sexes fed the highest dose of mancozeb, but neoplastic changes were not significantly different. It should be noted, however, that neoplastic changes observed in rats in the previous study were at the 750 ppm dose level, while the highest dose level used in this study was 400 ppm. The hyperplasia observed in rats at 400 ppm in this study could be indicative of pre-neoplastic changes.

In another toxicity study, a dose-dependent increase in hyperplasia in thyroid glands was observed in male albino rats (strain unspecified) fed 500, 1,000 or 1,500 mg/kg mancozeb for 90 days. The increase in hyperplasia in these rats (measured as the ratio of the thyroid gland and body weight), correlated with decreased levels of serum thyroxine. A decrease in serum thryoxine levels causes a compensatory increase in thyroid stimulating hormone (TSH) (Trivedi et al., 1993). A dose-dependent increase in the relative weight of the thyroid gland was also observed in male Wistar rats fed 0, 10, 50, 75, 113, 169, 253 or 379 mg/kg Dithane M-45 (80% mancozeb) for 12 weeks in another study (Szepvolgyi et al., 1989). Histological examination of the enlarged thyroid glands confirmed hyperplasia in the animals that had received 253 or 379 mg/kg Dithane.

Results from the above studies in rats indicate an increase in thyroid gland hyperplasia, which could be induced due to mancozeb's interference with the functioning of thyroid enzymes and a resultant feedback induction of the TSH. In one study that used the highest dose level of 750 ppm of mancozeb, thyroid gland hyperplasia was accompanied by a significantly increased incidence of follicular cell carcinomas in male rats [Stadler et al., 1990, as reviewed in (FAO/WHO, 1993)]. Hence, the increase in hyperplasia in short-term studies may be indicative of pre-neoplastic changes. EPA's Office of Prevention, Pesticides and Toxic Substances (OPP) has developed a science policy for the assessment of thyroid follicular cell tumors and concluded that rodent thyroid tumors

are relevant for assessment of human carcinogenicity. On reviewing the data on mancozeb's carcinogenic action in rodents, OPP has concluded that mancozeb's inhibitory action on thyroid enzymes induces the feedback stimulation of TSH producing cells, causing significant increase in tumor induction in the thyroids of male and female rats (Hurley et al., 1998). Historically, in cancer bioassays, male rats have been found to be more sensitive to thyroid carcinogens than females, and rats more sensitive than mice. Consistent with this pattern, male rats were found to be most sensitive to thyroid tumors following treatments with mancozeb, or its metabolite ETU. TSH levels are higher in male rats than in females, but how the increased TSH may contribute to sensitivity to thyroid tumors is not well understood (Hurley et al., 1998).

3. Summary, Experimental Animal Studies:
Mancozeb exposure in the diet for two years was not found to cause any carcinogenic effects in Charles River CD-1 mice in two independent studies [Everett et al., 1992 and Schellenberger, 1991, as reviewed in (FAO/WHO, 1993)]. Other studies in mice provide evidence for skin tumor promotion caused by mancozeb treatments: topical applications of mancozeb in female mice were found to increase the incidence of benign skin tumors (Shukla et al., 1990) and increase the tumor response to TPA (Mehrotra et al., 1987). In studies in rats, significant increases in non-neoplastic changes were observed in the thyroid glands of mancozeb-treated male and female rats [Stadler, 1990 and Hooks et al., 1992, as reviewed in (FAO/WHO, 1993)]. At the highest dose level used (750 ppm) in one study, mancozeb was toxic and caused a significant increase in incidence of thyroid follicular cell adenomas and/or carcinomas [Stadler, 1990, as reviewed in (FAO/WHO, 1993)]. Mancozeb's anti-thyroid action and the resultant feedback stimulation of TSH enzyme secreting cells has been proposed as the mechanism for mancozeb's mode of carcinogenic action in the thyroid glands of rodents (Hurley et al., 1998). However, the carcinogenicity of mancozeb at only very high doses could be an indicator of the threshold level for the carcinogenicity of its contaminant and metabolite ETU.

C. Current Classification of Carcinogenicity by Other Agencies

1. IARC Classification:
Mancozeb itself has not been classified as to its carcinogenic potential by IARC (Vettorazzi et al., 1995). However, ETU has been classified in Group 2B, possibly carcinogenic to humans, based on sufficient evidence for its carcinogenicity in experimental animals (IARC, 1987).

2. NTP Classification:
Mancozeb has not been classified as to its carcinogenicity by the National Toxicological Program (NTP) (USDHHS, 1998). In its eighth report on Carcinogens, NTP has listed ETU as having sufficient evidence for carcinogenicity in experimental animals but inadequate evidence for carcinogenicity in humans (USDHHS, 1998).

3. EPA Classification:
Mancozeb has not undergone a complete review for its cancer classification by EPA (USEPA, 1989; USEPA, 1987a).

IV. Critical Evaluation of Breast Cancer Risk

A. Human Studies:
Studies comparing the levels of mancozeb in tissues of women with breast cancer to women without breast cancer are not available. Such studies cannot be done retrospectively since there are no persistent biomarkers to indicate past exposures to mancozeb. The breast cancer incidence rates of women occupationally exposed to mancozeb in the past have not been compared to the rates in women with no exposure to mancozeb. Hence, there is no direct evidence for whether or not mancozeb exposure has affected breast cancer risk in women who may have been exposed to this fungicide.

B. Experimental Animal Studies:
We do not have any direct evidence from studies in experimental animals on mancozeb's mammary carcinogenicity. Studies on effects of long-term exposure to mancozeb have been reviewed (FAO/WHO, 1993). Incidences of mammary tumors have not been reported in the review. Since the unpublished studies were not available to us, we do not know if the mammary tumor incidences in mancozeb-treated rats were evaluated, but not reported due to a lack of significant change.

C. Other Relevant Data on Breast Cancer Risk

1. Evidence of Endocrine Disruption:
Breast cancer is a hormone-modulated cancer. We thus include in our evaluations any studies that indicate endocrine disruption or an effect on estrogen synthesis, function or blood levels.

An *in vitro* assay, E-SCREEN, has been designed to test for the ability of chemicals to mimic estrogen and induce proliferation of estrogen-dependent breast cancer cells (MCF-7). Many EBDC fungicides, including the closely related maneb, have been found to be non-estrogenic by this assay (Soto et al., 1995). Mancozeb specifically has not been tested.

In another study, endocrine glands of five random-bred male Wistar rats fed 0 or 100 ppm mancozeb in the diet for 90 days were evaluated for any effects on the circadian pattern of DNA, protein and RNA content (Nicolau, 1982). The protein content of the adrenal and thyroid glands was increased ($p < 0.05$) in mancozeb-treated rats. The investigators of this study detected temporal changes in RNA content of the thyroid in mancozeb-treated animals and point this out as evidence for endocrine disruption caused by

mancozeb treatments (Nicolau, 1982). However, hormone levels per se were not measured in the treated rats. An increased protein and RNA content in the thyroid can also be indicative of hyperplasia and excessive cell growth, an effect that has been observed in response to mancozeb treatments in other animal studies (see Section III.B.2). Although presented as a study of mancozeb's endocrine disruption, this study does not determine how hormone levels are affected by mancozeb treatments and is not very useful for an evaluation of breast cancer risk from mancozeb.

