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

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JUN 6 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Two Mutagenicity Studies on Maneb
Caswell #539. Project No. 443 (partial)

TO: Jodi Bakst
SPRD

FROM: Byron T. Backus Byron T. Backus 05/30/86
Toxicology Branch
HED (TS-769C)

THROUGH: Marcia van Gemert, Ph.D., Section Head
Review Section III
and
Ted Farber, Ph.D., Chief
Toxicology Branch
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Compound: Maneb

Action Requested:

The Toxicology Branch has been asked to review eight mutagenicity studies and comment as to their acceptability in satisfying data gaps on the subject chemical. This memorandum covers the reviews of two of these studies, with six remaining.

Comments and Recommendations:

1. The study by Ivett and Lebowitz, titled "Clastogenic evaluation of Maneb technical lot MT 01 (88.1% a.i.) conducted by Litton Bionetics Inc. has been classified as acceptable.

   Under the conditions of this assay, acute (one dose) oral ingestion of 4.9 g/kg and subacute (daily x 5 days) exposure to 1.64 g/kg in male rats did not cause a significant increase in chromosomal aberrations in bone marrow cells sampled over a complete mitotic cycle.

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2. The study by Loveday titled "Salmonella/microsome mutagenesis assay on technical grade Maneb" conducted by Bioassay Systems Corporation has been classified as unacceptable.

Under the conditions of the assay, Maneb, tested at six doses ranging from 1 to 20 ug/plate, did not cause a mutagenic response in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 or TA1538 without S9 activation or in the presence of rat or mouse S9. However, the range-finding study defining the toxicity of the test material (and highest dose used in the assay) was conducted only in the absence of S9 fraction (or under non-activated conditions).

The assay should be repeated, with preliminary range-finding assays defining the toxicity of the test material with separately conducted rat and mouse S9 exposures. The report should include information as to cell densities of cultures used. Cytotoxicity should be evident in results from at least the highest dose level. Additionally, statistical methods should be used to give levels of significance (where appropriate) to differences between means. It may be appropriate to do plating in triplicate, rather than duplicate, for calculation of standard deviations.

Based on numbers of mean revertants/plate, there were no indications of cytotoxicity for TA98, TA100 or TA1537 in the nonactivated assay conducted concurrently with the rat liver S9 assay. From these results, the test material should have been tested even in the nonactivated assay at an additional dose level that would have been higher than 20 ug/plate.

Data Evaluation Reports (attached)

1. Ivett, J. L. and Lebowitz, H. Clastogenic evaluation of Maneb technical lot MT 01 (88.1% a.i.). (Unpublished study no. 22202, LBI Assay No. 7892, prepared by Litton Bionetics, Inc., Kensington, MD, for the Maneb Task Force; dated August, 1985). Acc. no. 259071.

2. Loveday, K. S. Salmonella/microsome mutagenesis assay on technical grade Maneb. (Unpublished study no. 840014-40 conducted by Bioassay Systems Corporation, 225 Wildwood Ave., Woburn, MA 01801, for George Pazianos; dated June 17, 1985). Acc. no. 259018.

Data Evaluation Report (I)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb technical, lot MT 01, 88.1% a.i., described as a pale green powder.

3. STUDY/ACTION TYPE: Mutagenicity—in vivo bone marrow cytogenetics assay in rats.

4. STUDY IDENTIFICATION: Ivett, J. L. and Lebowitz, H. Clastogenic evaluation of Maneb technical lot MT 01 (88.1% a.i.). (Unpublished study no. 22202, LBI Assay No. 7892, prepared by Litton Bionetics, Inc., Kensington, MD, for the Maneb Task Force; dated August, 1985). Acc. no. 259071.

5. REVIEWED BY:

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Toxicologist
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6. APPROVED BY:

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7. CONCLUSIONS:

Under the conditions of this assay, the acute (one dose) oral exposure of 4.9 g/kg and subacute (daily x 5 days) oral exposure to 1.64 g/kg/day in male rats did not cause a significant increase in chromosomal aberrations in bone marrow cells sampled over a complete mitotic cycle.

