FILE COPY

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

NOV 19 1992

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of MANCOZEB

FROM: Irving Mauer, Ph.D.
Toxicology Branch-I
Health Effects Division (H7509C)

09-30-92

and
Esther Rinde, Ph.D.
Manager, Carcinogenicity Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Susan Lewis, PM 21
Registration Division (H7505c)
and

Walter Waldrop
Special Review and Reregistration Division (H7508W)

The Health Effects Division Carcinogenicity Peer Review Committee met on 6/3/92 to discuss and evaluate the weight-of-the-evidence on mancozeb with particular reference to its carcinogenic potential.

The Peer Review Committee agreed that mancozeb should be classified as Group B2- probable human carcinogen with inadequate evidence in humans.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Karl Baetcke

Marcia Van Gemert

Reto Engler

William L. Burnam

Marion Copley

Printed on Recycled Paper

Kerry Dearfield _____

George Ghali _____

Hugh Pettigrew _____

Esther Rinde _____

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Irving Mauer[1] _____ 09-30-92

Bernice Fisher _____

for Michael Stedham[2] (PAI/Clement) _____

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penelope Fenner-Crisp _____

Julie Du _____

Richard Hill _____

Jean Parker _____

John Quest _____

William Sette _____

Yin-Tak Woo _____

4. Other Attendees:

Eve Andersen (Clement)

Lori Brunsman (HED)

---

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with the pathological findings.

B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Irving Mauer; tables and statistical analysis by Bernice Fisher. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by Du Pont and Rohm and Haas.

C. Background Information:

Mancozeb (chemically, manganese ethylene bisdithiocarbamate [EBDC] complex with zinc salt) is a broad-spectrum fungicide registered for use to prevent damage to rac, as well as to protect harvested products from deterioration. It (as well as its EBDC congeners) is unstable in the presence of moisture and oxygen, as well as in biological systems, degrading principally to ethylenethiourea (ETU), a production contaminant and metabolite common to all EBDCs. EBDC residues (including mancozeb) also convert readily to ETU during commercial processing or (home) cooking of treated rac or food.

In 1977, the Agency initiated a Special Review (formerly referred to as Rebuttable Presumption Against Registration [RPAR]) of this class of fungicides, based upon the presumption that the EBDCs, and their common metabolite, ETU, pose several risks to human health and/or the environment, specifically: Oncogenicity, teratogenicity and acute toxicity to aquatic organisms. This list of concerns was expanded to include thyroid toxicity, mutagenicity and skin sensitization. In 1982, this review was concluded in a Decision Document reporting the following Agency's conclusions:

1. The potential risk of acute toxicity to aquatic organisms resulting from use of mancozeb on commercially grown wild rice would be mitigated through current cultivating practices, and the addition of a statement to the label warning users of a hazard to fish.

2. Potential risk of teratogenicity and thyroid toxicity to commercial and agricultural applicators would be adequately reduced by requiring protective clothing.

3. Potential dietary exposure resulting from consumption of home-grown produce could be reduced by highlighting preharvest intervals on labels of noncommercial products used by home gardeners.

4. The issues of whether mancozeb and other EBDCs or ETU pose a potential risk of oncogenicity, mutagenicity, teratogenicity, and thyroid effects to man were subject to many uncertainties. Available data on oncogenicity and

mutagenicity were not adequate to resolve key scientific issues such as their mechanism of action, and consequently additional data on the EBDCs as well as ETU were needed for the Agency to determine their mutagenic potential and to assess human exposure and oncogenic risk. Some data would be required at the termination of the special review while further data needs, with particular emphasis on chronic studies, dietary residues and exposure, would be identified during a later reregistration review. Data determined to be needed at that time (1982) were:

a. Metabolism studies designed to define the in vivo conversion of mancozeb and other EBDCs to ETU as well as other derivatives.

b. Dermal absorption studies designed to demonstrate the dermal penetration of mancozeb (as well as other EBDCs), and of ETU.

c. A battery of mutagenicity studies on each of the six registered EBDCs.

d. Mammalian cell transformation assays on each of the six EBDCs, and on ETU.

With the issuance of the 1982 Decision Document, the Agency concluded the special review and returned the EBDCs to the registration process, on the condition that registrants comply with the label changes and data requirements specified therein.

