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

005229

JUN 29 1986

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Mutagenicity Studies on Maneb
Caswell #539 Project No. 443

TO: Jodi Bakst
SPRD

FROM: Byron T. Backus
Toxicology Branch
HED (TS-769C)

THROUGH: Marcia van Gemert, Ph.D.
Section Head
Review Section III

Judith Hauswirth, Ph.D.
Toxicologist
Mission Support Staff

and
Ted Farber, Ph.D.
Chief
Toxicology Branch
HED (TS-769C)

Compound: Maneb

Action Requested:

The Toxicology Branch has been asked to review eight mutagenicity studies and comment as to their acceptability in satisfying a series of 6 data gaps specified in the Maneb Data Call-in Notice of January 17, 1983. This memorandum covers two mutagenicity studies reviewed June 6, 1986, as well as the six remaining studies from the submitted material.

Comments and Recommendations:

1. The first mutagenicity study requirement specified in the Data Call-in Notice of January 17, 1983 was for an assay for gene mutation in Salmonella typhimurium (TA strains), or Escherichia coli WP2, performed with and without the use of S9 mammalian metabolic activation from Arochlor 1254 induced liver microsomes from both mouse (B6C3F1) and rat (Fisher 344) strains.

This data requirement has not been satisfied.

The study by Loveday entitled "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). There is no indication that cytotoxicity occurred with strains TA98, TA100, TA1535 or TA1538 (and possibly TA1537 either) in the rat S9 part of this study. From this it appears that higher dosage levels of Maneb should have been tested.

Also, there is no indication (reduction in number of revertants/plate) of any cytotoxicity for TA1535 in the assay with mouse S9.

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, Maneb should have been tested even in the nonactivated assay at additional dose levels higher than 20 ug/plate demonstrating cytotoxicity.

2. The second requirement specified a host-mediated assay in either B6C3F1 mice or Fisher 344 rats, by the oral route of administration, with appropriate microbial or mammalian indicator cells.

This data requirement has been satisfied.

The study by McCarroll, N. E., Burlow, P. and Phipps, N. entitled "Host Mediated Assay in Mice with Compound Maneb Technical Study No. 2" conducted by Hazleton Biotechnologies Corporation has been classified as acceptable.

Under the conditions of this assay, there was no indication of a mutagenic response in Salmonella typhimurium strain TA 1530 when host B6C3F1 mice were orally dosed with Maneb at 0.5, 2.0 and 5.0 gm/kg.

In the other host mediated study submitted (McCarroll, N. E. and Phipps N. Host Mediated Assay in Mice with Compound Maneb Technical Study No. 1 conducted by Hazleton Biotechnologies Corporation) there was no indication of a mutagenic effect in S. typhimurium strain TA1530 when host B6C3F1 mice were orally dosed with Maneb at 0.5, 2.0 or 5.0 mg/kg.

Because there was no indication of toxicity in the host mice, or evidence of cytotoxicity to the bacteria, and the highest dosage level administered was under 5 gm/kg, this particular study was considered unacceptable. (However, as noted above, the data requirement was satisfied by the second host-mediated assay).

3. The third assay specified was an in vitro mammalian gene mutation assay, using the mouse lymphoma cell line L5178Y (for TK) or Chinese Hamster Ovary Cells (CHO) for HGPRT, performed with and without the use of mammalian metabolic activation systems (S-9) derived from Arochlor 1254 induced liver microsomes from both Fisher 344 and B6C3F1 mice.

This requirement has been satisfied.

The study by Thomas, M. and Loveday, K. S. entitled "CHO/HGPRT In vitro mammalian cell mutation assay on technical grade Maneb" conducted by Bioassay Systems Corporation has been classified as marginally acceptable.

Under the conditions of this assay, exposure to Maneb at concentrations ranging from 0.5 to 10 ug/ml in the absence of S9 activation, at 1 to 30 ug/ml in the presence of rat S9 and from 1 to 20 ug/ml in the presence of mouse S9, did not result in an increased incidence of forward mutations at the HGPRT locus in CHO cells.

The reviewer is of the opinion that the data from this study should have included presentation of a statistical analysis; also, there are no values reported relating to normal variability (either from the laboratory historical data base or from referenced sources) in this assay. This is particularly relevant because on p. 7 of the report it is stated that one test sample at 1 ug/ml without activation had a mutation frequency noticeably (significantly?) higher than that of the negative controls, but the mutant frequency in the duplicate flask was much lower. According to the report: "This response is considered to be within the variability of the assay."

Also, there were some differences between runs with respect to relative survivals at what were ostensibly identical dose levels with no explanation as to why this

may have happened. However, the results are sufficiently unequivocal in indicating a lack of mutagenic response at dosage levels at or near cytotoxicity that the study is marginally acceptable.

4. The fourth specification was for at least two studies consisting of in vitro and/or in vivo assays for both gross chromosome aberrations (clastogenesis) and for sister-chromatid exchanges (SCEs); with the stipulation that in vitro assays could be conducted with any recognized, established mammalian cell line or primary cell str[illegible] but had to be performed both with and without th[illegible] of metabolic activation from induced strains or mi[illegible] and rats.

The requirement for a clastogenic assay has been satisfied by the study of Ivett and Lebowitz titled "Clastogenic evaluation of Maneb technical lot MT 01 (88.1% a.i.)" conducted by Litton Bionetics Inc.

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.

This study was classified as acceptable.

The requirement for a sister chromatid exchange assay has not been satisfied.

The study by Thomas, M. and Loveday, K. S. "In vitro sister chromatid exchange assay on technical grade Maneb" conducted by Bioassay Systems Corporation has been classified as unacceptable, and a full repeat assay is necessary.

While there was no indication or evidence of an increase in SCE as a result of nonactivated or S9 (both rat and mouse) activated exposure to Maneb in CHO cells, this study was deficient in a number of respects, including the following:

i. Most of this study was run with no duplication. In the actual assays exposure to the test material at a specific concentration involved only contents of a single flask. Also, most of the work was done with no concurrent or subsequent confirmatory assays. It was therefore not demonstrated that the findings of this assay were reproduciple.

Where there were duplicate runs (mouse S9 activation assay) there was a striking "anomaly" in that cells at 40 and 60 ug/ml could be analyzed in the first run, but in the second run 7.5 ug/ml was the highest concentration

from which cells could be analyzed. This "anomaly" does not inspire confidence in the reproducibility of this study.

ii. All values for SCE/cell per dosage level are simply presented as means, with no further statistical information (standard deviation and/or standard error of the mean). In order to evaluate a study of this type, we should have some information as to the level of inherent variability associated with the data as presented. There is always the possibility that the lack of a statistically significant difference between values associated with a dosage level and those of a negative control may be due to a high level of variability. Another problem is that there was an insufficient number of cells scored (30) at each dosage level in each assay run.

iii. In the reporting of the nonactivated SCE assay it is indicated (p. 10) that, at 1 ug/ml Maneb, 28 cells were in M1 and 72 were in M2. On p. 11 the respective values are 4 and 96. A clarification as to why these values (and others at different dose levels) are not the same or similar should be made. A similar situation exists with respect to some of the numbers of cells in M1 and M2 as given on pages 12 and 13.

iv. From the data on p. 11 it is noted that at 1 ug/ml in the nonactivated study there was still a considerable level of mitotic activity (mitotic index = 0.036, or about 50% that of 0.070 for solvent control). According to the data on p. 11 96% of the metaphases were M2. From this it appears that the test material should have been further evaluated for cytotoxicity at concentrations between 1 and 5 ug/ml.

v. In the initial mouse S9 activation assay, there is no information reported for dosages between 5 and 40 ug/ml. Also, what exactly is meant by "a cell between first and second metaphase"?

vi. While not something that can be readily linked to a specific protocol or set of guidelines for the conduct of this type of assay, this reviewer does not feel that the preliminary work adequately defined the cytotoxicity of the test material, or conditions (including concentrations of, and length of exposure to the test material) appropriate for testing, particularly in the nonactivated part of the assay. As an example of this, on p. 10 it is indicated that no metaphase cells were present at any of the doses (15 to 1500 ug/ml) of the initial range-finding study. The second run ("experiment no. 2") is identified as the nonactivated SCE assay, with no indications of any attempt to resolve cytotoxicity in a second preliminary assay.

With respect to the protocols used in this study, the Agency has accepted nonactivated SCE assays in which exposure to the test material was for 2 to 4 hours, followed by cell washing, suspension in fresh medium containing BrdU, and subsequent 25 to 28 hour incubation. If the test material causes mitotic delay it is sometimes appropriate to extend the post-exposure incubation period.

vii. Additionally (but not so critically) the types of chromosomal aberrations reported as having occurred in M1 cells at 50 and 60 ug/ml in the rat S9 activation assay, and at 60 ug/ml in the first mouse S9 study, were not specified. Also, instead of doses such as 0.5, 1, 5 and 10 ug/ml it would have been more appropriate to test values corresponding to half-log increments (i.e., something like 0.3, 1, 3 and 10 ug/ml).

5. The fifth specification was for a primary hepatocyte repair assay for unscheduled DNA synthesis (UDS).

   This requirement was not satisfied.