In summary, the one study that evaluated the influence of mancozeb on endocrine glands did not provide evidence to conclude on its estrogenicity or breast cancer risk. Results of the E-SCREEN assay with closely related EBDCs have not indicated estrogen-like effects; however, mancozeb itself has not been tested.

2. Reproductive and Teratogenic Effects:
Studies reporting reproductive toxicity or teratogenic effects of mancozeb are reviewed for any indicators of endocrine disruptive events that can affect breast cancer risk.

A study of 2,951 men and 5,916 women flower growers in Colombia with potential for occupational exposure to pesticides, including mancozeb, indicated increased rates of spontaneous abortions, Odds Ratio (OR) = 2.20 (95% CI, 1.82-2.66), and premature birth, OR = 1.86 (95% CI, 1.59-2.17) in pregnancies in women workers. Recall bias was suspected since the rates of induced abortions were also higher, OR = 1.98, (95% CI, 1.47-2.67) (Restrepo et al., 1990). Although mancozeb was among the ten most commonly used pesticides by these workers, its role in the adverse reproductive outcomes is unclear since the highest OR was found to be associated with jobs with little or no exposure to any pesticides.

In study of mancozeb's reproductive toxicity in experimental animals, male albino rats (Druckery strain, 50/group) were fed 500, 1,000 and 1,500 mg/kg mancozeb in peanut oil for one year (Kackar et al., 1997). Mancozeb treatments caused an increase in the relative testicular weights, accompanied with histopathological changes indicative of gonadal damage. Clinical changes and increased mortality were also observed at all doses of mancozeb used in this study, indicating that the dose levels were toxic. Another study (reported in Russian) found gonadal toxicity in rats exposed to 140 to 1,400 mg/kg mancozeb [Ivanova-Chemishanka et al., 1975, as reported in (IPCS, 1988)]. Details on whether these doses were toxic to the animals were not available.

The teratogenic potential of mancozeb has been evaluated in two studies in rats, through inhalation exposure and oral exposure, respectively. In the first study, Crl:CD rats were exposed to mancozeb by inhalation at 0, 1, 17, 55, 110, 890, or 1,890 mg/m^3 for six hours/day from day six through 15 of gestation. The highest dose exposure on day six caused severe weight loss and mortality. The dose was subsequently reduced to 500 mg/m^3. Reproductive and teratogenic effects were observed at dose levels that were also toxic to the dams (Lu and Kennedy, 1986). The study of mancozeb's teratogenic potential through oral administration gave similar results. Mancozeb was found to be teratogenic to Sprague-Dawley rats that were fed a high dose (1,320 mg/kg). The teratogenic effects were preventable with zinc acetate supplements, indicating that high doses of mancozeb were interfering with the absorption of zinc during fetal development (Larsson et al., 1976).

In another study reported as a meeting abstract only, groups of female New Zealand rabbits (20/dose) were fed 0, 10, 30, or 80 mg/kg mancozeb on days seven to 19 of gestation. There were no treatment-related effects on the maternal reproductive parameters in the groups fed 10 or 30 mg/kg. There were two deaths and five abortions among the dams treated with the highest dose (80 mg/kg) of mancozeb, indicating toxicity to the dams (Solomon and Lutz, 1989).

In summary, mancozeb has been associated with reproductive and teratogenic effects in animals. However, these effects have been observed only at doses that were toxic to the animals and do not indicate gonad-specific toxicity or an effect on hormone-mediated events.

3. Tests of Mutagenicity and Genotoxicity:
Studies indicating any mutagenic and clastogenic potential of a chemical in animals, isolated cells, bacteria and yeast are evaluated for the potential of the chemical to cause genotoxic effects, which could lead to an increased risk of breast cancer. We have outlined below the results of the available studies of mancozeb's genotoxic potential in different systems.

a. Chromosome Aberrations in Occupationally Exposed Humans:
A cytogenetic analysis was conducted on blood samples from men exposed to EBDC, including mancozeb, during fungicide application on tomatoes in Mexico. The study was controlled for age and smoking. Urine samples and blood samples were analyzed for ETU to document exposure. ETU levels in urine confirmed that fungicide exposure levels were highest among sprayers. Landowners had moderate levels of exposure to EBDC/ETU. An increase in sister chromatid exchange (SCE) frequency ($p = 0.03$) and chromosome translocation ($p = 0.05$) was observed for the group of highly exposed backpack sprayers ($n = 49$) when compared to non-exposed controls ($n = 31$) (Steenland et al., 1997). In the group of 13 lightly exposed landowners, the frequency of chromosome aberrations was increased, but not significantly. While these results indicate an increased frequency of chromosome

aberrations in men in association with EBDC exposure, whether or not mancozeb caused the increase is not clear since these workers were also exposed to many EBDC fungicides as well as other pesticides.

In another study, the chromosome aberration rates in the peripheral blood lymphocytes of 44 workers (30 men and 14 women) occupationally exposed to mancozeb were compared with 30 non-exposed control workers at a manufacturing plant. Cytogenetic analysis of blood lymphocytes indicated a significantly elevated rate of chromosome aberrations among the mancozeb-exposed workers compared to non-exposed workers (2.07% vs. 1.13%, $p < 0.05$). The frequency of SCE was increased in the mancozeb-exposed groups, but was significant only among those who smoked ($p < 0.05$) (Jablonicka et al., 1989; Vargova et al., 1987). Information on past exposure histories of workers or exposure to other chemicals was not available.

The above studies suggest that high exposure to EBDC and mancozeb may be associated with a small increase in risk of chromosome aberrations.

b. Studies in Animals:
Results of cytogenetic analysis of bone marrow cells after mancozeb treatment of experimental rodents at high doses have been positive. Cytogenetic analysis are predictive of mutagenicity. The one *in vivo* study that has evaluated the frequency of mutations induced by mancozeb treatments directly, found mancozeb to be non-mutagenic in insects (Vasudev and Krishnamurthy, 1980). The study design and results are summarized below.

In a study of genotoxic potential of mancozeb, Wistar rats (4/dose) were fed 0 or 1.7 mg/kg mancozeb in the diet for 280 days (Georgian et al., 1983). A significantly increased level ($p < 0.001$) of chromosome damage was observed in bone marrow cells of rats that were chronically exposed to mancozeb through the diet. The report did not mention if there were any other toxic effects or if the above dose was well tolerated.