Since issuance of the Decision Document, the Agency has issued four data call-in (DCI) notices for mancozeb, as follows:

1. January 17, 1983, which required the submission of the metabolism, dermal penetration and mutagenicity data identified in the 1982 Decision Document.

2. July 20, 1984, which advised registrants of the Agency's concern about the existence of pesticides in ground water and the designation of a number of chemicals, including mancozeb, which may have the potential to contaminate ground water. These chemicals were identified based upon such factors as chemical structure, solubility, and use patterns. This notice required submission of certain environmental fate and product chemistry data.

3. The October 19, 1984 notice required dietary exposure, product chemistry and toxicological (subchronic feeding and inhalation) data, considered necessary to reassess the registration status of mancozeb.

4. Finally, the April 30, 1985 notice required additional data, not identified in the October 19, 1984 DCI, but considered necessary for risk reassessment of these chemicals, namely, additional toxicological (subchronic feeding and inhalation) and residue data for ETU, in addition to the above on mancozeb.

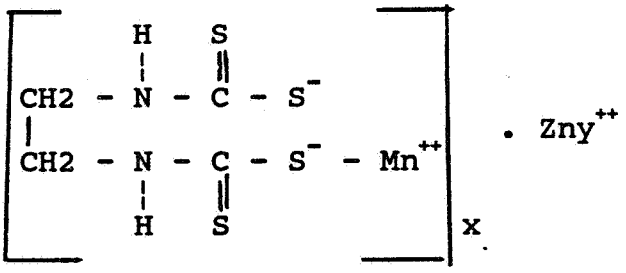
The data required by these call-in notices have since been received and considered by the Agency in its evaluation of mancozeb, as presented in the assessment section of the mancozeb Registration Standard (April, 1987).

Since the issuance of the Standard, further data from long-term (chronic feeding) as well as other CORT studies, have been submitted by the registrants and evaluated by the Agency, essentially completing the tox. data base required for re-registration of mancozeb.

This data base is summarized below, and documented by relevant DERs appended.

The Caswell (or Tox Chem) Number of mancozeb is 913A.
The Chemical Abstracts Registry Number (CAS No.) is 8018-01-07.
The Shaughessy Number is 014504

The structure of mancozeb is



D. Evaluation of Carcinogenicity Evidence:

1. Rat Carcinogenicity Study

Reference: Combined Chronic Toxicity/Oncogenicity Study with Mancozeb: Two-year Feeding Study in Rats, performed at du Pont's Haskell Laboratories, Newark, DE, Project #7859-001/Report No. 259-89, dated September 13, 1990 (EPA MRID 41903601).

a. Experimental Design

Mancozeb technical (83.8% ai) was fed to male and female Crl:CD(BR) rats for 24 months at dietary levels of 0, 20, 60, 125, or 750 ppm (providing 0, 0.77, 2.33, 4.38, or 30.90 mg/kg/day for males; 0, 1.06, 3.06, 6.72, or 40.20 mg/kg/day for females).

b. Discussion of Tumor Data

Male rats had a significant dose-related, increasing trend ($p \leq 0.01$) in thyroid follicular cell adenomas and carcinomas, as well as in the combined thyroid follicular cell adenomas and/or carcinomas. The three tumor rate categories also were significantly increased in the pair-wise comparison of controls at the highest (750 ppm) dose group ($p<0.01$).

Females also had a significant dose-related, increasing trend ($p<0.01$) in thyroid follicular cell adenomas and carcinomas as well as in the combined thyroid follicular cell adenomas and/or carcinomas. The combined thyroid follicular cell adenoma and/or carcinoma tumor rate at the HDT was significantly ($p<0.01$) increased over the control value. The tumor rates in adenomas and in carcinomas had only borderline significant increases in pair-wise comparisons of controls and the highest (750 ppm) dose group ($p<0.052$, 0.056).