   The study by Thomas and Loveday titled "In Vitro Unscheduled DNA synthesis assay in rat hepatocytes using technical grade Maneb" conducted by Bioassay Systems Corporation has been classified as unacceptable.

   While there was no indication, under the conditions of this assay, of any increased incidence of UDS as a result of exposure to Maneb at concentrations ranging from 0.15 to 15 ug/ml, based on net nuclear counts, there was no quantitative assessment of cytotoxicity at these dose levels, no statistical analysis of the data, and no additional criteria for UDS (such as number of nuclei per exposure level showing 6 or more net nuclear grains; number of nuclei per exposure level with 20 or more net nuclear grains) were used in the evaluation of the findings. Also, the reproducibility of this assay should have been demonstrated in a repeat assay, particularly as negative results were obtained.

6. The sixth requirement was for a mammalian cell transformation assay on Maneb, with and without metabolic activation (as appropriate) in a cell system capable of detecting initiation, as well as in one capable of detecting enhancement of transformation by chemicals (promotion).

   The study by Tu, Sivak, Hatch and Breen titled "Evaluation of Maneb in the C3H-10T 1/2 cell transformation assay" conducted by Arthur D. Little Inc. has been classified as acceptable for nonactivated conditions.

Under the conditions of this cell transformation assay Maneb at five concentrations ranging from 0.05 to 0.20 ug/ml did not induce neoplastic transformation in C3H-10T 1/2 cells in the absence of metabolic (S9) activation.

While the C3H-10T 1/2 cell transformation assay is often performed without S9 activation because this cell line can metabolize certain chemicals to active carcinogens, it has not been demonstrated that C3H-10T 1/2 cells are capable of metabolizing Maneb to any significant extent, or that the metabolites (if any) are the same as those which might be formed under S9-activated conditions. Therefore, a data gap remains for a cell transformation assay in the presence of metabolic activation.

Additionally, Maneb was not tested under a protocol for detecting enhancement of transformation (promotion), so this requirement has not been satisfied. Although there are no established guidelines for promotion assays, the use of at least five different dose levels is recommended, since promotors may induce erratic and non-dose effects.

Data Evaluation Reports (attached)

3. Tu, A. S., Sivak, A., Hatch, K. and Breen, P. Evaluation of Maneb in the C3H-10T 1/2 cell transformation assay (unpublished study no. ADL 88720-44 (1-0860) prepared by Arthur D. Little, Inc., Cambridge, MA for the Maneb Task Force; dated July 31, 1985). Acc. no. 259073.

4. Thomas, M. and Loveday, K. S. CHO/HGPRT In vitro mammalian cell mutation assay on technical grade Maneb (unpublished study no. 840014-10 prepared by Bioassay Systems Corporation for George Pazianos; dated July 31, 1985). Acc. no. 259070.

5. Thomas, M. and Loveday, K. S. In vitro unscheduled DNA synthesis assay in rat hepatocytes using technical grade Maneb (unpublished study project no. 840014-30 prepared by Bioassay Systems Corporation for George Pazianos; dated August 5, 1985). Acc. no. 259072.

6. Thomas, M. and Loveday, K. S. In vitro sister chromatid exchange assay on technical grade Maneb (unpublished study project no. 840014-20 prepared by Bioassay Systems Corporation for George Pazianos; dated July 30, 1985 with revision of August 22, 1985). Acc. no. 259327.

7. McCarroll, N. E. and Phipps, N. Host-mediated assay in mice with compound Maneb technical study no. 1 (unpublished study project no. 2325-100 prepared by Hazleton Biotechnologies Corp. for Pazianos Associates; dated June 14, 1985). Acc. no. 259019.

8. McCarroll, N. E. and Phipps, N. Host-mediated assay in mice with compound Maneb technical study no. 2 (unpublished study project no. 2325-100 prepared by Hazleton Biotechnologies Corp. for Pazianos Associates; dated June 14, 1985). Acc. no. 259020.

8

Data Evaluation Report (III)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb technical, lot MT 01, 88.1% a.i., described as an off-white powder.

3. STUDY/ACTION TYPE: Mutagenicity--Transformation assay in C3H-10T 1/2 cells.

4. STUDY IDENTIFICATION: Tu, A. S., Sivak, A., Hatch, K. and Breen, P. Evaluation of Maneb in the C3H-10T 1/2 cell transformation assay (unpublished study no. ADL 88720-44 (1-0860) prepared by Arthur D. Little, Inc., Cambridge, MA for the Maneb Task Force; dated July 31, 1985). Acc. no. 259073.

5. REVIEWED BY:

Byron T. Backus, M.S.
Toxicologist
Toxicology Branch, HED

Byron T. Backus 06-20-86

6. APPROVED BY:

Marcia van Gemert, Ph.D.
Section Head, Review Section III
Toxicology Branch, HED

Marcia van Gemert 6.23.86

7. CONCLUSIONS AND RECOMMENDATIONS:

A. Under the conditions of the cell transformation assay Maneb at five concentrations ranging from 0.05 to 0.20 ug/ml did not induce neoplastic transformation in C3H-10T 1/2 cells in the absence of metabolic activation.

B. The study is acceptable for nonactivated conditions.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials:

1. Test Material: Maneb, lot # MT 01, described as an off-white powder. While no percentage of active ingredient is given in this report, other studies utilizing Maneb from this lot number have reported it as containing 88.1% a.i.

2. Indicator Cells: Mouse embryo fibroblast C3H-10T 1/2 (clone 8) cells, obtained from Dr. Charles Heidelberger, University of California Comprehensive Cancer Center, Los Angeles. These had been expanded in culture, frozen and stored in liquid N_2 in sealed ampules at approximately 10^6 cells/ampule. They were cultured in Basal Medium Eagle supplemented with 10% heat

inactivated fetal calf serum. Stock cells were grown in the absence of antibiotics at 37° C, >90% relative humidity, and 5% CO_2 in air atmosphere.

3. Positive Control: 3-methylcholanthrene (MCA) was used at a concentration of 5 ug/ml.

B. Methods:

1. Range-Finding Cytotoxicity Tests: Cytotoxicity was determined by reduction in cloning efficiency after a 24-hr exposure of 200 cells/plate to 9 doses (in triplicate) of the test material at half-log increments of from 0.1 to 1000 ug/ml. An untreated control was also evaluated. After exposure, the cells were incubated for approximately 10 days to allow colonies to develop. The plates were then fixed in methanol, stained with Giemsa, and the number of colonies counted. Percent survival was calculated by comparing cloning efficiency of treated cells to that of the untreated control.

   At 0.1 ug/ml, the lowest concentration, less than 10% of the cells survived. In a repeat assay, Maneb was less cytotoxic at this concentration, with 76% cell survival. Based on the results of the cytotoxicity range-finding assays, six levels (0.01, 0.025, 0.05, 0.075, 0.1 and 0.25 ug/ml) of Maneb were evaluated in the first experiment, and five (0.05, 0.075, 0.10, 0.15 and 0.20 ug/ml) in the second.

2. Cell Transformation Assay: 20 - or in some cases, 25 - (60 mm) plates were each seeded with 2000 C3H-10T 1/2 cells from frozen stock. Approximately 24 hrs later the appropriate amount of test compound was added to each plate; 24 hrs later the test material was removed, the plates were rinsed and fresh medium was added. The plates were incubated for approximately 6 weeks, with the medium changed twice a week during the first 2 weeks, and weekly thereafter. At the end of the incubation period, plates were fixed in methanol and stained with 2-3% Giemsa.

   The positive control was 3-methylcholanthrene (MCA). In the first experiment it was tested at 5 ug/ml; in the second it was tested at 2 and 5 ug/ml.

3. Scoring of Transformed Foci: Foci were scored and classified into three types as described by Reznikoff, Brankow and Heidelberger (Cancer Res. 33 [1973]:3231-3239). Types II and III foci were recorded separately. Any plates with foci too numerous to count were included in calculation of plates with foci but not in the calculation of foci/plate.

4. Evaluation Criteria: According the report: "A test compound is considered positive if:

   a) a minimum of 5 plates in the 4 test concentrations contain Type II or Type III foci; and

b) Type II or Type III foci are present in more than one test concentration.

These criteria are not absolutes; other extenuating factors may enter in the final interpretation of the results."

5. Statistical Analysis: There were no statistical tests for significance, although there were calculations of the standard error of the mean for foci/plate/dose level.

9. REPORTED RESULTS:

A. Cytotoxicity Test:

1. Initial Assay: At 0.1 ug/ml (lowest dose tested) Maneb there were 1.6 ± 1.5 (S.D.) colonies/plate, compared to 22.8 ± 4.5 colonies/plate for untreated controls. No colonies were present at concentrations of the test material of 0.3 ug/ml or more.