In another study, inbred Swiss albino mice (18/group) were fed 0 or 1,000 mg/kg mancozeb and injected interperitoneally (i.p.) with 0, 10, 20 or 40 mg/kg of ascorbic acid. Mancozeb treatments alone were found to significantly increase the frequency of chromosome aberrations ($p < 0.01$) in the bone marrow cells but did not cause an increase in mitosis disruptions (Khan and Sinha, 1993; Khan and Sinha, 1994). Co-treatments with ascorbic acid caused a significant decrease in the frequency of chromosome damage ($p < 0.01$).

In another paper, the same investigators also reported a decrease in sperm count and a higher frequency of sperm with aberrant head morphology in mice treated with mancozeb (Khan and Sinha, 1996). The sperm toxicity in mice was also reduced with co-treatments of ascorbic acid. Ascorbic acid is a known antioxidant with the potential to prevent oxidative damage to DNA. However, the observations in this study were based on morphological examination of the sperm alone and are not sufficient evidence for genotoxicity. It should be noted also that in an unrelated study discussed in Section III.B, a much lower dose of mancozeb, at 100 mg/kg, was found to be highly toxic to mice (Shukla et al., 1990). It is possible that the dose used by Khan and Sinha was higher than the MTD, causing cytotoxic damage.

In contrast, mancozeb treatments of *Drosophila* did not indicate mutagenic activity. The frequency of autosomal or sex-linked recessive lethal mutations was not changed among offspring of *Drosophila* males that had been treated with Dithane M-45® (Mancozeb) as larvae (Vasudev and Krishnamurthy, 1980).

c. Studies in Isolated Cells:
In one study, mancozeb was tested for its DNA-damaging effects on peripheral blood lymphocytes from healthy human donors. Mancozeb treatments induced an increase in SCE, but only at a cytotoxic dose (25 μg/ml) that reduced cell viability to 40% (Perocco et al., 1989). The cytotoxicity and SCE rate from mancozeb exposure was eliminated by the presence of metabolic activation with S-9 (rat liver microsomal fraction), indicating a lower risk for SCE caused by mancozeb *in vivo*.

Another study found a dose-dependent increase in frequency of chromosome aberrations in cultured human blood lymphocytes treated with 4, 10 and 20 μg/ml mancozeb (Georgian et al., 1983). Metabolic activation was not used in this study. While the above cytogenetic studies of isolated cells treated with mancozeb have indicated its potential to cause chromosomal damage, mancozeb did not induce cell transformations in two other assays using C3H/10T cells (FAO/WHO, 1993).

d. Studies in Bacteria and Yeast:
Results of the mutagenicity of mancozeb from studies in bacteria and yeast are equivocal. Mancozeb was not observed to be mutagenic using the Ames test in two different strains of *Salmonella*, in the presence or absence of activation using S-9 (De Lorenzo et al., 1978) as well as in an assay for gene conversion in *Saccharomyces* (Siebert et al., 1970). In another study that was reported in an abstract only, Dithane M-45 (80% mancozeb) was found to cause more gene conversion in *Saccharomyces*, *Samonella* and *Escherichia* tester strains than other EBDC fungicides (Warren et al., 1976).

A review article that has compared the mutagenicity of different pesticides has grouped mancozeb among pesticides that have been

largely negative for genetic activity in different assays (Garrett et al., 1986).

4. Evidence of Tumor Promotion:
An agent that promotes the number, size, or progression of tumors can affect cancer risk. We have outlined below studies of tumor promotion ability of mancozeb in experimental animals.

In one study, groups of female Swiss albino mice (20/group) initiated for tumors with a topical application of 0.52 mg of 7,12-dimethylbenz[*a*]nthracene (DMBA) were observed to have no skin tumors at the end of 17 weeks. However, the group of DMBA-treated mice that also received cutaneous treatments of 100 mg/kg mancozeb three times a week, 13/20 (65%) developed skin tumors that were visible at 17 weeks, indicating a tumor promotion effect of mancozeb (Shukla et al., 1988). In another two-stage tumor initiation-promotion assay, groups of DMBA-initiated mice were topically treated with 0.005 mg TPA (DMBA + TPA group), or TPA and mancozeb (DMBA + TPA + mancozeb group) for three times a week (Mehrotra et al., 1990). The first skin tumor was observed earlier in the DMBA + TPA + mancozeb group, compared to the DMBA + TPA group. The number of tumors per mouse was also higher in the DMBA + TPA + mancozeb group than in the DMBA + TPA group ($p < 0.01$). The ornithine decarboxylase activity and radioactive thymidine incorporation was found to be increased following a single topical application of 2 mg mancozeb in 0.2 ml DMSO to mouse skin (Gupta and Mehrotra, 1992). These results, indicative of an induction of cell growth, were similar to the effects observed after the application of a known skin tumor promoter, TPA. Results of the above studies indicate the skin tumor promotion ability of mancozeb in female mice and are in agreement with results of studies described under III.B. 1 (Shukla et al., 1990, Mehrotra et al., 1987).

In another study of tumor promotion in rats, Wistar rats (17 to 25/group) treated with a single i.p. injection of 50 mg/kg nitrosomethylurea (NMU) at day three of age were fed 100 mg/kg mancozeb. At week 24, all rats treated with NMU had focal acinar cell hyperpalsia in the pancreas. However, rats that were treated with NMU and mancozeb had dysplastic foci (9/14; 64%) and carcinoma *in situ* (5/14; 36%), suggesting more advanced progression of the NMU-initiated tumors in the presence of mancozeb (Monis and Valentich, 1993). Dysplastic foci and carcinoma *in situ* were not observed in groups of rats treated with NMU or mancozeb alone. An investigation of the tumor histology of these rats indicated an alteration in the expression pattern of dynamin in the NMU + mancozeb treated rats that had more advanced tumors (Valentich et al., 1996). However, whether the altered expression pattern for dynamin is the mechanism through which mancozeb may be promoting pancreatic tumors is not known.

The above studies indicate that mancozeb has the potential to promote skin tumors in mice and pancreatic tumors in rats. Studies testing the ability of mancozeb to promote mammary tumors were not located.

5. Immunological Effects:
An immune system that is compromised for its ability to detect and destroy cancer cells can increase the risk of cancer. We have evaluated below the evidence for immuno-toxicity of mancozeb for any effects that could compromise the body's defense against cancer. Immune system suppressants may suppress the response of the body against cancer cells. However, whether chemicals that trigger hypersensitivity reactions also affect the ability of immune system to respond efficiently to cancer cells is not known.

In an epidemiological survey, an increased mitogen-triggered lymphocyte proliferative response was observed in the blood samples from 14 mancozeb-exposed workers at a chemical manufacturing plant. However, there were no clinical symptoms of immune-mediated diseases in the exposed workers. The authors propose that the changes observed in *in vitro* immune responses in mancozeb-exposed workers may be early predictors of immune-related disorders (Colosio et al., 1996). A very small number of workers were evaluated in this survey, but these workers should be followed for any health effects indicative of a compromised immune system. The results of this small study are not sufficient evidence to indicate that mancozeb adversely affects the human immune system or increases breast cancer risk, but they do suggest the need to monitor immune function in mancozeb-exposed populations.