Mancozeb - Sprague-Dawley Male Rats Thyroid Follicular Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	20	60	125	750
Tumors					
Adenomas (%)	0/70 (0)	1/72 (1)	1/71 (1)	0/68 (0)	20[a]/71 (28)
p=	0.00**	0.507	0.504	1.000	0.000**
Carcinomas (%)	0/70 (0)	1[b]/72 (1)	2/70 (3)	2/68 (3)	14/71 (20)
p=	0.00**	0.507	0.248	0.241	0.000**
Both (%)	0/70 (0)	2/72 (3)	3/70 (4)	2/68 (3)	34/71 (48)
p=	0.00**	0.255	0.122	0.241	0.000**

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died before 52 weeks.

[a] First adenoma observed at week 63, dose 750 ppm.
[b] First carcinoma observed at week 52, dose 20 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then p<.05 and if ** then p<.01.

Mancozeb - Sprague-Dawley Male Rats, Thyroid Follicular Cell Hyperplasia Only Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	20	60	125	750
Hyperplasia only (%)	1/70 (1)	1/72 (1)	2/71 (3)	3[a]/68 (4)	25/71 (35)
p=	0.0**	0.745	0.505	0.299	0.000**

[+] Number of animals with hyperplasia/Number of animals examined, excluding those that died before 52 weeks.

[a] First hyperplasia observed at week 52, dose 125 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then p<.05 and if ** then p<.01.

Mancozeb - Sprague-Dawley Female Rats, Thyroid Follicular Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	20	60	125	750
Tumors					
Adenomas (%)	1[a]/62 (2)	1/60 (2)	1/62 (2)	1/61 (2)	6/60 (10)
p=	0.001**	0.744	0.752	0.748	0.052
Carcinomas (%)	0/62 (0)	0/60 (0)	0/62 (0)	1/61 (2)	4[b]/60 (7)
p=	0.000**	1.000	1.000	0.496	0.056
Both (%)	1/62 (2)	1/60 (2)	1/62 (2)	2/61 (3)	10/60 (17)
p=	0.000**	0.744	0.752	0.494	0.004**

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before 54 weeks.

[a] First adenoma observed at week 83, dose 0.
[b] First carcinoma observed at week 99, dose 750 ppm.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Mancozeb - Sprague-Dawley Female Rats, Thyroid Follicular Cell Hyperplasia Only Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	20	60	125	750
Hyperplasia only (%)	1/72 (1)	0/71 (0)	1/72 (1)	0/71 (0)	27[a]/72 (38)
p=	0.000**	0.504n	0.752	0.504n	0.000**

[+] Number of animals with hyperplasia/Number of animals examined, excluding those that died before observation of the first lesion.

[a] First hyperplasia observed at week 44, dose 750 ppm.

n Negative change from control.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then p<.05 and if ** then p<.01.

c. Non-neoplastic Lesions and Other Observations

In both sexes of rats receiving the high dose, increases in both absolute and relative thyroid weights were seen at 24 months. An increase in the incidence and severity of bilateral retinopathy was also noted in the high-dose rats at 24 months. Granular yellowish-brown pigment was seen microscopically in the kidneys of males and females receiving 125 or 750 ppm at both 12 and 24 months; however, there were no accompanying dose-related increases in histopathological lesions in the kidney. Reductions in body weight gains were noted in the high-dose males during the first year and throughout most of the second year of the study.

The incidence of diarrhea was lower in the high-dose males as compared to controls. No effects on survival, food consumption, hematology, ophthalmology, or urinalysis were observed.

Statistical evaluation of mortality indicated no significant dose-related differences in survival in either sex.

Decreased levels of thyroxine (T4) were seen in high-dose males and females ($p<0.05$). Thyroid stimulating hormone (TSH) was increased in both high-dose males and females ($p<0.05$). Triiodothyronine (T3) was not affected consistently.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered adequate for assessment of carcinogenic potential. A loss in body weight gain was noted in high-dose female rats during the first 91 days of treatment; although these females initially recovered from the loss in body weight gain, body weight gains were still lower than controls at the end of 1 year. The PRC determined that the dosing was adequate for assessment of carcinogenic potential.