2. Second Assay:

Chemical	ug/ml	Clonal Counts	Mean colonies/ Plate ± S.D.	Relative Survival
Control	-	30,38,37,57,30	38.4 ± 11.1	-
Maneb	0.001	33,40.40,33,34	36.0 ± 3.7	0.94
	0.0025	39,3 ,28,25,32	31.4 ± 5.3	0.82
	0.005	27,27,44,39,36	34.6 ± 7.5	0.90
	0.01	28,35,33,30,37	32.6 ± 3.6	0.85
	0.025	33,39,27,34,47	36.0 ± 7.5	0.94
	0.05	32,38,34,41,28	34.6 ± 5.1	0.90
	0.10	27,25,38,25,†	28.8 ± 6.2	0.75

† contaminated

According to the report: "The variability in cytotoxicity may have resulted from the limited solubility of the test chemical and from the different primary stock concentrations used in the two assays (20 mg/ml and 50 ug/ml respectively)."

B. Cell Transformation Assays:

1. First Assay: There were no Type II or Type III foci on any of the Maneb-exposed or negative control plates. By contrast, 7/23 MCA-exposed (5 ug/ml) plates showed Type III foci (with a total of 10 Type III foci on these plates), and 14/23 positive control plates showed Type II and/or Type III foci. Relative survival at 0.1 ug/ml Maneb was 0.85; at the next higher dose (0.25 ug/ml) it was 0.005.

This assay was judged unacceptable by the laboratory because many plates of the assay (including negative and positive controls) showed small areas without cells. Although the reason for this was not known, it can be prevented by substituting Eagle Minimal Essential Medium (MEM) for the Eagle Basal Medium (BME), which was done in the second assay.

005229

2. *Second Assay:* Maneb exhibited no transformation activity under the conditions of this assay. From Table 3, p. 9:

	Chemical & Concentration (ug/ml)							
	Control	Maneb					MCA	
	-	0.05	0.075	0.10	0.15	0.20	2.0	5.0
Type III foci/ total plates	0/23	0/19	0/20	0/18	0/17	0/23	3/20	8/18
Foci/plate ± S.E.M.	0	0	0	0	0	0	0.15±0.08	0.44±0.12
Type II + III foci/ total plates	0/23	0/19	0/20	0/18	0/17	0/23	7/20	12/18
Foci/plate ± S.E.M.	0	0	0	0	0	0	0.35±0.11	0.72±0.19
Colonies/plate ± S.E.M.	32±2.0	23±1.3	23±1.1	22±2.5	7±2.2	2±0.7	32±1.8	26±1.1
Survival	-	0.72	0.72	0.69	0.22	0.06	1.00	0.81

S.E.M. = standard error of the mean.

10. STUDY AUTHORS' CONCLUSION/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "The test compound, Maneb, produced no transformation response in the C3H-10T 1/2 cells under the conditions of the assay. The test compound was quite cytotoxic to the C3H-10T 1/2 cells despite a limited solubility in water."

B. A quality assurance statement was signed and dated July 31, 1985.

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

Under the conditions of this study (which did not involve activation) Maneb did not cause an increase in the number of transformed foci. The highest dose levels (0.15 and 0.20 ug/ml) resulted in cytotoxicity, so that the dose range was adequate. The positive control, MCA at 2.0 and 5.0 ug/ml, demonstrated the sensitivity of the assay to detect a genotoxic effect.

Data Evaluation Report (IV)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb technical, lot MT 01, 88.1% "technical grade Maneb" (presumably active ingredient, as it is stated that there was 11.9% inerts), described as a yellow powder.

3. STUDY/ACTION TYPE: Mutagenicity--Chinese hamster ovary (CHO) HGPRT forward mutation assay.

4. STUDY IDENTIFICATION: Thomas, M. and Loveday, K. S. CHO/HGPRT In vitro mammalian cell mutation assay on technical grade Maneb. (unpublished study no. 840014-10 prepared Bioassay Sytems Corporation for George Pazianos; dated July 31, 1985). Acc. no. 259070.

5. REVIEWED BY:

Byron T. Backus, M.S. Byron T. Backus 06-19-86
Toxicologist
Toxicology Branch, HED

6. APPROVED BY:

Marcia van Gemert, Ph.D.
Section Head, Review Section III M. van Gemert 6.23.86
Toxicology Branch, HED

7. CONCLUSIONS AND RECOMMENDATIONS:

A. Under the conditions of this assay, exposure to Maneb at concentrations ranging from 0.5 to 10 ug/ml in the absence of S9 activation, at 1 to 30 ug/ml in the presence of rat S9 and from 1 to 20 ug/ml in the presence of mouse S9 did not result in an increased incidence of forward mutations at the HGPRT locus in CHO cells.

B. The study is marginally acceptable.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials:

1. Test Material: Maneb, BSC No. 85-11, lot/batch no. MT 01, described as a yellow powder. While the statement is made that the purity was "88.1% Technical Grade Maneb" other studies utilizing Maneb from this lot number have reported it as containing 88.1% active ingredient. The test sample was assayed in DMSO solution. "Concentrations were based on the weight of the test sample as received; no corrections were made using the purity of the active ingredient."

2. Positive Controls: Ethylmethane sulfonate (EMS) at 234 ug/ml was used in the assays without S9 activation, and 9,10-dimethyl-1,2 benzanthracene (DMBA) at 15 ug/ml was used in assays with rat or mouse S9.

3. Cell line: CHO cells, obtained from Dr. Samuel Latt at the Children's Hospital Medical School, Boston, MA., which in turn had been obtained from Dr. Arthur Pardee at the Sydney Farber Cancer Center, Boston, MA. Master vials were stored in liquid nitrogen or in a freezer at -70° C. Stock cultures were replaced from frozen vials. "All the frozen cultures have been presecreened for mycoplasma contamination and the spontaneous background mutant frequency is acceptably low." Working cultures were maintained in incubators in F12 medium without hypoxanthine containing 5% dialyzed calf serum. The exposure medium was supplemented with HEPES 20 mM buffer.

4. S9 Fractions: The activated assay was performed with S9 fractions prepared from livers of Sprague-Dawley rats and B6C3F1 mice (sexes unspecified in both cases) induced with Arochlor 1254 at 500 mg/kg. Both were commerical preparations as the rat liver S9 was from Litton Bionetics, Kensington, MD and the mouse S9 was from Microbiological Associates, Bethesda, MD.

B. Methods:

1. Range-finding Cytotoxicity Tests: Cultures seeded at 5 x 10^5 cells/flask were exposed 24 hours later to different Maneb concentrations. Exposure without S9 (range of 0.5 to 200 ug/ml Maneb) was for 16 hrs; with activation (range of 10 to 100 ug/ml Maneb) was 4 hrs. Controls (with and without S9) were exposed to 1% DMSO. Only the rat S9 was tested in the range-finding study; concentrations used with mouse S9 were based on information from an *in vitro* CHO SCE assay conducted at about the same time in this laboratory. Immediately after exposure in nonactivated assays and the day following exposure in activated assays 200 cells were seeded in duplicate petri dishes. "After at least one week of incubation the cells were fixed, stained and scored."

2. Mutation Assay: Based on the cytotoxicity results, at least seven dose levels were selected for both of the activated assays (1 to 50 ug/ml in the first experiment, and 0.1 to 30 ug/ml in the second experiment), and the non-activated assay (range of 0.5 to 25 ug/ml).

   a. Treatment: Cultures were seeded at about 1.5 x 10^6 cells per flask and were exposed to the appropriate concentration of Maneb (or control material) 24 hrs later. Exposure times were 4 hrs with S9 and 16 hrs without. Maneb concentrations and the positive control were tested in duplicate; negative controls were run using two sets of duplicate flasks. Immediately after exposure in the non-

activated assay and the day following exposure in the activated assay cells were replated for toxicity and expression time. "Due to the large number of flasks used in the nonactivated assays, all of the flasks were not trypsinized immediately after chemical removal," but in some cases were seeded six hours later. Expression flasks were replated (usually every 2-3 days) to maintain maximum growth rate.

b. Mutant Selection: After 7 days 2 x 10^5 cells were plated per dish (usually six dishes/flask) into medium containing 2 ug/ml 6-thioguanine (6TG). Cell survival at selection for each treatment group was measured by plating 200 cells from each flask into duplicate petri dishes without 6TG. Incubation was for at least 7 days; survival and selection plates were then fixed, stained and colonies counted.

3. Calculations: The following were calculated:

$$PE \text{ (plating efficiency)} = \frac{\text{Avg. No. colonies}}{\text{No. of plated cells}}$$

$$\text{Mutant Frequency} = \frac{\text{Number of Mutants}}{\text{No. of cells plated in selective medium} \times PE}$$

$$\text{Relative Percent Survivors} = \frac{\text{Avg. No. Colonies of Sample} \times 100}{\text{Avg. No. Colonies in Negative Control}}$$

4. Evaluation Criteria: Not directly stated; from the way the report is presented the implication is that a positive response would consist of a mutant frequency significantly different from (and presumably higher than) negative controls, although whether this could be at any dose level or would have to be in a dose-response fashion is not certain.

Although it is stated (p. 8) that the positive controls EMS (without S9) and DMBA (with rat or mouse S9) induced significant increases in mutant frequency, no values for probability associated with these increases, or even indications that statistical significances were calculated, are given.

9. REPORTED RESULTS:

A. Cytotoxicity Test:

1. Initial Range-finding without S9: There was no evidence for a toxic effect of Maneb at concentrations of 0.5 or 1.0 ug/ml. At 5 ug/ml survival was slightly reduced (89.1% of control value) and at 10 ug/ml it was reduced to 7.5% of the control level. At higher concentrations of test material (50 ug/ml and above) no colonies were formed.