8. RECOMMENDATIONS:

Both the acute and subacute studies are acceptable.

9. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Test Material: Maneb, lot MT 01, 88.1% a.i., described as a pale green powder.

2. Test Animals: Random-bred adult male Fischer 344 rats, purchased from Charles River Breeding Laboratories, Inc., Kingston, N.Y.

3. Animal Maintenance: Rats were housed 2/cage. Purina certified Laboratory Chow #5002 and water were available ad libitum. Animals were quarantined for at least 2 weeks prior to the start of the study.

4. Assignment to Groups: One hundred and seventy rats were randomly assigned to dosage groups according to the testing facility's standard operating procedures. Each rat was individually identified by ear tag; treatment groups were identified by cage card. Prior to study initiation, individual rats were weighed to calculate the amount of test material each would receive. Weights of rats receiving Maneb ranged from 178.5 to 212.4 grams at the start of the study.

5. Compound Preparation/Dosing Procedures:

Suspensions were prepared by mixing 35.0 g of Maneb technical with 85 ml 0.25% (low viscosity) carboxymethylcellulose (CMC). The resulting suspension contained 328 mg/ml Maneb, and was administered directly (at 15 ml/kg, or 4.92 g Maneb technical per kg) to high-dose rats in the acute and subacute groups. The medium dose level in the subacute study had a concentration of 109 mg/ml (x 15 ml = 1.64 g/kg).

6. Dose Selection:

The sponsor recommended high-dose level for both the acute and subacute study was 5 g/kg.

7. Compound Administration:

a. Acute Cytogenetics Study: Thirty male rats per dosage level were fasted 18 hours before oral administration of the test material or vehicle control. Rats were weighed before being dosed.

Ten males from each dosage level, as well as ten males from the vehicle control group, were sacrificed at 6, 24 and 48 hrs after administration of the test material. A positive control group (triethylenemelamine at 1.0 mg/kg administered once IP) of ten males was sacrificed 18 hrs after being injected.

b. Rats in the subchronic portion of the study were weighed each day before being dosed. They were sacrificed 6 hrs after receiving the last dose of test material.

8. Animal Sacrifice/Bone Marrow Harvest: Animals were weighed and 2.0 mg/kg colchicine was injected IP three hours prior to sacrifice (since CO_2 is mentioned this was presumably by asphyxiation). The marrow was removed from both tibiae and transferred to Hanks' balanced salt solution. The marrow button was collected by centrifugation and then resuspended in 0.075M KCl. The centrifugation was repeated and the cells were resuspended in methanol:acetic acid (3:1) fixative. The fixative was changed once and cells were left overnight at 4° C. These cells were then dropped onto glass slides, air-dried, and stained with 5% Giemsa at pH 6.8.

9. Slide Analysis: Slides were coded to avoid bias and then scored for chromosomal aberrations. A list of aberrations includes:

chromatid gap	pulverized chromosome
chromatid break	pulverized cells
isochromatid gap	ring chromosome
chromosome break	dicentric chromosome
chromatid deletion	minute chromosome
fragment	double minute chromosome
acentric fragment	abnormal metacentric chromosome
translocation	greater than 10 aberrations
triradial	polyploidy
quadriradial	both hypo- and hyperploidy
complex rearrangement	

Gaps were not counted as significant aberrations.

A maximum of 50 well-spread metaphases/animal were scored for the presence of the above abnormalities.

Mitotic indices were based on at least 500 cells/animal.

The highest dose groups (4.9 g/kg for the acute phase of the study, and 1.64 g/kg/day for the subacute) from which data could be obtained, along with their respective vehicle controls, were analyzed for chromosomal aberrations.

10. Evaluation Criteria: "The type of aberration, its frequency, the statistical significance of any increase and its correlation to dose in a given time period were all considered in evaluating the clastogenic potential of the test article. The criteria for a positive response are generally a statistically significant dose-related increase in the number of structural aberrations at three dose levels. The final decision was based upon scientific judgment."