2. Mouse Carcinogenicity Study

Reference: (83-2) Oncogenicity Study --- Mancozeb: 18 Month Dietary Oncogenicity Study in Mice, performed for the registrant by Tegeris Laboratories, Inc., Temple Hills, MD, Study No. 85051; Final Report dated June 04, 1991 (EPA MRID No. 41981801).

a. Experimental Design

Mancozeb was administered for 18 months in the diet of CD-1 mice (94 animals per sex per group) at concentrations of 0, 30, 100 and 1000 ppm, which provided average compound intakes for males/females of, respectively: 0/0, 4/5, 13/18 and 131/180 mg/kg/day.

b. Discussion of Tumor Data

No statistically significant dose-related increases over control values for tumors overall or in any tumor type were recorded in mice.

c. Non-neoplastic Lesions and Other Observations

Incidences of some non-neoplastic endpoints were found to be significantly increased. There were calculus of the urinary bladder in mid-dose males, cystic follicles of the thyroid in mid-dose females and lymphocytosis of the urinary bladder in high-dose females. However, no dose-related patterns or trends were observed in these responses. The following changes in thyroid function also were observed: In males, triiodothyronine (T3) levels were significantly increased in the high-dose group, whereas in females, levels of thyroxine (T4) and T3 were significantly decreased. No significant changes in thyroid-stimulating hormone (TSH) levels were reported.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be inadequate for assessing the carcinogenic potential of mancozeb in mice. Body weight gains were decreased 13% in males, but only 9% in females.

E. Additional Toxicology Data on Mancozeb:

1. Metabolism

Fifty percent of orally administered single doses of 1.5 or 100 mg/kg mancozeb was rapidly absorbed, and excreted equally in feces and urine. The parent chemical was rapidly metabolized to ETU and (other) intermediates (ETD, EBIS, EDA, etc.). Absorbed doses accumulate in major organs, to the greatest extent in thyroid (residue analysis for ETU = 1 ppm during 24 hours after the 100 mg/kg dose, but undetectable thereafter).

2. Mutagenicity

Testing requirements for both mancozeb as well as for ETU have been satisfied. Acceptable data from the full initial battery of mutagenicity studies are inconsistent and equivocal. There are several studies with negative results, and there were some studies with positive results.

For mancozeb, repeated (adequate) negatives have been registered for both bacterial (Salmonella) and mammalian cell gene mutation

assays. Mancozeb was positive for aberrations in CHO cells, but negative in rat bone marrow and in a mouse micronucleus assay. Mancozeb was positive in a sister-chromatid exchange (SCE) assay in CHO cells. A weak positive result was found for unscheduled DNA synthesis (UDS) in HeLa cells, but negative results were found in primary rat hepatocytes.

It should be noted that in many instances the induced genotoxic effects by mancozeb are not extremely substantial, for example the SCE assay. However, in some instances, the response is significantly large, for example the in vitro cytogenetics assay. Overall, it appears that mancozeb has some genotoxic activity that may contribute to a mutagenic concern. ETU itself, while also producing some genotoxic activity, does not appear to be a highly genotoxic agent. Another concern may be the aspect of nitrosation of ETU. Nitrosated ETU and ETU in combination with sodium nitrite have been demonstrated to induce potent genotoxic effects in gene mutation assays and in in vivo micronucleus and aberration assays. However, there is not enough information currently available to clearly discern a role for the "weak" genotoxicity of mancozeb and ETU in the induction of thyroid tumors.

The body of evidence for ETU suggests that ETU is capable of inducing a variety of genotoxic endpoints. These include responses to gene mutation assay (e.g. Salmonella and mouse lymphoma assays), structural chromosomal assays (e.g. aberrations in cultured mammalian cells as well as a dominant lethal assay) and other genotoxic effects (e.g. bacterial rec assay and yeast conversion assay).

A major consideration that should be taken into account when examining the genotoxicity of ETU is the magnitude of these positive responses. While ETU induces a variety of genotoxic endpoints, it does not appear to be a potent genotoxic agent. For example, it is considered a weak bacterial mutagen in the Salmonella assay without activation in strain TA1535 at concentrations usually at or above 1000 ug/plate. Activation conditions generally do not alter the mutagenic response. This type of activity is usually seen in most of the assay with positive results. It should be noted that since ETU does not appear to be very potent, and that it is not extremely toxic to test cells and organisms, it is not surprising to find that ETU does not induce effects in many of the assays reviewed. Therefore, in many instances, positive and negative results in the same assay are reported from different investigators, but these results may be dependent upon the test conditions in each individual laboratory. However, usually there are problems with many of the negative assay in protocol or reporting, and in many studies, the concentration levels used are not high enough for an adequate test.