2. Range-finding with Rat Liver S9: At 10 and 20 ug/ml there was 71.5 and 35.8% relative survival respectively in terms of the control level. At 40 ug/ml there was 0.8% relative survival and at 60, 80 and 100 ug/ml there were no colonies.

3. Range-finding with Mouse S9: According to the report (p. 7) "concentrations used in the activated assay using mouse liver S9 were based on the results of the activated . . . assay using rat liver S9 and information obtained using Maneb in a Sister Chromatid Exchange Assay with mouse liver S9 (BSC Project No. 8400014-20)." An check of the latter report (in Acc. no. 259069) indicates that the source of the CHO cells in the SCE assay was different from that of the HGPRT study and that, in the presence of mouse S9, some survival occurred in one run at 60 ug/ml of Maneb.

4. Mutation Assay without S9: There was no indication of either significant differences in mutation frequencies between cells exposed to any of the different concentrations of Maneb and the negative controls, or of a trend involving an increase in mutation frequency with higher doses of Maneb. Exposure to the positive control (EMS) resulted in a 240x and 400x increase in mutation incidence at the HGPRT locus relative to DMSO controls:

TABLE 1. Representative Replicate Results from CHO Assay with Maneb without S9 activation

	Dose (ug/ml)	Run	Relative Survival after Dosing (% survival)	6TG^r Mutant Frequency per 10^6 survivors
Solvent Control				
1% DMSO	-	1	100	1.6
	-	2	100	2.2
Positive Control				
EMS	234	1	62.6	393.7
	"	2	57.9	886.6
Test Material				
Maneb	10	1	17.3	<1.2
	10	2	0.2	<1.2
"	7.5	1	57.6	1.4
	7.5	2	4.8	1.6
"	5	1	76.8	2.9
	5	2	50.4	3.9

005229

5. Mutation Assay with Rat S9: There was no indication of either significant differences in mutation frequencies between the negative controls and cells exposed to any concentration of Maneb, or of a trend involving higher incidences in mutation frequency at increased dose levels of Maneb. Exposure to the positive control (DMBA) at 15 ug/ml with rat S9 resulted in 52x and 42x the mean incidence of mutation at the HGPRT locus observed in solvent control cells.

TABLE 2. Representative Replicate Results from CHO Assay with Maneb in the Presence of Rat S9

	Dose (ug/ml)	Run	Relative Survival after Dosing (% survival)	6TG^r Mutant Frequency per 10^6 survivors
Solvent Control				
1% DMSO + rat S9	-	1	100	4.3
	-	2	100	4.7
Positive Control				
DMBA + rat S9	15	1	62.6	233.9
	"	2	57.9	192.4
Test Material				
Maneb + rat S9	30	2	0.6	3.1
" "	20	1	2.0	<1.7
	20	2	13.1	6.8
" "	15	1	31.4	2.5
	15	2	24.6	<1.6
" "	10	1	60.2	1.0
	10	2	58.7	<1.3

6. Mutation Assay with Mouse S9: There appeared to be (in the absence of calculations) no significantly increased in mutation frequency in cells exposed to any concentration of Maneb with respect to their negative controls; also, there was no consistent indication of a trend of increased incidences in mutation frequency correlating with increased dose levels of Maneb. Exposure to DMBA at 15 ug/ml in the presence of mouse S9 resulted in a 38x and 45x higher incidence of the mean mutation rate occurring at the HGPRT locus in solvent controls:

005229

TABLE 3. Representative Replicate Results from CHO Assay with Maneb in the Presence of Mouse S9

	Dose (ug/ml)	Run	Relative Survival after Dosing (% survival)	6TG^r Mutant Frequency per 10^6 survivors
Solvent Control				
1% DMSO + mouse S9	-	1	100	3.7
	-	2	100	5.1
Positive Control				
DMBA + mouse S9	15	1	62.6	167.8
	"	2	46.0	199.2
Test Material				
Maneb + mouse S9	20	2	7.6	7.3
" "	10	1	39.0	<1.7
	10	2	6.1	12.7
" "	5	1	77.8	1.8
	5	2	52.4	11.9
" "	3	1	111.3	1.9
	3	2	148.5	2.7

10. STUDY AUTHORS' CONCLUSION/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Maneb is non-mutagenic under the conditions of the CHO/HGPRT . . . mutagenesis assay."

B. A quality assurance statement was signed and dated July 31, 1985.

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

Under the conditions of this study, Maneb did not cause an increased incidence in mutations at the HGPRT locus in CHO cells. This reviewer is of the opinion that the data from this study should have been presented to include statistical analysis; also, there are no values reported relating to normal variability (either from the laboratory historical data base or from referenced sources) in this assay. On p. 7 it is stated that one test sample at 1 ug/ml without activation had a [redacted] mutation frequency noticeably [significantly?] higher than that of the negative controls, but the mutant frequency in the duplicate flask was much lower. "This response is considered to be within the variability of the assay."

In the non-activated part of the study the positive control (EMS) was used at a concentration (234 ug/ml; no rationale or reference for this dose was given) considerably above that of the test substance (maximum concentration for which results were obtained for Maneb: 10 ug/ml). However, in the activated assays the positive control (DMBA) was used at 15 ug/ml, a concentration within the same range as that for Maneb.

Also, there were some differences between runs with respect to relative survivals at what were ostensibly identical dose levels with no explanation as to why this may have happened. However, the results are sufficiently unequivocal in indicating a lack of mutagenic response at levels at or near cytotoxicity that the study is marginally acceptable.

Data Evaluation Report (V)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb technical, lot MT 01, BSC No. 85-11A, described as a yellow powder.

3. STUDY/ACTION TYPE: Mutagenicity--Unscheduled DNA Synthesis Assay in Rat Hepatocytes.

4. STUDY IDENTIFICATION: Thomas, M. and Loveday, K. S. In Vitro Unscheduled DNA Synthesis Assay in Rat Hepatocytes Using Technical Grade Maneb (unpublished study project no. 840014-30 prepared by Bioassay Systems Corporation, 225 Wildwood Ave., Woburn, MA 01801 for George Pazianos, 211 Ninth St. NE, Washington, D.C.; dated August 5, 1985). Acc. no. 259072.

5. REVIEWED BY:

Byron T. Backus, M.S. Byron T. Backus 06-19-86
Toxicologist
Toxicology Branch, HED

6. APPROVED BY:

Marcia van Gemert, Ph.D.
Section Head, Review Section III M. van Gemert 6.23.86
Toxicology Branch, HED

7. CONCLUSIONS AND RECOMMENDATIONS:

A. While there was no indication, under the conditions of this assay, of any increased incidence of UDS as a result of exposure to Maneb at concentrations ranging from 0.15 to 15 ug/ml, based on net nuclear grain counts, there was no quantitative assessment of cytotoxicity at these dose levels, no statistical analysis of the data, and no additional criteria for UDS (such as number of nuclei per exposure level showing 6 or more net nuclear grains; number of nuclei per exposure level with 20 or more net nuclear grains) were used in the evaluation of the findings. Also, the reproducibility of an assay of this type should be demonstrated in a repeat assay, particularly as negative results have been obtained.

B. The study is not acceptable.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials:

1. Test Material: Maneb, BSC No. 85-11, lot/batch no. MT 01, described as a yellow powder. While the statement is made that

the purity was "88.1% Technical Grade Maneb" other studies utilizing Maneb from this lot number have reported it as containing 88.1% active ingredient. The test sample was assayed in DMSO solution. "Concentrations were based on the weight of the test sample as received; no corrections were made using the purity of the active ingredient."

2. Hepatocyte Source: An 8-week old male Sprague-Dawley rat was obtained from Charles River Breeding Laboratories, Wilmington, MA. and used for this purpose.

B. Methods:

1. Hepatocyte Culture Preparation: The rat liver was perfused with modified Hanks' balanced salt solution containing 0.5 mM Ethyleneglycol-bis (B-aminoethylether), N,N-tetraacetic acid and 10 mM Hepes Buffer, followed by Williams' Medium E (WME). The liver was dissected out, trimmed of fat and connective tissue, and the capsule was peeled away. Hepatocytes were put into suspension using a stainless steel comb. The suspension was centrifuged, and cells were resuspended in WME supplemented with 10% fetal bovine serum (WMES). A viability count was taken using trypan blue; aliquots of 5×10^5 viable cells were seeded onto 25 mm round coverslips in WMES and incubated at 37° C. Two hours after seeding cells were washed leaving only attached viable cells on the coverslips.

2. Exposure to Maneb: Cells were exposed to Maneb at 12 different concentrations (while not all levels were reported, they were probably 0.005, 0.015, 0.05, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500 and 1510 ug/ml) for 18 hrs. Negative controls were exposed to 1% DMSO for 18 hrs; positive controls were exposed to 9,10-dimethyl-1,2-benzanthracene (DMBA) at 0.125 mg/ml for an unspecified period of time. Six coverslips were used for each concentration of Maneb and each control. The exposure medium included tritiated thymidine at 4 uCi/ml.