11. Statistical Methods: Analyses were performed for the following:

    1) number of structural aberrations/cell/animal
    2) number of numerical aberrations/cell/animal
    3) number of cells with at least one structural aberration
    4) number of cells with 2 or more structural aberrations

"The Kruskal-Wallis test was performed at the alpha = 0.01 level to determine whether any of the mean values between the negative control and dose levels were significantly different from one another."

B. Protocol: Provided in the information above.

10. REPORTED RESULTS:

A. Acute Cytogenetics Study: Data from the low (0.5 g/kg) and mid-dose (1.7 g/kg) animals were not reported. Some of the high-dose animals had difficulty in breathing, and two of the animals previously designated to be sacrificed at 24 hours as well as one scheduled at 48 hours died before their scheduled terminations. Lethargy was noted in all high-dose animals.

There were no significant differences between frequencies of structural or numerical aberrations in the 4.9 g/kg rats and those of their respective negative controls. A significant positive response in structural (but not numerical) aberrations was elicited in animals which had received 1.0 mg/kg TEM. Representative means are shown in Table 1. There is a slight discrepancy in that on p. 25 of the report animal 3816 (5 g/kg Maneb, 6 hr kill) is reported as having a chromatid gap in one of its cells which is reported as being a structural aberration. This should not have been

reported in number of cells with structural aberrations.

B. Subacute Cytogenetics Study: The only cytogenetic data reported are from those animals receiving 1.64 g/kg Maneb/day (medium dose) for 5 days and their negative (vehicle) controls, as 9/10 of the high-dose (4.9 g/kg/day) rats had died during the dosing period. The mid-dose animals "looked scruffy" at the end of the dosage period, but no other symptoms are reported.

There were no significant differences between frequencies of structural or numerical aberrations in the 1.7 g/kg/day rats and those of their respective negative controls.

11. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded: "The test article, Maneb Technical, Lot MT 01 (88.1% a.i.), was considered negative for inducing chromosomal aberrations in bone marrow cells of male rats under the acute and subchronic conditions of this assay."

B. A quality assurance inspection statement was signed and dated August 13, 1985.

12. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Since animal toxicity was demonstrated in both the acute and subacute portions of this study, and there was no indication of any increased incidence of chromosomal aberrations, we concur with the study authors' conclusions.

The sensitivity of the system to detect clastogenic agents was adequately demonstrated by the statistically significant increase in chromosomal aberrations in rats treated with the postive control (triethylenemelamine, 1.0 mg/kg, IP).

TABLE 1. Results of the Rat Bone Marrow Cytogenetics Assay with Maneb

Treatment/Dose	Exposure Time	No. of Male Animals Analyzed	No. of Metaphases Analyzed	Number of aberrant cells		% of aberrant cells		Mean Mitotic Index % ± S.D.¶
				Structural[a]	Numerical	Structural[a]	Numerical	
Vehicle control								
0.25% carboxymethylcellulose in water 15 ml/kg	6 h	9	450	2	7	0.4	1.6	3.6 ± 1.5
	24 h	10	483	3	8	0.6	1.7	5.5 ± 1.2
	48 h	10	500	3	10	0.6	2.0	6.3 ± 2.0
15 ml/kg/d x 5d	5 d	10	500	4	8	0.8	1.6	5.3 ± 1.5
Positive control								
Triethylenemelamine 1.0 mg/kg	18 h	6†	184	163	1	88.6*	0.5	1.2 ± 0.5
Test material								
Maneb 4.92 g/kg	6 h	6	450	6	5	1.3	1.1	4.4 ± 1.3
	24 h	8	400	7	6	1.8	1.5	5.7 ± 0.7
	48 h	8	400	0	10	0.0	2.5	4.8 ± 1.2
1.64 g/kg/d x 5d	5 d	7	350	3	5	0.9	1.4	5.2 ± 2.3

[a] Excluding gaps
† Animals with 16 or more scorable metaphases; in all other groups with at least 25 scorable metaphases
¶ Only for those animals in which there were a sufficient number of analyzable metaphases
* Significant at $p \leq 0.01$ by Kruskall-Wallis test

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Data Evaluation Report (II)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb, lot no. MT01, described as a yellow powder with 88.1% active ingredient.