A case report has documented contact dermatitis of face, lower neck and arms in a 34-year-old worker exposed to Rondo-M® (pyrifenox and mancozeb mixture) and alfacron®(azamethiphos). In testing for allergic reaction to individual components, the patient had a positive reaction with mancozeb (Iliev and Elsner, 1997). Sensitivity to mancozeb has also been reported in other case reports of workers exposed to mancozeb, ETU and other EDBC fungicides (Bruze and Fregert, 1983; Koch, 1996). These case reports indicate that mancozeb can trigger immune responses and allergic reactions. However, whether such reactions are accompanied with effects compromising the body's ability to fight cancer cells is not known.

Similar to the effects documented in case reports, Wistar rats treated with a subcutaneous injection of mancozeb were found to develop allergic reactions (Matsushita et al., 1976). Further, exposure to EDBC of these rats caused them to develop cross-sensitization to other fungicides in the family. These investigators have rated mancozeb as an extremely potent irritant, based on the reaction observed in rats (Matsushita et al., 1976).

In summary, one study has indicated that occupational exposure to mancozeb may lead to increased mitogen-triggered lymphocyte proliferative response in humans (Colosio et al., 1996). Other studies have indicated that mancozeb acts as a strong antigen and irritant, triggering immune response and contact dermatitis in exposed workers, but do not provide evidence for a compromised ability of the immune system to fight cancer cells. These results indicate immune toxicity and the need to follow immune function in mancozeb-exposed people.

6. Summary of Other Relevant Data on Breast Cancer Risk:
Mancozeb has not been tested for estrogenic effects in animals or *in vitro*. Closely related EBDC fungicides have not been found to be estrogenic by the *in vitro* E-SCREEN assay (Soto et al., 1995). Results of studies of mancozeb's reproductive toxicity in experimental animals do not indicate gonad-specific toxicity or disruption of estrogen-mediated events. In humans, occupational exposure to mancozeb and other pesticides was found to be associated with an increased frequency of chromosome aberrations (Jablonicka et al., 1989; Steenland et al., 1997; Vargova et al., 1987). In studies in animals, mancozeb was found to cause an increased frequency of chromosome aberrations in the bone marrow of treated rodents (Georgian et al., 1983; Khan and Sinha, 1993; Khan and Sinha, 1994). Mancozeb treatments did not induce mutations in *Drosophila* (Vasudev and Krishnamurthy, 1980). Results of tests for mutagenicity of mancozeb in bacteria and yeast have been equivocal (De Lorenzo et al., 1978; Siebert et al., 1970; Warren et al., 1976). Mancozeb treatments were found to promote skin tumors in mice and pancreatic tumors in rats (Gupta and Mehrotra, 1992; Mehrotra et al., 1987; Shukla et al., 1990; Shukla et al., 1988; Valentich et al., 1996). Mancozeb has not been tested for its ability to promote mammary tumors in experimental animals. Mancozeb has been reported to cause immune reactions and hypersensitivity in humans and animals (Bruze and Fregert, 1983; Colosio et al., 1996; Iliev and Elsner, 1997; Koch, 1996; Matsushita et al., 1976), but evidence from studies done so far do not determine whether mancozeb compromises the immune system in a way that would increase cancer risk.

V. Other Information

A. Environmental Fate and Potential for Human Exposure:
Dermal and inhalation exposure to mancozeb has been documented in studies of manufacturing workers and applicators. Exposure levels vary, with the highest exposures being through the dermal route during the weighing, mixing and loading operations. The major concern for human exposure is ETU rather than mancozeb. While a one percent dermal absorption rate is used as an estimate of exposure for mancozeb, it is estimated that 24% of ingested mancozeb may be metabolically converted to ETU; ETU is water soluble and readily absorbed (USEPA, 1987b).

1. Occupational Exposure:
In one study, dermal and inhalation exposure to mancozeb was evaluated for applicators and mixer-loaders during field trials in different states and using different application techniques. These included aerial applications in Michigan, Minnesota and Oregon, airblast spraying in Ohio, and applications using compressed air sprayers in a home yard setting (Mumma et al., 1985). Absorbent pads, respirators, gloves and urine samples were analyzed for mancozeb and ETU residues. Forearms of mixer-loaders were found to be most exposed to mancozeb (0.93 to 7.7 $\mu g/cm^2$). Pilots had relatively lower levels of exposure during aerial spraying, mostly on hands (0.03 to 1.7 $\mu g/cm^2$). Home gardeners experienced little to no exposure, except on their ankles and thighs. Protective clothing was found to greatly reduce exposure in all cases.

Inhalation and dermal exposure was evaluated in four groups of workers with different potential for exposure to EBDC fungicides through their work in potato fields or pine tree nurseries (Kurttio et al., 1990). Group I consisted of nine male applicators at potato farms in 1986; Group II consisted of 29 male applicators at potato farms in 1987; Group III had five male applicators at pine nurseries in 1986; and Group IV had 15 women who weeded in pine tree nurseries in 1987. Protective equipment was poor to non-existent for all, except applicators in Group III. A preliminary analysis by same investigators had revealed that ambient air levels of EBDC were highest during the weighing operations (Savolainen et al., 1989). Urine analyses of the workers indicated highest levels of ETU in samples from the potato field workers (Group I and II), followed by Group III and IV. ETU residues were highest in urine during the first 60 hrs following exposure, dropping to trace amounts, detectable in only the highest exposed workers by 21 days (Kurttio and Savolainen, 1990). The range of concentrations of ETU in the air for the workers in Group II were 0.004 to 3.3 $\mu g/m^3$ in the breathing zone and 0.006 to 0.8 $\mu g/m^3$ in the tractor cabin (Kurttio and Savolainen, 1990). Only one to 10% of the ETU on clothes was estimated to have reached the skin of the workers. Exposure to workers was greatest through inhalation, but did not exceed the acceptable daily intake values recommended by FAO (0 to 0.03 mg/kg bdwt). These results indicate again that use of protective clothing and respirators are effective, and considerably reduce or even eliminate exposures to mancozeb.

Workers (n = 57) at a manufacturing plant were monitored for mancozeb, ETU and dimethoate, the three main active ingredients present at the plant. Urine levels indicated higher exposure levels in workers who packaged powder mancozeb (35% AI). All workers wore gloves during the work shift. Workers involved in mixing powders wore cotton gloves (waterproofed), while those involved in bottling liquid formulations wore latex. Inhalation and dermal exposure through hand contamination were the two main routes of exposure observed among these workers. Skin contamination

under the clothes was negligible. Handwash samples and urinary analyses indicated that all workers were exposed to all the AIs manufactured at the plant, although to a different extent. This result suggested that pesticides were present throughout the factory and not just in the formulation areas (Aprea et al., 1998).