3. Post-exposure Treatment: Cells were washed 3X with WME and treated for 15 minutes with 1% sodium citrate solution. Cells were fixed in 3:1 ethanol-glacial acetic acid, and coverslips were mounted (presumably cell side up) on glass slides. Three of the six slides from each Maneb and control concentration were dipped in Kodak NTB-2 emulsion and stored at 4° C in a light-proof box. After 7 days slides were photographically developed and fixed, followed by staining in Harris' hematoxylin.

4. Analysis: Fifty cells (no indication these were randomly selected) from each of two slides of the negative control, the 0.125 mg/ml DMBA, and from four Maneb concentrations ranging from 0.15 to 15 ug/ml were analyzed. (At a fifth Maneb concentration, 1.5 ug/ml, only one coverslip was suitable for analysis, and so 100 cells were examined from this coverslip). Nuclear grains were counted using a Biotran III automatic colony counter with a microscope and

T.V. screen attachment. Background counts were obtained from three nuclear sized areas adjacent to each nucleus (counted?). Net grain counts were calculated by subtacting the average background count from the nuclear count.

5. Statistical Analysis: Although it is stated (p. 7) that the positive control induced a significant increase in average net grains/nucleus, no indication is given as to how this calculation was made or what specific probability value is associated with the increase.

6. Evaluation Criteria: Not given.

9. REPORTED RESULTS:

A. Cytotoxicity:

No quantitative measurements of cytotoxicity are given. It is simply stated (p. 7) that "signs of toxicity (no cytoplasm surrounding the nucleus) were observed at concentrations higher than 15 ug/ml" and that, based on this "cytotoxicity", only the data from 0.15, 0.5, 1.5, 5 and 15 ug/ml were analyzed.

B. UDS Study:

The data, as reported (p. 8) are the following:

Material	Concentration (ug/ml)	Average Grains/Nucleus	Average Background	Average Net Grains/Nucleus
Maneb	0.15	8.02	6.11	1.9
		7.28	5.26	2.0
	0.5	5.80	4.85	1.0
		5.84	5.08	0.8
	1.5†	9.46	6.59	2.9
	5.0	5.58	3.65	1.9
		4.80	4.21	0.6
	15.0	2.92	1.47	1.5
		2.04	1.41	0.6
1% DMSO (negative control)	-	4.42	3.67	0.8
		5.36	4.60	0.8
DMBA (positive control)	125.0	18.42	4.74	13.7
		13.60	2.83	10.8

† only one slide suitable for analysis

005229

10. STUDY AUTHORS' CONCLUSION/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Technical grade Maneb does not have the potential to induce unscheduled DNA Synthesis in rodent hepatocytes."

B. A quality assurance statement was signed and dated August 5, 1985.

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

There are a number of serious problems involving both the conduct and presentation of this study.

Foremost of these is the lack of quantitative cytotoxicity data. In studies of this type relative survival indices are usually calculated from preliminary and/or concurrent (parallel to the UDS assay) runs. Normally, the dosage levels of test compound should be associated with at least a 10-50% range of relative survival indices, but from the presentation of this report I have no idea whether this was true in this case.

I am also concerned about the way in which the average net grains/nucleus values were calculated, and the absence of statistical measurements associated with these given values. A net grain count is usually calculated for each evaluated nucleus by subtracting the mean count of 3 adjacent nuclear-sized areas from the count obtained for the nucleus. Many laboratories consider the lowest possible value for any one nucleus then to be zero (even when the 3 adjacent nuclear-sized areas have a greater mean number of grains than the nucleus). Average net nuclear grain counts of six or more above control average are usually assumed to constitute a positive response in this type of assay, since six is normally more than twice the standard deviation associated with the control count. However, in this case no standard deviation is reported for the negative control (or any other exposure group either). In any case, the statistical resolution of the data should be such that definite conclusions can be drawn.

No additional criteria for UDS were utilized (number of nuclei per exposure level showing 6 or more net nuclear grains; number of nuclei/exposure level showing 20 or more net nuclear grains). These are indicated in Brusick, D. Principles of Genetic Toxicology, Plenum Press, 1980, where it is stated (p. 227):

> The test article should be considered active in the UDS assay at applied concentrations that cause (1) an increase in the mean nuclear grain count to at least six grains per nucleus in excess of the concurrent negative control;

and/or (2) the percentage of nuclei with six or more grains to increase above 10% of the examined population, in excess of the concurrent negative control; and/or (3) the percentage of nuclei with 20 or more grains to reach or exceed 2% of the examined population.

This reference continues:

Generally, if the first condition is satisfied, the second and often the third condition will also be met. However, satisfaction of only the second or third condition can also indicate UDS activity...all three of the above conditions should be considered in the evaluation.

Additionally, the reproducibility of an assay of this type should be demonstrated, particularly if negative results have been obtained.

For these reasons, the study, as reported, is classified as unacceptable.

Data Evaluation Report (VI)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb technical, lot MT 01, BSC No. 85-11A, described as a yellow powder.

3. STUDY/ACTION TYPE: Mutagenicity--In Vitro Sister Chromatid Exchange Assay (CHO Cells).

4. STUDY IDENTIFICATION: Thomas, M. and Loveday, K. S. In Vitro Sister Chromatid Exchange Assay on Technical Grade Maneb (unpublished study project no. 840014-20 prepared by Bioassay Systems Corporation, 225 Wildwood Ave., Woburn, MA 01801 for George Pazianos, 211 Ninth St. NE, Washington, D.C.; dated July 30, 1985 with revision of August 22, 1985). Acc. no. 259327.

5. REVIEWED BY:

Byron T. Backus, M.S. Byron T. Backus 06-19-86
Toxicologist
Toxicology Branch, HED

6. APPROVED BY:

Marcia van Gemert, Ph.D.
Section Head, Review Section III M. van Gemert 6.23.86
Toxicology Branch, HED

7. CONCLUSIONS AND RECOMMENDATIONS:

A. The study is unacceptable. The major noteworthy finding was the occurrence of "chromosomal aberrations" at 50 and 60 ug/ml Maneb in the presence of rat S9 and at 60 ug/ml with mouse S9. A full repeat assay is necessary.

B. While there was no indication or evidence of an increase in SCE as a result of nonactivated or S9 (both rat and mouse) activated exposure to Maneb in CHO cells, this study was deficient in a number of respects, including the following:

1. Most of this study was run with no concurrent or subsequent confirmatory assays. Exposure to the test material at a specific concentration involved only contents of a single flask. It was therefore not demonstrated that most of the findings of this study were reproducible.

In the one run which was duplicated (mouse S9 activation assay) there was a striking "anomaly" in that cells at 40 and 60 ug/ml could be analyzed in the first run, but in the second run 7.5 ug/ml was the highest concentration from which cells could be analyzed. This "anomaly" does not inspire confidence in the reproducibility of this study.

2. All values for SCE/cell per dosage level are simply presented as means, with no further statistical information (standard deviation and/or standard error of the mean). In order to evaluate a study of this type, we should have some information as to the level of inherent variability associated with the data as presented. There is always the possibility that the lack of a statistically significant difference between values of a dosage level and those of negative controls may be due to a high level of variability. Another problem is that there was an insufficient number of cells scored (30) in each assay run.

3. In the reporting of the nonactivated SCE assay it is indicated (p. 10) that, at 1 ug/ml Maneb, 28 cells were in M1 and 72 were in M2. On p. 11 the respective values for the same dose are 4 and 96. A clarification as to why these values (and others at different dose levels) are not the same or similar should be made. A similar discrepancy exists with respect to some of the numbers of cells in M1 and M2 as given on pages 12 and 13.

4. From the data on p. 11 it is noted that at 1 ug/ml in the nonactivated study there was still a considerable level of mitotic activity (mitotic index = 0.036, or about 50% that of 0.070 for solvent control). According to the data on p. 11 96% of the metaphases were M2. From this, it appears that the test material should have been further evaluated for cytotoxicity at concentrations between 1 and 5 ug/ml.

5. In the initial mouse S9 activation assay, there is no information reported for dosages between 5 and 40 ug/ml (or whether there were even dosages between these values). Also, what exactly is meant by "a cell between first and second metaphase"?

6. While not something that can be readily linked to a specific protocol or set of guidelines for the conduct of this type of assay, this reviewer does not feel that the preliminary work adequately defined the cytotoxicity of the test material, or conditions (including concentrations of, and length of exposure to the test material) appropriate for testing, particularly in the nonactivated part of the assay. As an example of this, on p. 10 it is indicated that no metaphase cells were present at any of the doses (15 to 1500 ug/ml) of the initial range-finding study. The second run ("experiment no. 2") is identified as the nonactivated SCE assay, with no indications of any attempt to resolve cytotoxicity in a second preliminary assay.

With respect to the conduct of this study, the Agency has accepted nonactivated SCE assays in which exposure to the test material was for 2 to 4 hours, followed by cell washing, suspension in fresh medium containing BrdU, and subsequent 25 to 28 hour incubation. If the test material causes mitotic delay it is sometimes appropriate to extend the post-exposure incubation period.