3. STUDY/ACTION TYPE: Mutagenicity—Reverse mutation in Salmonella (Ames study).

4. STUDY IDENTIFICATION: Loveday, K. S. Salmonella/microsome mutagenesis assay on technical grade Maneb. (Unpublished study no. 840014-40 conducted by Bioassay Systems Corporation, 225 Wildwood Ave., Woburn, MA 01801). Acc. no. 259018.

5. REVIEWED BY:

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6. APPROVED BY:

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7. CONCLUSIONS:

A. Under the conditions of the assay, Maneb, tested at six doses of from 1 to 20 ug/plate, did not cause a mutagenic response in the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 or TA1538 without S9 activation or in the presence of rat or mouse S9. However, the range-finding study defining the toxicity of the test material (and highest dose used in the assay) was conducted only under non-activated conditions.

B. The study is unacceptable.

8. RECOMMENDATIONS: It is recommended that the assay be repeated, with preliminary range-finding studies defining the toxicity of the test material with both rat and mouse liver S9 activation. The report should include information as to cell densities of cultures used. Cytotoxicity should be evident in results from at least the highest dose. Additionally, statistical methods should be used to give levels of significance (where appropriate) to differences between

means. It may be appropriate to do plating in triplicate, rather than duplicate, for calculation of standard deviations.

Based on numbers of mean revertants/plate, there were no indications of cytotoxicity for TA98, TA100 or TA1537 in the nonactivated assay conducted concurrently with the rat liver S9 study. It seems appropriate then that the test material should have been tested at an additional dose level higher than 20 ug/plate.

9. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Test Material: Technical Maneb, lot/batch no. MT01, 88.1% "Maneb" (presumably, 88.1% active ingredient); identified as a yellow powder slightly soluble in water. Stock solutions of the test material were made in dimethylsulfoxide (DMSO).

2. Bacterial Strain: S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were used. The source of these strains is identified as Dr. Bruce Ames of the University of California, Berkeley, CA. Bacterial strains were checked the day of the assay to ensure that all were sensitive to crystal violet and that TA98 and TA100 contained the R-factor.

3. S9 Fraction: The rat liver S9 used in the study was prepared from Arochlor 1254 induced Sprague-Dawley rats. It was obtained from Microbiological Associates, lot/batch no. R192. The S9 mix from this species consisted of 3% rat liver S9 fraction in a cofactor mix of 8mM $MgCl$, 33mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM Na_2HPO_4, pH 7.4.

The mouse liver S9 used in the study was prepared from Arochlor 1254 induced B6C3F1 mice. It was obtained from Microbiological Associates, lot/batch no. M107. The S9 mix from this species consisted of 1% mouse liver S9 fraction in the same cofactor mix as that given above for rat S9.

4. Plate Incorporation: The test material was assayed according to the method of Ames, McCann and Yamaski (1975).

5. Evaluation Criteria: Not specifically indicated, but the text refers to a "concentration-dependent positive response."

6. Statistical Evaluation: There is no mention in the text of any statistical methods or procedures. Although the number of revertants for positive controls are obviously elevated over their corresponding controls (tables 3 and 5), there is no indi-

cation as to the levels of statistical significance associated with these elevations.

B. A protocol was not provided.

10. RESULTS:

Cytotoxicity: A preliminary assay was conducted using S. typhimurium strain TA100 with seven doses of the test material ranging from 20.1 ug/plate to 20.1 mg/plate without S9 activation. At 20.1 ug/plate the bacterial lawn was thinner than the control; at 60.3 ug/plate there were only microcolonies. No growth occurred at 201 ug/plate or above. Precipitate was observed only at 20.1 mg/plate. A subsequent assay indicated no evidence for toxicity at concentrations of 15 ug/plate or less. Based on these findings the highest dose level selected for the assay was 20 ug/plate.