In another study, blood samples of 23 male workers at a mancozeb manufacturing plant in Italy were analyzed for hemoglobin-ETU adducts. ETU adducts were detected in six of the 15 men who were occupationally exposed to average air concentrations of mancozeb at 1 mg/m^3, indicating a high potential for exposure to ETU during mancozeb manufacturing processes (Pastorelli et al., 1995).

Results from studies of occupational exposure to mancozeb have documented exposure to this fungicide during manufacture and field operations. Studies of exposure among applicators suggest that inhalation is the major route of exposure, and weighing and mixing operations contribute considerably to the exposure levels. Use of protective equipment and clothing has been found to greatly reduce or eliminate exposure to mancozeb (Aprea et al., 1998; Kurttio and Savolainen, 1990; Mumma et al., 1985). California EPA has identified 370 carcinogens and 112 reproductive/ developmental toxicants as a result of the State's Safe Drinking Water and Toxic Enforcement Act of 1986. Of these toxicants, a list of 33 potential priority carcinogens and reproductive/ developmental toxicants has been compiled, based on review of published data and technical reports from NTP, IARC and EPA. Mancozeb is listed among these 33 high priority pesticides (Hooper et al., 1992). A similar study has considered direct occupational exposure measurements and dose estimates for pesticides used in California, along with dermal absorption values and toxicological indices. This study has also classified mancozeb as high priority, based on chronic exposure dose estimates and cancer risk (Woodruff et al., 1994).

A study of agricultural workers has compared data on pesticide exposure histories when collected from workers asked to simply recall exposures, versus exposure history constructed from use of "circumstantial determinants" of pesticide use. The rationale behind this study was that workers may be more likely to recall circumstantial determinants such as the crops cultivated, the surface areas treated, and the kind of crop infestations, rather than the names of pesticides used. Specific chemical use recall was found to be lower compared to recall of specific determinants associated with chemical use. When data on circumstantial determinants was used, the number of workers suspected to be exposed to mancozeb was ten, compared to only four workers having recalled using the specific fungicide on the questionnaire (Nanni et al., 1993). Results of this study, while documenting mancozeb exposure in workers, indicate that the numbers of workers exposed to pesticides are usually underrepresented in studies that rely on workers to recall specific chemical exposures.

2. Potential for Exposure for the General Population:
The main potential for mancozeb exposure to the general population is through the low levels of residues that are sometimes found in food (IPCS, 1988).

a. Mancozeb Exposure Through Food and Water:
Mancozeb residues in food vary depending on the food surface characteristics. In a field experiment conducted in Canada, mancozeb residues were found to persist for 28 days after treament of tomato crops, but no residues were found after 28 days on potatoes that had been similarly treated. Small amounts of ETU were found to persist up to 28 days at low levels (< 0.04 ppm) on tomatoes, but not in potato tubers (Newsome, 1979). Different rates of dissipation of mancozeb residues on different fruits has also been observed by other investigators. Field spraying experiments on apricot fields in Greece indicate that mancozeb residues dissipate faster on green apricots than on ripe ones in the initial 21 days after application. These results indicate that the persistence of mancozeb residues on fruits and vegetables can vary, depending on the fruit surface characteristics. Washing the apricots removed 35 to 75% of mancozeb residues (Patsakos et al., 1992). In another study, washing mancozeb-treated tomatoes and spinach in water was found to significantly reduce the load of mancozeb and ETU residues. More residues remained in injured leaves (Lentza-Rizos, 1990).

The main concern regarding the toxic effects of mancozeb is its degradation product ETU. The residues levels of ETU are expected to be below 0.1 mg/kg product, even when EBDCs are applied at the maximum recommended levels (IPCS, 1988). However, studies have indicated that mancozeb converts to ETU more readily under some food processing conditions (Lentza-Rizos, 1990). Boiling tomatoes for 10 minutes, and spinach for 15 minutes caused 48.8%, and 13 to 26% of mancozeb to convert to ETU, respectively. Boiling apples treated with mancozeb caused 5.3 to 8.9% of the mancozeb to convert to ETU. In another study, ETU residues were analyzed in canned baby food made from mancozeb-contaminated apples after processing and a storage period of nine months. The highest conversion of mancozeb to ETU was found to occur after 30 minutes of heat processing. Levels of ETU were reduced by 26 to 70% after nine months of storage. Levels of ETU were reduced to a greater extent at a lower pH (Hajslova et al., 1986). In a study of grain samples, 30% of mancozeb residues were found to convert to ETU after cooking (Rosenberg and Siltanen, 1979). No EBDC residues were found in any commercial beer and wine samples analyzed in a study conducted in France. However, ETU residues were detected in beer made experimentally from mancozeb-treated hops, indicating conversion to ETU during the brewing process.

ETU residues were below the level of detection (0.01 ppm) in 77.4% of commercial beer and 91.8% of commercial wine samples from different regions that were tested by these investigators (Casanova and Guichon, 1988).

EBDCs decompose rapidly in water, with a half-life of less than one day in sterile water (IPCS, 1988). Studies on environmental fate of EBDC compounds submitted to EPA by the registrants had indicated the potential of these fungicides to leach into ground water under certain conditions. In 1988, EPA requested ground water monitoring data for EBDCs. Data submitted in response to this call indicated the occurrence of ETU residues (levels not stated) in the groundwater in Maine, in areas where maneb and mancozeb were the most widely used pesticides (USEPA, 1989). Further studies on ground water levels of EBDCs or ETU were not found. ETU residues are known to be rapidly photolyzed in water in the presence of sunlight.

In summary, mancozeb residues in food vary depending on the food surface characteristics. Washing is effective in reducing the levels of mancozeb by 35 to 75%. The conversion of mancozeb to ETU can vary greatly depending on the food processing steps used. Although water is unlikely to be a major source of mancozeb for the general population, a small survey has indicated ETU residues in ground water in Maine, in areas where maneb and mancozeb were used.

b. Mancozeb Exposure Through Air:

In an indirect study of the potential for human exposure, non-migratory pigeons raised in the vicinity of orchards and vineyards where mancozeb was sprayed repeatedly, were examined and compared to pigeons raised in areas where no agrochemicals were used. The presence of zinc and histopathological lesions in the lungs and trachea of the pigeons in the first group were reported as indicators of exposure to mancozeb around the farms (Roperto and Galati, 1998). However, the possibility of other industrial pollutants which may have contributed to the zinc residues or histopathological lesions of the lungs was not discussed in this study.

3. Storage, Metabolism and Excretion of Mancozeb in Mammals:

In rats fed radioactively labeled mancozeb, 71% of the label was found to be excreted in the feces and 15.5% in the urine (Paulson, 1977). Parent compound was detected in the feces, but not in the urine, indicating that mancozeb is not readily absorbed through the gastrointestinal tract. Metabolites detected in urine and feces included ethyleneuria, ethylenethiourea, and ethylenebisisothyocyanate sulfide. The concentration of radioactivity was found to be higher in the thyroid glands than in other tissues. In cows that were similarly treated, ethyleneurea and ethylenethiourea were detected in the urine and milk (Paulson, 1977).