7. Additionally (but not so critically) the types of chromosomal aberrations reported as having occurred in M1 cells at 50 and 60 ug/ml in the rat S9 activation assay, and at 60 ug/ml in the first mouse S9 study, were not specified. Also, instead of doses such as 0.5, 1, 5 and 10 ug/ml it would have been more appropriate to test values corresponding more to half-log factors (i.e., something 0.3, 1, 3 and 10 ug/ml).

C. The study should be completely redone in such a way as to demonstrate reproducibility of the findings, adequate resolution (either by preliminary or concurrent assays) of the cytotoxicity of the test material, and the appropriate concentrations of (and length of exposure to) the test material.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials:

1. Test Material: Maneb, BSC No. 85-11, lot/batch no. MT 01, described as a yellow powder. While the statement is made that the purity was "88.1% Technical Grade Maneb" other studies utilizing Maneb from this lot number have reported it as containing 88.1% active ingredient. The test sample was assayed in DMSO solution. "Concentrations were based on the weight of the test sample as received; no corrections were made using the purity of the active ingredient."

2. Positive Controls: Cyclophosphamide (CP) at 2.5 ug/ml was used in the assays with S9 activation, and Mitomycin C at 0.01 ug/ml was used in assays without S9 activation.

3. Cell Line: CHO cells were obtained from Dr. Sheila Galloway at Litton Bionetics, Kensington, MD. Master vials were stored in liquid N_2 or at in a freezer at -70° C. The CHO cells used in this assay were maintained in McCoy's 5A medium with fetal calf serum.

4. S9 Fractions: The activated assays were performed with S9 fractions prepared from livers of Sprague-Dawley rats and B6C3F1 mice induced with Aroclor 1254 at 500 mg/kg. Both were apparently commercial preparations as the rat liver S9 was from Litton Bionetics, Kensington MD and the mouse S9 was from Microbiological Associates, Bethesda, MD.

B. Methods:

1. Preliminary Cytotoxicity Tests Without Activation: CHO cells were seeded at a density of 1.5×10^6 in plastic flasks (uncertain whether this means 1.5×10^6 cells per flask, or per some unit area of each flask). One day (24 hrs?-not specifically stated) later cells in McCoy's 5A Medium supplemented with 10% fetal calf serum were exposed to concentrations of Maneb

ranging from 15 to 1500 ug/ml, as well as to positive and negative (solvent) controls. Two hrs later BrdU was added (no indication is given that exposure to the test material ended at this time since it is stated on p. 5: "BrdU...was added to the exposure medium and cells were incubated for an additional 28 hrs."). Vinblastine sulfate (instead of colcemid or colchicine which are usually used as mitotic arresting agents) was added to the exposure medium 2-2 1/2 hrs before cells were harvested. Cells were harvested, treated with hypotonic solution, fixed in 3:1 methanol:acetic acid and slides were prepared. Slides were photoactivated by exposure to UV for 30 minutes, immersed twice (15 minute intervals) in 65° C 0.015 M sodium citrate and 0.15 M NaCl solution, rinsed in distilled water and stained with 5% Giemsa. One hundred metaphase cells/treatment were counted, and the numbers of these in M1 and M2 were noted.

Because no metaphase cells were observed in the first range-finding assay, Maneb was subsequently tested at concentrations of from 0.01 to 20 ug/ml for both toxicity and evidence of cell cycle delay.

2. Preliminary Cytotoxicity Tests With Activation: The same procedure as that for without activation was used with the following modifications: exposure time was 2 hrs in serum-free medium. The test sample was removed, cells were washed with phosphate-buffered saline, and grown for 28 hrs in medium containing serum and BrdU. Cells were harvested as in the nonactivated assay (however, it is not stated when the metaphase arresting agent was added to the medium).

3. SCE Assay: The same procedures as indicated above for cytotoxicity assays were followed. Without activation, eight concentrations of Maneb ranging from 0.01 to 20 ug/ml were tested, and results from four levels (0.05, 0.1, 0.5 and 1 ug/ml) were analyzed in a single assay. With rat S9 activation, there was a single assay in which eight concentrations of Maneb ranging from 1 to 80 ug/ml were tested, and four (5, 10, 20 and 40 ug/ml) were analyzed.

   The mouse S9 activation study involved two assays. In the first eight concentrations of Maneb ranging from 1 to 80 ug/ml were tested, and four (1, 5, 40 and 60 ug/ml) were analyzed. In the second seven concentrations of Maneb ranging from 1 to 50 ug/ml were tested; four (1, 2.5, 5 and 7.5 ug/ml) were analyzed.

4. Evaluation Criteria: Not stated. The implication from the summary (p. 1) is that a positive response might consist of a reproducibly statistically significant increase in SCE at a single dose level.

5. Statistical Analysis: The only thing reported is that analysis of variance was performed on the results of the different assays.

9. REPORTED RESULTS:

A. Cytctoxicity:

1. Non-activation: In the first assay, no metaphase cells were observed at any concentrations of Maneb (15 to 1500 ug/ml). At 300 ug/ml and above Maneb precipitated out of solution.

In the second run, which is identified as the nonactivated SCE assay, no cells in metaphase were obtained at concentrations of 5 ug/ml and above. From Table 1, p. 10:

Test material and Concentration (ug/ml)		Cell Cycle M1	M2
Maneb	20	No cells	
	10	No metaphase cells	
	5	No metaphase cells	
	1	28	72
	0.5	6	94
	0.1	12	88
	0.05	1	99
	0.01	0	100
1% DMSO (negative control)		0	100
	"	100*	0
0.01 ug/ml Mitomycin C		14	86
	"	0	100

*Technical error, no BrdU added.

2. Activation - rat S9: In the first (range-finding) assay no cells in metaphase were found at concentrations of 110 ug/ml or more of Maneb. From Table 3, p. 12:

Test material and Concentration (ug/ml)		Cell Cycle M1	M2
Maneb + rat S9	110	No metaphase cells	
	75	Sparse metaphase	
	30	67	33
	15	36	64
1% DMSO + rat S9		4	96
	"	1	99

In the second experiment (identified as the activated SCE assay) the following values are given (Table 3, p. 12):

Test material and Concentration (ug/ml)		Cell Cycle M1	M2
Maneb + rat S9	80	No metaphase cells	
	60	89	11
	50	90	10
	40	46	54
	20	9	91
	10	14	86
	5	2	98
	1	31	69
1% DMSO + rat S9		28	72
	"	0	100
2.5 ug/ml CP + S9		9	91
	"	12	83

3. Activation - mouse S9: No cytotoxicity assays are reported. 005229

B. Sister Chromatid Assays:

1. Non-activation: None of the Maneb concentrations induced a significant increase in SCE compared to the negative control. Exposure to Mitomycin C at 0.01 ug/ml caused a significant increase in SCE/cell. From Table 2, p. 11:

Test Material and Concentration (ug/ml)	Mitotic Index	Cell Cycle M1	Cycle M2	Number of Cells Scored	Number of SCEs	SCE/ cell
Solvent Control 1% DMSO	0.070	0	100	30	327	10.9
Positive Control 0.01 ug/ml Mitomycin C	0.043	0	100	10	414	41.4
Maneb 1 ug/ml	0.036	4	96	30	357	11.9
Maneb 0.5 ug/ml	0.040	2	98	30	266	8.9
Maneb 0.1 ug/ml	0.056	2	98	30	220	7.3
Maneb 0.05 ug/ml	0.041	0	100	30	242	8.1

2. Activation - Rat S9: No M2 cells suitable for analysis were present above 40 ug/ml; chromosomal aberrations were observed at 50 and 60 ug/ml (on p. 13 it is stated aberrations were present in - but it is not stated they were limited to - M1 cells). There was no evidence of an increase in SCEs as a result of exposure to levels of 40 ug/ml or less of Maneb; from p. 13:

Test Material and Concentration (ug/ml)	Mitotic Index	Cell Cycle M1	Cycle M2	Number of Cells Scored	Number of SCEs	SCE/ cell
Solvent Control 1% DMSO + rat S9	0.120	2	98	30	313	10.4
Positive Control rat S9 + 2.5 ug/ml Cyclophosphamide	0.101	4	96	10	379	37.9
Rat S9 + Maneb 40 ug/ml	0.046	34	66	30	301	10.0
20 ug/ml	0.082	10	90	30	312	10.4
10 ug/ml	0.079	2	98	30	264	8.8
5 ug/ml	0.029	0	100	30	222	7.4

3. Activation - Mouse S9: There were 2 SCE assays. In the first mitotic indices >5 ug/ml were low. At 5 ug/ml a ratio of about 1:1::M1:M2 cells was obtained. From p. 14:

Test Material and Concentration (ug/ml)	Mitotic Index	Cell Cycle M1	Cell Cycle M2	Cell Cycle M1+	Number of Cells Scored	Number of SCEs	SCE/cell
Solvent Control 1% DMSO + mouse S9	0.092	4	96		30	265	8.8
Positive Control mouse S9 + 2.5 ug/ml Cyclophosphamide	0.097	0	100		10	859	85.9
Mouse S9 + Maneb 60[a] ug/ml	0.006	62[b]	28[b]	11[b]	30	313	10.4
40 ug/ml	0.004	68[c]	29[c]	3[c]	7[d]	80	11.4
5 ug/ml	0.010	48	52		30	369	12.3
1 ug/ml	0.078	4	96		30	273	9.1

[a]Chromosomal aberrations were observed in some M1 cells.
[b]Cell cycle stages determined from 47 cells instead of 100.
[c]Cell cycle stages determined from 31 cells instead of 100.
[d]Because of the low mitotic index only 7 cells were scored.
M1+ - between first and second metaphase

Analysis of variance indicated the number of SCEs (in the whole set of concentrations analyzed?) for Maneb with mouse S9 was statistically "different" from that of negative controls at $p = 0.05$.