3. Developmental Toxicity

Mancozeb produced developmental toxicity but only above the dose level producing maternal toxicity (A/D ratios were <1.0).

In rat litters, dilated ventricles, spinal cord hemorrhage and delayed ossification, accompanied by increased resorptions and decreased pup weight were encountered at a dose of 512 mg/kg/day (NOEL = 128 mg/kg), compared to the maternal toxicity (decreased food consumption and weight) at 128 mg/kg/day (NOEL = 32 mg/kg). In rabbits, developmental toxicity was not registered at the HDT, 80 mg/kg/day, a dose level resulting in maternal ataxia, abortion and death (maternal NOEL = 30 mg/kg/day).

In an acceptable reproduction study in CD:BR (Sprague-Dawley) rats, dietary mancozeb at doses up to 1200 ppm fed over two generations resulted in increased liver weights in P2 males, and renal pigment in both parental sexes (NOEL = 30 ppm, equivalent to intakes of 1.5-2.5 mg/kg/day), but no reproductive effects at the HDT.

4. Structure-Activity Correlations

A major toxicological concern from exposure to mancozeb is the hazard to the human thyroid from the presence of ethylenethiourea (ETU), a contaminant, degradation product and metabolite present in mancozeb and other EBDC products. In addition to the thyroid effects, systemic effects have been observed in both the kidney and prostate gland.

ETU has caused developmentally toxic/teratogenic effects in rats and hamsters. However, available data indicate that mancozeb is not a primary developmental toxicant or teratogen.

ETU has been classified as a Group B2 carcinogen in accordance with the Agency's Guidelines for Carcinogen Risk Assessment (September 26, 1986, 51 FR 33992), based on studies which show that it induced an increased incidence of thyroid adenomas and adenocarcinomas in rats, and thyroid and liver tumors in mice.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on mancozeb in a weight-of-the-evidence determination of carcinogenic potential:

1. Mancozeb was associated with statistically significant dose-related increasing trends in thyroid follicular cell adenomas, carcinomas and combined adenomas/carcinomas in both sexes of the Sprague-Dawley rat when fed in the diet at doses up to 750 ppm (HDT). In male rats, at the HDT, the incidences of adenomas, carcinomas and combined adenoma/carcinomas were also significantly increased ($p<0.01$) in pair-wise comparison with controls. In female rats, at the HDT, only the combined adenoma/carcinoma incidence was significantly increased ($p<0.01$) in pair-wise comparisons with controls; the increases in adenomas and in carcinomas were of borderline significance ($p=0.052$, 0.056, respectively).

The incidence of tumors at the HDT was outside the range reported for historical controls at the testing facility. The HDT was considered adequate, based on increased thyroid weight coupled with increase in incidence and severity of retinopathy and renal pigment (at both mid and high-dose) in rats. While a hormonal influence for thyroid gland tumors has been suggested, conclusive evidence is lacking.

2. Mancozeb was not associated with increases in neoplasms when fed in the diet to CD-1 mice at doses up to 1000 ppm. The HDT in this study was considered to be inadequate to assess carcinogenic potential, since body weight loss in females was only 9%.

3. There was some evidence of genotoxicity from both mancozeb and ETU, but the data were inconsistent and equivocal to consider either chemical a positive mutagen.

4. The thyroid tumor response is consistent with that seen with other members of this class of compounds. Both maneb and ETU, with structures closely related to mancozeb, were associated with thyroid toxicity and/or tumors. Mancozeb is known to be converted to ETU, which is classified as a B2 carcinogen. The types of tumors associated with mancozeb are the same as those associated with ETU.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that the classification for mancozeb should be Group B2- probable human carcinogen with inadequate evidence in humans.

This decision was based on statistically significant increases in thyroid follicular cell tumors in both sexes of the rat, in a study which used adequate doses for the determination of carcinogenic activity. Acceptable data from the full initial battery of mutagenicity studies have indicated ETU and Mancozeb are (at best) very weak mutagens.

Mancozeb is converted to ETU which is a Group B2 carcinogen. For this reason, the PRD determined that the q_1^* should be the same as for ETU.