Studies of occupational exposure of workers in potato fields and pine nurseries have indicated levels of ETU in urine of EBDC-exposed workers to be higher than exposure level to ETU alone, indicating conversion of EBDC to ETU in the body (Kurttio and Savolainen, 1990). ETU and its metabolites were found to have a half-life of 28 hrs in monkeys, 9 to 10 hours in rats and 5 hrs in mice (IPCS, 1988).

VII. Summary and Recommendations for Breast Cancer Risk Classification

Our evaluation on mancozeb leads us to classify it in Group 3, *not classifiable to its breast carcinogenicity in humans* (please see Appendix B for an explanation of the BCERF Cancer Risk Classification Scheme). This is based on the following:

- Human Studies: Breast cancer rates of women exposed to mancozeb in the past have not been studied.
- Animal Studies: Studies that have been done so far have not reported on incidences of mammary tumors in mancozeb-treated animals.
- Related Mechanisms: There is very limited evidence for mancozeb's potential to affect breast cancer risk through other mechanisms. There is evidence that high doses of mancozeb cause chromosomal aberrations in human and animal cells (Georgian et al., 1983; Jablonicka et al., 1989; Khan and Sinha, 1993; Khan and Sinha, 1994; Steenland et al., 1997; Vargova et al., 1987). However, mancozeb was not found to be a strong mutagen in bacteria, yeast or *Drosophila* (Garrett et al., 1986; Vasudev and Krishnamurthy, 1980). Mancozeb has been found to promote skin tumors in mice and pancreatic tumors in rats (Gupta and Mehrotra, 1992; Shukla et al., 1988; Valentich et al., 1996). Mancozeb has not been tested for its ability to promote mammary tumors in animals.

High levels of mancozeb have been associated with carcinogenic effects in rodents (Hurley et al., 1998), and potentially genotoxic effects in humans and animals (Georgian et al., 1983; Jablonicka et al., 1989; Khan and Sinha, 1993; Khan and Sinha, 1994; Steenland et al., 1997; Vargova et al., 1987). Fortunately, the potential for such high exposures to mancozeb for the general population is low. Occupational exposure to mancozeb has been observed. In almost all these studies, protective clothing and use of respirators have been found to be effective in reducing the potential for occupational exposures (Aprea et al., 1998; Kurttio and Savolainen, 1990; Mumma et al., 1985).

VII. Identification of Research Gaps, and Other Recommendations

- Several studies have documented exposure to mancozeb. An ecological study has suggested an increased mortality from cancers of bone, prostate and thyroid in regions where mancozeb was widely used. Large-scale epidemiological studies are needed to evaluate cancer incidences in men and women known to have been exposed to this fungicide through their occupations.
- Studies of experimental animals exposed to mancozeb that have been done have not reported on incidence of mammary tumors. Comparative data of mammary tumors in mancozeb-treated animals and in non-exposed controls is needed for an evaluation of mancozeb's breast carcinogenicity.
- Mancozeb has been found to promote skin tumors in mice and pancreatic tumors in rats. Mancozeb should be tested for its ability to promote mammary tumors in experimental animals that have been treated with a known breast carcinogen.
- Mancozeb should be tested for ability to mimic estrogen in *in vitro* assays.

VIII. Summary of New Human Studies Currently Being Conducted

Health Effects of Exposure in Agriculture
Principal Investigator: Sandler, D., National Institute of Environmental Health (from CRISP Database)
Cancer risk and health effects of farm workers in Iowa and North Carolina will be evaluated in association with agricultural exposures. Health effects of spouses and children of farmers will also be followed in this study. Enrollment for this study includes 83% of the private pesticide applicators and a cohort of 1,000 African-American farm workers.

Pesticides—Health Fertility and Reproductive Risk
Principal Investigator: Garry, V., University of Minnesota, Twin Cities (from CRISP Database)
Male mediated infertility cases in the Red River Valley, Minnesota, will be studied for endocrine disruption, spermatotoxicity, and chromosome aberrations, in association with exposure to pesticides, including fungicides.

Epidemiology of Parkinsons Disease and Farm Risk Factors
Principal Investigator: Strickland, D., University of Nebraska Medical Center (from the CRISP Database)
This case-control study will evaluate specific agricultural exposures and any associations with risk of Parkinsons disease among 300 cases and 600 controls from 66 counties of Nebraska.

Mechanism of Action of Environmental Antiandrogens
Principal Investigator: Wilson, E., University of North Carolina, Chapel Hill (from CRISP Database)
Industrial chemicals and pesticides, including fungicides, will be evaluated for their ability to interact and/or interfere with androgen receptors in an *in vitro* assay. The results of the ability of chemicals to interact with the receptor will be used to evaluate their potential to impact reproductive capacity in wildlife populations. This study would also serve as a screen for chemicals with potential to cause endocrine disruption in humans.

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X. Appendix A. Common Abbreviations, Acronyms and Symbols

AI	active ingredient
ATSDR	Agency for Toxic Substances and Disease Registry
BaP	benzo[*a*]pyrene
BCERF	Program on Breast Cancer and Environmental Risk Factors in New York State, based in the Cornell Center for the Environment, Institute for Comparative and Environmental Toxicology
bdwt	body weight
CAS	Chemical Abstract Service
CDC	Center for Disease Control and Prevention
CfE	Cornell University's Center for the Environment
CI	confidence interval
cm	centimeter
CRISP	Computer Retrieval of Information on Scientific Projects; database of scientific intra and extramural projects supported by the Dept. of Health and Human Services (i.e., NIH, EPA, USDA)
DMBA	7,12-dimethylbenz[*a*]nthracene
DMSO	dimethyl sufoxide
DNA	deoxyribonucleic acid
EBDC	ethylene bisdithiocarbamates
EPA	Environmental Protection Agency
ETU	ethylenethiourea
E-SCREEN	screening assay for estrogenicity that measures proliferative response in estrogen-dependent breast tumor cells
FAO	World Food and Agricultural Organization
FDA	Food and Drug Administration
ft	feet
GUP	General Use Pesticide
hr	hour
IARC	International Agency for Research on Cancer, headquartered in Lyon, France
ICET	Institute for Comparative and Environmental Toxicology
i.p.	interperitoneal
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	kilogram
L	liter
lbs	pounds
m	meter
MCF-7	Michigan Cancer Foundation; cells derived from human breast tumor
µg	microgram
mg	milligram
MTD	maximum tolerated dose
n	number of subjects/animals in the group
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NIH	National Institutes of Health
NMU	nitrosomethylurea
NTIS	National Technical Information Service; repository for federal agency technical reports
NTP	National Toxicology Program
NY	New York
NYS	New York State
OPP	Offices of Prevention, Pesticides and Toxic Substances, Environmental Protection Agency
OR	Odds Ratio
OSHA	Occupational Safety and Health Administration
ppb	parts per billion
ppm	parts per million
REI	Restricted Entry Interval
RfD	reference dose
RNA	ribonucleic acid
RR	relative risk
SCE	sister chromatid exchange
SLRL	sex-linked recessive lethals
SMR	standardized mortality ratio, the ratio of deaths among a cohort, to the expected number of deaths, multiplied by 100
SRR	age-standardized mortality rate ratio
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TSH	thyroid stimulating hormone
TWA	time-weighted average
US	United States
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Symbols:

α	alpha
β	beta
γ	gamma
µg	microgram
µM	micromolar
ng	nanogram
<	less than
>	greater than
%	percent
p	p value
±	plus or minus
=	equal to
®	registered trademark

XI. Appendix B. Critical Evaluations of Breast Cancer Risk

This includes an overview of the Critical Evaluations and explanation of the BCERF Breast Cancer Risk Classification Scheme

The Process

Starting Point - Existing Critical Evaluations on Evidence of Carcinogenicity
IARC Monographs (**I**nternational **A**gency for **R**esearch on **C**ancer)
NTP ARC (**N**ational **T**oxicology **P**rogram, **A**nnual **R**eport on **C**arcinogens)
ATSDR (**A**gency for **T**oxic **S**ubstances and **D**isease **R**egistry)

Conduct **Literature Searches** using databases to obtain historical and the most recent information; i.e. Toxline, Medline, Biosis, Cancerlit

- Peer-reviewed scientific literature-available through Cornell libraries and interlibrary loans.
- Technical Reports-NTIS-National Technical Information Service
- TOXNET databases—EPA's IRIS database source of oncogenicity and regulatory status information
- Grey literature—Studies submitted to EPA that are not published:
  - -Industry generated oncogenicity studies
  - -Some abstracts (short summaries) are on line (IRIS database)
  - -Request reports from industry
  - -Request reports from EPA through Freedom of Information Act

The Critical Evaluation will include some general background information, including chemical name, CAS#, trade name, history of use, and current regulatory status.

Evidence of cancer in other (non-breast) organ systems will be provided in synopsis form with some critical commentary, along with the current overall carcinogenicity classification by international (IARC) and US Federal Agencies (NTP, EPA).

Human epidemiological studies, animal studies, and other relevant studies on possible mechanisms of carcinogenesis are critically evaluated for evidence of exposure to agent and breast cancer risk based on "strength of evidence" approach, according to a modification of IARC criteria as listed in the IARC Preamble. (See below for a more detailed explanation of the BCERF Breast Cancer Risk Classification scheme)

The **emphasis of the document** is the critical evaluation of the evidence for breast cancer carcinogenicity, classification of the agent's breast cancer risk, identification of research gaps, and recommendations for future studies. A section will also be devoted to brief summaries of new research studies that are in progress. A bibliography with all cited literature is included in each critical evaluation. Major international, federal and state agencies will be provided with copies of our report.

General Outline of BCERF Critical Evaluations

I. Introduction
    A. History of Use and Nomenclature
    B. Usage
    C. Chemical Information
II. Current Regulatory Status
    A. Regulatory Status, EPA
    B. Other Sections As Applicable
III. Summary on Evidence of Overall Carcinogenicity (Non-Breast Sites)
    A. Human Studies
    B. Animal Studies
    C. Current Classification of Carcinogenicity by other Agencies
        1. IARC (International Agency for Research on Cancer)
        2. NTP (National Toxicology Program)
        3. EPA (Environmental Protection Agency)
IV. Critical Evaluation of the Scientific Evidence for Breast Carcinogenicity
    A. Humans Studies will include:
        1. Case-Studies
        2. Human Epidemiological Cohort Studies
        3. Human Epidemiological Case-Control Studies
    B. Experimental Animal Studies
    C. Other Relevant Information, including mechanisms by which exposure may affect breast cancer risk (examples: co-carcinogenicity, estrogenicity, endocrine disruptor, mutagenicity, tumor promotion, cell proliferation, oncogene/tumor suppressor gene expression, immune function, etc.)
V. Other Relevant Information
    A. Specific for the pesticide; (i.e. may include information on environmental fate)
    B. When available will summarize information on detection /accumulation in human tissues / and validation of biomarkers
VI. Summary, Conclusions, Recommendation for Classification
VII. Identification of Research Gaps, and Other Recommendations
VIII. Brief Summaries of New Human Studies Currently Being Conducted
IX. Bibliography
X. Appendix A. Common Abbreviations, Acronyms and Symbols
XI. Appendix B. Critical Evaluations of Breast Cancer Risk
XII. Appendix C. Trade Names
XIII. Appendix D. Public Comment Received

BCERF Breast Cancer Risk Classification Scheme (adapted from the IARC Preamble by S.M.Snedeker)

Group 1: **Human breast carcinogen;** *sufficient evidence* of carcinogenicity to humans is necessary. *Sufficient evidence* is considered to be evidence that a **causal** relationship has been established between exposure to the agent and human breast cancer.

Group 2A: **Probable breast carcinogen**; this category generally includes agents for which there is *1) limited evidence* of breast carcinogenicity in humans and *sufficient evidence* of mammary carcinogenicity in experimental animals. The classification may also be used when there is *2) limited evidence* of breast carcinogenicity in humans and strong supporting evidence from other relevant data, or when there is 3) *sufficient evidence* of mammary carcinogenicity in experimental animals and strong supporting evidence from other relevant data.

Group 2B: **Possible breast carcinogen;** this category generally includes agents for which there is 1) *limited evidence* in humans in the absence of *sufficient evidence* in experimental animals; 2) *inadequate evidence* of carcinogenicity in humans or when human data is nonexistent but there is *sufficient evidence* of carcinogenicity in experimental animals, 3) *inadequate evidence* or no data in humans but with *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data.

Group 2C: **Potential to affect breast cancer risk**; this category includes agents for which there is **inadequate or nonexistent human and animal data**, but there is **supporting evidence from other relevant data** that identifies a mechanism by which the agent may affect breast cancer risk. Examples are, but are not limited to: evidence of agent's estrogenicity, disruption of estrogen metabolism resulting in potential to affect exposure to estrogen; evidence of breast tumor promotion, progression or co-carcinogenicity; increased expression of proto-oncogenes or oncogenes; evidence of inactivation of tumor suppressor gene associated with breast cancer; evidence of adverse effect on immune function; or evidence of a structural similarity to a known breast carcinogen (structure-activity relationship).

Group 3: **Not classifiable** as to its breast carcinogenicity to humans. Agents are placed in this category when they do not fall into any other group.