Mutation Assay: Overnight broth cultures of each tester strain were dosed with 1, 2, 5, 10, 15 or 20 ug/plate in the absence or presence of rat or mouse S9. Solvent control (DMSO) and positive controls were also assayed. The assay was performed in duplicate.

The data, as presented, give only the number of revertants per plate (as well as a mean). There is no indication (reduction in number of revertants/plate) of any cytotoxicity for strains TA98 or TA1537 in the nonactivated assay conducted concurrently with the rat S9 assay. Additionally, there was no indication of cytotoxicity for strains TA98, TA100, TA1535 or TA1538 (and possibly not for TA1537 either) in the rat S9 part of this study (refer to table 4, p. 10).

There is no indication (reduction in number of revertants/plate) of any cytotoxicity for TA1535 in the mouse S9 part of the study (table 6, p. 12).

The positive controls (without S9: 10 ug/plate 2-nitrofluorene for TA98 and TA1538, 2.5 ug/plate sodium azide for TA100 and TA1535 and 100 ug/plate 9-aminoacridine for TA1537; with S9: 1 ug/plate of 2-anthramine for all tester strains) in each case yielded a dramatic increase in number of revertants; however, there was no indication of any computation as to level of statistical significance associated with these increases. It is stated (p. 6) that the negative control values were within historical limits, but these limits (and whether they were from the literature or represent data from this laboratory alone) are not reported.

Maneb did not appear to induce any increases in revertants in any of the tester strains with or without S9 (rat or mouse) activation.

Representative results are presented in table 1.

STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded: "...no evidence of a positive response was observed under activated or nonactivated conditions. Based on the results of the Salmonella/Microsome Mutagenesis assay, Technical Grade Maneb can be considered non-mutagenic."

B. A quality assurance statement was signed and dated June 17, 1985.

12. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The most serious deficiency in this study is the lack of cytotoxicity data associated with concurrent exposure of the tester strains to Maneb and rat or mouse S9 fractions. This should have been developed independently for both S9 fractions. Without these data, we have no assurance that Maneb was tested at the highest possible dose level.

Of additional concern is the question of whether Maneb was in fact tested at the highest possible level even in the nonactivated assays. Based on numbers of mean revertants/plate, there were no indications of cytotoxicity for TA98, TA100 or TA1537 in the nonactivated assay conducted concurrently with the rat liver S9 study. It seems appropriate then that the test material should have been tested at an additional dose level higher than 20 ug/plate even in the nonactivated phase of the study.

Statistical analyses of the data would be appropriate in a repeat study. Additional information that would be relevant would include historical control data for negative responses.

Substance	Dose/ Plate	S9 Activation	Mean Number Revertants/Plate of Bacterial Tester Strains TA98	TA100	TA1535	TA1537	TA1538
RAT S9 ASSAY:							
Solvent control (DMSO)	—	-	27	86	30	11	19
Positive control**		-	795	750	790	1211	1728
Maneb	20 ug/ plate	-	31	70	11	13	9
Activated Assay							
Vehicle control		+(rat)	28	96	11	8	22
Positive control***		+(rat)	2735	1830	219	268	2380
Maneb	20 ug/ plate	+(rat)	31	76	13	6	22
MOUSE S9 ASSAY:							
Solvent control (DMSO)		-	41	112	44	8	19
Positive control**		-	879	949	903	1134	1673
Maneb	20 ug/ plate	-	10	87	23	5	9
Activated Assay							
Vehicle control		+(mouse)	41	154	47	13	42
Positive control***		+(mouse)	1005	2242	311	123	2810
Maneb	20 ug/ plate	+(mouse)	16	31	22	17	18

**Positive controls without S9: 10 ug/plate 2-nitrofluorene for TA98 and TA1538; 2.5 ug/plate sodium azide for TA100 and TA1535; 100 ug/plate 9-aminoacridine for TA1537.
***Positive controls with S9: 1 ug/plate 2-anthramine for all strains.