In a repeat assay, no metaphase cells were obtained at concentrations >7.5 ug/ml. From p. 14:

Test Material and Concentration (ug/ml)	Mitotic Index	Cell Cycle M1	Cell Cycle M2	Number of Cells Scored	Number of SCEs	SCE/cell
Solvent Control 1% DMSO + mouse S9	0.090	0	100	30	233	7.8
	0.080	0	100	30	237	7.9
Positive Control mouse S9 + 2.5 ug/ml Cyclophosphamide	0.032	6	94	10	508	50.8
	0.120	2	98	10	577	57.7
Mouse S9 + Maneb 7.5 ug/ml	0.012	30	70	30	245	8.2
5 ug/ml	0.036	36	64	30	283	9.4
2.5 ug/ml	0.116	0	100	30	243	8.1
1 ug/ml	0.062	4	96	30	211	7.0

"None of the test sample concentrations resulted in significant increases in the number of SCE/cell when compared to the negative control. The 19% increase observed at 5 ug/ml is not high enough to be significant..."

"Analysis of variance...indicated no statistically significant differences between the number of sister chromatid exchanges induced by the test sample and the negative controls."

10. STUDY AUTHORS' CONCLUSION/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Under the conditions of the assay employed, Maneb did not cause a significant increase in sister sister chromatid exchanges under nonactivated or activated conditions."

B. A quality assurance statement dated July 30, 1985 was signed.

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

This study has been very difficult to interpret, even with the revisions of August 22, 1985.

However, it is still difficult to state whether the major problems with this report involve its clarity or the protocol used and/or the data as reported. The following comments relate to some of the major deficiences of this study:

1. Most of this study was run with no duplication. Exposure to the test material at a specific concentration involved only cells within a single flask. Also, most of the runs were done with no concurrent or subsequent confirmatory assays. The reproducibility of the findings of this assay was therefore not demonstrated.

   Where there were duplicate runs (mouse S9 activation assay) there was a striking "anomaly" in that cells at 40 and 60 ug/ml could be analyzed in the first run, but in the second run 7.5 ug/ml was the highest concentration from which cells could be analyzed. This "anomaly" does not inspire confidence in the reproducibility of this study.

2. All values for SCE/cell are simply presented as means for each dosage level (based on values from 30 cells, an insufficient number) with no further statistical information (standard deviation and/or standard error of the mean). In order to evaluate a study of this type, we should have some information as to the level of inherent variability associated with the data. There is always the possibility that the lack of a statisti-

cally significant difference between values obtained at a dosage level and background (from negative controls) may be due to a high level of variability.

3. In the reporting of the nonactivated SCE assay, it is indicated (p. 10) that, at 1 ug/ml Maneb, 28 cells were in M1 and 72 in M2. On p. 11 the respective values are 4 and 96. A clarification as to why these values (and others at the different dose levels) are not the same or similar should be made. A similar discrepancy exists with respect to some of the numbers of cells in M1 and M2 as given on pages 12 and 13.

4. From the data on p. 11 it is noted that at 1 ug/ml in the nonactivated study there was still a considerable amount of mitotic activity (mitotic index = 0.036, or about 50% that of 0.070 for solvent control). From this, it appears that the test material should have been further evaluated for cytotoxicity at concentrations between 1 and 5 ug/ml.

5. In the initial mouse S9 activation assay, there is no information reported for dosages between 5 and 40 ug/ml. Also, what exactly is meant by "a cell between first and second metaphase"?

While not something that can be readily linked to a specific protocol or set of guidelines for the conduct of this type of assay, this reviewer does not feel that the preliminary work adequately defined the cytotoxicity of the test material, or conditions (including concentrations of, and length of exposure to the test ted part of the assay. As an example of this, on p. 10 it is indicated that no metaphase cells were present at any of the doses (15 to 1500 ug/ml) of the initial range-finding assay. The second run ("experiment no. 2") is identified as the nonactivated SCE assay.

The Agency has accepted nonactivated SCE assays in which exposure to the test material has been for 2 to 4 hours, followed by cell washing, suspension in fresh medium containing BrdU, and subsequent 25 to 28 hour incubation. Where the test material causes mitotic delay it is sometimes appropriate to extend the post-exposure incubation period.

Additionally (but not so critically) the types of chromosomal aberrations reported as having occurred in M1 cells at 50 and 60 ug/ml in the rat S9 activation assay, and at 40 and 60 ug/ml in the first mouse S9 study, were not specified. Also, instead of doses such as 0.5, 1, 5, then 10 ug/ml it would have been more appropriate to use values corresponding to half-log increments (i.e., something like 0.3, 1, 3, 10 ug/ml).

Overall, this reviewer considers the study to be unacceptable as a SCE assay. The major noteworthy finding of this study was the occurrence of "chromosomal aberrations" at 50 and 60 ug/ml Maneb in the presence of rat S9, and at 60 ug/ml Maneb with mouse S9. A full repeat assay is necessary.

Data Evaluation Report (VII)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb technical, lot MT01, LH No. 21,838A, described as a pale green (other studies have identified it as yellow) powder with a purity of 88%.

3. STUDY/ACTION TYPE: Mutagenicity--Host Mediated Assay in Mice.

4. STUDY IDENTIFICATION: McCarroll, N. E. and Phipps, N. Host Mediated Assay in Mice with Compound Maneb Technical Study No. 1 (unpublished study project no. 2325-100 prepared by Hazleton Biotechnologies Corp., 9200 Leesburg Turnpike, Vienna, VA 22180 for Pazianos Associates, 211 Ninth St. NE, Washington, D.C.; dated June 14, 1985). Acc. no. 259019.

5. REVIEWED BY:

Byron T. Backus, M.S. Byron T. Backus 06-19-86
Toxicologist
Toxicology Branch, HED

6. APPROVED BY:

Marcia van Gemert, Ph.D. Marcia van Gemert 6.23.86
Section Head, Review Section III
Toxicology Branch, HED

7. CONCLUSIONS:

A. While there was no indication of a mutagenic response in Salmonella typhimurium TA 1530 when host mice were orally dosed with the test substance at 0.5, 2.0 and 5.0 mg/kg, there was no evidence, either from toxic effects in the mice or cytotoxicity in S. typhimurium, that the test material was administered at a sufficiently high dosage level.

B. The study is unacceptable.

8. RECOMMENDATIONS:

The study should be repeated with a dose range such that the highest level is either 5 g/kg, or demonstrates some toxic or cytotoxic response.

9. MATERIALS AND METHODS (PROTOCOLS):

A. Materials:

1. Test Material: Maneb technical, 88% active ingredient, described as a pale green powder, stored at room temperature. The test material was suspended in corn oil for purposes of administration to the host animals.

2. Positive Control Material: Dimethylnitrosamine, with an assumed purity of 100%, from Aldrich.

3. Test Animals: Seven to eight week old male B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Kingston, NY., on February 13, 1985. Sixty of these mice (randomly assigned with 20 per solvent control group, 10 per each of 3 Maneb dose groups, and 10/positive control group) were subsequently used in the assay 6 days later.

4. Indicator Organism: Salmonella typhimurium LT-2 strain TA1530. This strain is unable to synthesize histidine due to a lesion in the phosphoribosyl-ATP-pyrophosphorylase gene in the histidine operon. Colonies can be formed in the absence of exogenous histidine only through mutational reversion to a non-histidine-deficient state.

B. Methods:

1. Administration: The test material was administered at dosage levels of 0.5, 2.0 and 6.0 mg/kg (in terms of the active ingredient) as a suspension in corn oil; negative (solvent) control mice were dosed with vehicle only. In each of these four groups the material was administered via oral gavage at a dosing volume of 10 ml/kg. Positive control mice received approximately 0.1 ml of a 10% dimethylnitrosamine solution via intramuscular injection.

2. Bacterial Inoculation: Immediately following compound administration, an aliquot of two mls of a suspension of Salmonella typhimurium strain TA1530 containing a total of 7×10^8 cells was administered via a single IP injection.

3. Animal Sacrifice: All mice were sacrificed by cervical dislocation. The positive control group was sacrificed 2 hours after bacterial inoculation; the solvent control and 3 test material groups were sacrificed 4 hours after inoculation.

4. Bacterial Recovery: Sacrificed mice were cleansed with 70% EtOH and skinned for laparotomy. A minimum of 1 ml sterile 0.85% NaCl solution was injected IP through the abdominal musculature of each mouse. The peritoneal cavity was aseptically opened, and the bacterial exudate was withdrawn.