Group 4: **Probably not a breast carcinogen in humans**: This category is used for agents for which there is evidence suggesting a lack of breast carcinogenicity in human studies and in animal studies, together with a lack of related evidence which may predict breast cancer risk. The absence of studies does **not** constitute evidence for a lack of breast carcinogenicity.

Brief Definitions of Sufficient, Limited, and Inadequate Evidence: (adapted for breast carcinogenicity from the IARC Preamble by S.M. Snedeker)

Human Studies

Sufficient evidence of carcinogenicity in humans: Must have established evidence between exposure to the agent and human breast cancer. Case-reports are given the least weight in considering carcinogenicity data in humans—they are suggestive of a relationship, but by themselves cannot demonstrate causality. Consistent, case-control studies which have controlled for confounding factors and have found high relative risks of developing breast cancer in relation to an identified exposure are given the most weight in determining a causal relationship.

Limited evidence of breast carcinogenicity in humans: A positive association has been observed between exposure to the agent and breast cancer, but chance, bias or confounding factors could not be ruled out.

Inadequate evidence of breast carcinogenicity in humans: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association.

Experimental Animal Studies

Sufficient evidence of breast carcinogenicity in animals: Evidence of malignant tumors or combination of benign and malignant tumors in (a) two or more species of animals, (b) or two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Limited evidence of breast carcinogenicity in animals: The studies suggest a carcinogenic effect, but are limited for making a definitive evaluation because: (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent increases the incidence of only benign neoplasms of lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains of animals.

Inadequate evidence of breast carcinogenicity in animals: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations.

XII. Appendix C. Trade Names of Mancozeb* (Meister, 1999)

Table 2. Trade names and formulators of mancozeb-containing products

Trade names	Producer/formulator
Mancotan®	Agrochemical Industries Co. Ltd.
Amicozeb®	Agrolex Pte. Ltd.
Mankotam M-45®	AGRO-SAN Kimya Sanayi ve Ticaret A.S.
Chemispor®, Man 50®	Chemiplant S.A.
Dhanuka M-45®	Dhanuka Pesticides Ltd.
Phytox® MZ80	Diachem S.p.A.
Manozin®, Manzin®	Dupocsa
Formanco®, Forthane®, Fothane®	Forward International Ltd.
Fumazin®, Hekmazin®	Hectas Ticaret T.A.S.
Hilthane®	Hindustan Insecticides Ltd.
Crittox®	Industrie Chimiche Caffaro S.p.A
Nemispor®	ISAGRO S.p.A.
Kilpest M-45®	Kilpest India Ltd.
Dikotan®	Karuma Tarim, S.A.
Top-Gun®	Ladda Co., Ltd.
Mangazeb®	Lainco, s.a.
Luxazeb®	Luxan B.V.
Mancomed®	Medmac Agrochemicals
Dikozeb®	Midiltipi Agro-Chemicals, Inc.
Belpron®	Probelte, S.A.
Lucazeb®	Quimica Lucava, S.A. de C.V.
Emthane-M-45®	Sabero Organics Gujarat Ltd.
Raze®	Searle India Ltd.
Wopromanzin®	B.V. Industrie-& Handelsonderneming Simonis
Grain Guard®	Trace Chemicals, Inc.
Mancothane®	VAPCO
Vimancoz®	Vietnam Pesticide Co.
Sparsh®	Wockhardt Ltd.
Pennflo®, Ziman®	

Ref: (Meister, 1999)

**Note: Trade names are used herein for convenience and informational purposes only. No endorsement of products is intended and no criticism of unnamed products is implied. Trade names of mancozeb and mixtures containing mancozeb listed are those currently in use.. Discontinued trade names are listed at the end of each table.*

Table 3. Trade names of pre-mixes containing mancozeb

Trade mixes	Other pesticides in pre-mix	Producer/formulator
Manzivex Forte®		Agrides, S.A.
Tricopper-M®	+ copper oxychloride + copper sulfate	Agrochemicals Industries Co. Ltd.
Cymotine®	+ cymoxanil	
Agromil-MZ®	+ metalaxyl	
Acrobat® MZ, Acrobat® Plus, Invader®	+ dimethomorph	American Cyanamid Co.
Talman Combi®	+ metalaxyl	Cequisa
Occidor Plus®	+ carbendazim	Chimac-Agriphar S. A.
Manzeb®	+ maneb	Crystal Chemical Inter-American and Dupocsa
Cimomanil®	+ cymoxanil	Diachem S.p.A.
Cuprofix® 30	+ copper sulfate	Elf Atochem North America, Inc.
Fortazeb®	+ metalaxyl	Forward International Ltd.
ManKocide®	+ copper hydroxide	Griffin Corp.
Trenetal®	+ chlorothalonil + metalaxyl	Helb USA, Inc.
Kendofort®	+ copper, fixed + iron	
Metazol®	+ metalaxyl + sufur	
Curzeb®	+ cymoxanil	Hectas Ticaret T.A.S.
Tattoo®	+ propamocarb hydrochloride	Hoechst Schering AgroEvo GmbH
Manage® M	+ imibenconazole	Hokko Chemical Industry Co., Ltd.
Crioram®	+ Bordeaux mixture	Industrie Chimiche Caffaro S.p.A.
Oxicob-mix®	+ copper oxychloride	Ingeniera Industrial, S.A. de C.V.
Galben® M	+ benalaxyl	ISAGRO S.p.A.
Tri FL + Karnak®	+ captan + cymoxanil	Lainco, s.a.
Laikenia®	+ cymoxanil	
Branda®	+ metalaxyl	
TriKubrazeb Forte-S®	+ basic copper sulfate	Midiltipi Agro-Chemicals, Inc.
Kuprosolor®	+ fixed copper + sulfur	Midiltipi Agrochemicals, Inc.
Mildifan M®	+ oxadixyl	
Midipost M®	+ oxadixyl + cymoxanil	
Superxyl®	+ metalaxyl	Nantong Dyes Chemical Factory
Pulsan®, Ripost® M	+ cymoxanil + oxadixyl	Novartis
Ridomil Gold MZ®	+ mefenoxam	
Acylon® Fubol® Ridomil® MZ	+ metalaxyl	
Trimiltox®	+ mixed copper	
Sandofan® M	+ oxadixyl	
Fosbel Plus®	+ fosetyl-aluminium	Probelte, S.A.
Otria Plus®	+ metalaxyl	
Rhodax®	+ fosetyl-aluminium	Rhone-Poulenc
Mexyl MZ®	+ metalaxyl	Saigon Pesticide Co.
Grain Guard Plus®	+ lindane	Trace Chemicals, Inc.
Mantox®	+ copper oxychloride	VAPCO
Curtine-V®	+ cymoxanil	
Vacomil® MZ	+ metalaxyl	
Vimonil®		Vietnam Pesticide Co

XIII. Appendix D. Public Comments Received

After technical internal and external peer-review, the Critical Evaluation will be posted on the BCERF web site for 30 days. If any public comments are received, they will be scanned as submitted, and become a part of Appendix D.