5. Plating of Bacterial Exudate: For revertant counts, undiluted peritoneal fluid was added in triplicate to complete top agar containing histidine and biotin, mixed, and poured over Vogle-Bonner E minimal agar. Similarly, for total cell counts, 10-fold dilutions (10^{-1} to 10^{-8}) of peritoneal fluid were prepared in 0.85% NaCl solution and the three highest dilutions were added to top agar and poured over Tryptone Soy Agar plates in triplicate. All plates were incubated at 37° C. Colonies were counted after 48 hrs incubation.

305229

6. Calculations: The total number of colony forming units (CFU) was calculated using the following formula:

$$\frac{\text{Number of colonies/plate}}{\text{Number of plates}} \times \text{dilution factor} = \text{CFU/ml sample plated}$$

The mutation frequency (MF) for each sample was calculated from:

$$MF = \frac{\text{Total Number of Mutant Colonies}}{\text{Total Number of Colonies}}$$

7. Evaluation Criteria: It was not stated what the criteria for a positive response would be.

10. REPORTED RESULTS:

Clinical observations were reported as normal for all animals, and no deaths occurred during the study.

No increase was observed in the mutation frequency of bacterial colonies from mice that were orally dosed with 0.5, 2.0 or 5.0 mg/kg of the test material. The overall mutation frequency in bacterial colonies from positive control mice (0.1 ml 10% DMN administered IM) was 24 times that of colonies derived from the solvent (negative) control animals.

The follcwing is from Table 3, p. 18:

Test Material and Dosage Level	MF x 10^7	Ratio of MF of Dosage group to that of the negative control
Corn oil	0.1	-
Maneb 0.5 mg/kg	0.1	1.0
Maneb 2.0 mg/kg	0.07	0.7
Maneb 5.0 mg/kg	0.1	1.0
DMN - 0.1 ml 10% IM	2.4	24

11. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Maneb Technical did not demonstrate a mutagenic response when tested in the host mediated assay using Salmonella typhimurium Strain TA1530 as the indicator strain and B6C3F1 mice as the host."

B. A quality assurance statement was signed and dated May 30, 1985.

12. *REVIEWER'S DISCUSSION:*

Administration of the test material by oral gavage to host mice did not cause a mutagenic response in S. typhimurium strain TA1530. By contrast, the positive control, administered by intramuscular injection, elicited a 24x increase in mutation frequency over that of the solvent (negative) control, demonstrating that the assay system was capable of detecting a mutagenic response. However, there is no reporting of any preliminary range-finding or cytotoxicity data supporting the dose range used in this assay, nor whether the compound was absorbed from the gastrointestinal tract and reached the indicator organism at an effective concentration. Since the highest dose tested, 5 mg/kg, did not elicit a toxic effect in the dosed mice or a cytotoxic response in S. typhimurium TA1530 it is concluded that the dose range selected was not high enough to fully assess the mutagenic potential of Maneb in the host-mediated assay.

Data Evaluation Report (VIII)

1. CHEMICAL: Maneb

2. TEST MATERIAL: Maneb technical, lot MT01, LH No. 21,838A, described as a pale green (other studies identify it as yellow) powder with a purity of 88%.

3. STUDY/ACTION TYPE: Mutagenicity--Host Mediated Assay in Mice.

4. STUDY IDENTIFICATION: McCarroll, N. E., Burlew, P. and Phipps, N. Host Mediated Assay in Mice with Compound Maneb Technical Study No. 2 (unpublished study project no. 2325-100 prepared by Hazleton Biotechnologies Corp., 9200 Leesburg Turnpike, Vienna, VA 22180 for Pazianos Associates, 211 Ninth St. NE Washington, D.C.; dated June 14, 1985). Acc. no. 259020.

5. REVIEWED BY:

Byron T. Backus, M.S. Byron T. Backus 6-19-86
Toxicologist
Toxicology Branch, HED

6. APPROVED BY:

Marcia van Gemert, Ph.D. M. van Gemert 6.23.86
Section Head, Review Section III
Toxicology Branch, HED

7. CONCLUSIONS:

A. There was no indication of a mutagenic response in Salmonella typhimurium strain TA 1530 when host mice were orally dosed with Maneb at 0.5, 2.0 and 5.0 gm/kg.

B. The study is acceptable.

*. MATERIALS AND METHODS (PROTOCOLS):

A. Materials:

1. Test Material: Maneb technical, 88% active ingredient, described as a pale green powder, stored at room temperature. The test material was suspended in corn oil for purposes of administration.

2. Positive Control Material: Dimethylnitrosamine, with an assumed purity of 100%, from Aldrich.

3. Host Animals: Six to seven week old male B6C3F1 mice were obtained from Charles River Breeding Laboratories, Inc., Kingston, NY., on April 3, 1985. Sixty of these mice (randomly assigned with 20 per solvent control

group, 10 per each of 3 Maneb dose groups, and 10 in the positive control group) were subsequently used in the assay 9 days later.

4. Indicator Organism: Salmonella typhimurium LT-2 strain TA1530. This strain is unable to synthesize histidine due to a lesion in the phosphoribosyl-ATP-pyrophosphorylase gene in the histidine operon. Colonies can be formed in the absence of exogenous histidine only through mutational reversion to a non-histidine-deficient state.

B. Methods:

1. Administration: The test material was administered at dosage levels of 0.5, 2.0 and 5.0 gm/kg (in terms of the active ingredient) as a suspension in corn oil; negative (solvent) control mice were dosed with vehicle only. In each of these four groups the material was administered via oral gavage at a dosing volume of 10 ml/kg. Positive control mice received approximately 0.1 ml of a 10% dimethylnitrosamine solution via intramuscular injection.

2. Bacterial Inoculation: Following compound administration, an aliquot of two mls of a suspension of Salmonella typhimurium strain TA1530 containing a total of 9.6×10^8 cells was administered via a single IP injection.

3. Animal Sacrifice: All mice were sacrificed by cervical dislocation. The positive control group was sacrificed 2 hours after bacterial inoculation; the solvent control and 3 Maneb groups were sacrificed 4 hours after inoculation.

4. Bacterial Recovery: Sacrificed mice were cleansed with 70% EtOH and skinned for laparotomy. A minimum of 1 ml sterile 0.85% NaCl solution was injected IP through the abdominal musculature of each mouse. The peritoneal cavity was aseptically opened, and the bacterial exudate was withdrawn.

5. Plating of Bacterial Exudate: For revertant counts, undiluted peritoneal fluid was added in triplicate to complete top agar containing histidine and biotin, mixed, and poured over Vogle-Bonner E minimal agar. Similarly, for total cell counts, 10-fold dilutions (10^{-1} to 10^{-8}) of peritoneal fluid were prepared in 0.85% NaCl solution and the three highest dilutions were added to top agar and poured over Tryptone Soy Agar plates in triplicate. All plates were incubated at 37° C. Colonies were counted after 48 hrs incubation.

6. Calculations: The total number of colony forming units (CFU) was calculated using the following formula:

$$\frac{\text{Number of colonies/plate}}{\text{Number of plates}} \times \text{dilution factor} = \text{CFU/ml sample plated}$$

The mutation frequency (MF) for each sample was calculated from:

$$MF = \frac{\text{Total Number of Mutant Colonies}}{\text{Total Number of Colonies}}$$

7. Evaluation Criteria: No specific criteria for a positive response were reported.

10. REPORTED RESULTS:

Clinical observations were reported as normal for all animals, and no deaths occurred during the study.

No results were obtained for one animal in the 2.0 gm/kg group "due to a toxic effect on the bacteria. This effect was not seen on any other animal in the study..."

No increase was observed in the mutation frequency of bacterial colonies from mice that were orally dosed with 0.5, 2.0 or 5.0 gm/kg of the test material. The overall mutation frequency in bacterial colonies from positive control mice (0.1 ml 10% DMN administered IM) was 16.6 times that of colonies derived from the solvent (negative) control animals.

The following is from Table 3, p. 18:

Test Material and Dosage Level	MF x 10^3	Ratio of MF of Dosage group to that of the negative control
Corn oil	0.7	-
Maneb 0.5 gm/kg	0.4	0.6
Maneb 2.0 gm/kg	0.5	0.7
Maneb 5.0 gm/kg	0.5	0.7
DMN - 0.1 ml 10% IM	11.6	16.6

11. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Maneb Technical did not demonstrate a mutagenic response when tested in the Host Mediated mutation assay using Salmonella typhimurium Strain TA1530 as the indicator strain and B6C3F1 mice as the host."

B. A quality assurance statement was signed and dated May 30, 1985.

12. REVIEWER'S DISCUSSION:

Administration of the test material by oral gavage to host mice did not cause a mutagenic response in S. typhimurium strain TA1530. By contrast, the positive control, administered by intramuscular injection, elicited a 16.6x increase in mutation frequency over that of the solvent (negative) control, demonstrating that the assay system was capable of detecting a mutagenic response. Since the test material was administered at a the limit (according to FIFRA Guidelines) dosage level of 5 gm/kg (according to the text the oral LD50 in rats of the test material is 6,750 mg/kg) it is concluded that the dose range was adequate to assess the mutagenic potential of Maneb in this host-mediated assay. The study is therefore acceptable for regulatory purposes.