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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mancozeb - Review of Mutagenicity Data Submitted on
Mancozeb and ETU in Response to Data Call-In Notice
of January 17, 1983 - ID #707-78, Accession No.
259044

Caswell No. 913A

FROM: Irving Mauer, Ph.D.
Toxicology Branch
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J. Mauer
6/4-30-86

TO: Arvella Farmer, PM 65
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and

Henry M. Jacoby, PM 21
Fungicide-Herbicide Branch
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THRU: Jane E. Harris, Ph.D.
Head, Section VI
Toxicology Branch
Hazard Evaluation Division (TS-769C)

J. E. Harris
4/30/86

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5/1/86

Registrant: Rohm & Haas

Action Requested:

(870) Review and evaluate the following mutagenicity studies in response to the Data Call-In Notice dated January 17, 1983. Individual Data Reviews are attached to this memorandum.

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Study Title (No. and Date)	Reported Result	Reviewer Evaluation
lian Cell Transformation Assay in C3H 10T 1/2 Cells with ethioureia (No. 84R-056, November 19, 1984).	Negative	Acceptable
lian Cell Transformation Assay for Promotion in C3H 10T 1/2 with Ethylenethioureia (No. 84R-298, May 29, 1985).	Negative	Unacceptable
cial Mutagen (Ames) Test with Dithane M-45 Fungicide (S-9 red from Aroclor 1254-induced Fischer 344 rats) (No. 84R-0059, 21, 1984).	Negative	(Incomplete)*
cial Mutagen (Ames) Test with Dithane M-45 Fungicide (S-9 red from Aroclor 1254-induced B6C3F1 mice) (No. 84R-0060, 21, 1984).	Negative	(Incomplete)*
Mediated Assay in Mice with Dithane M-45 Fungicide 84RC-25B, September 26, 1984).	Negative	Unacceptable
Mediated Assay in Mice with Dithane M-45 Fungicide (Repeat) (No. 84RC-48, July 1, 1985).	Negative	Acceptable
Mutation Assay in CHO Cells with Dithane M-45 Fungicide 84R-207, February 11, 1985).	Negative	(Incomplete)*
cheduled DNA Assay in Rat Hepatocytes with Dithane M-45 Fungicide 84R-280, May 29, 1985).	Negative	Inconclusive**
enetic Study in Fischer-344 Rats with Dithane M-45 Fungicide 84R-246, December 21, 1984).	Negative	Acceptable
r Chromatid Exchange Assay in CHO Cells with Dithane M-45 ide (No. 84RC-60, March 1985).	Positive	Acceptable
lian Cell Transformation Assay in C3H 10T 1/2 Cells with ne M-45 Fungicide (No. 84R-055, November 19, 1984).	Negative	Acceptable
lian Cell Transformation Assay for Promotion in C3H 10T 1/2 with Dithane M-45 Fungicide (No. 84R-297, May 29, 1985).	Negative	Unacceptable

ceptable with S-9 activation. Although initially declared "unacceptable under nactivated conditions" because no positive controls were included for that part of e assay, the sensitivity of the test system to respond was demonstrated adequately the activated assays. Hence these studies are ACCEPTABLE.
 esumptively positive; procedural problems indicate assay should be repeated.

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Two additional studies were included with this submission (Accession No. 259044), but were not reviewed at this time, namely (as listed in the registrant's Table of Contents):

"Section 2. Acute Oral LD50 in B6C3F1 Mice With Dithane M-45 Fungicide"
(R & H Report No. 83R-213A, dated September 24, 1984).

"Section 3. Acute Oral LD50 in Fischer-344 Rats With Dithane M-45 Fungicide" (R & H Report No. 83R-213B, dated September 24, 1984).

These two acute studies will be evaluated for inclusion in the MANCOZEB REGISTRATION STANDARD.

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DATA EVALUATION RECORD

MANCOZEB

Mutagenicity

STUDY IDENTIFICATION: Mutagenicity Overview on the Pesticide Mancozeb.

REVIEWED BY:

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Principal Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-3-86

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APPROVED BY:

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Signature: Jane Harris
Date: 04-04-86 JEH

TEST CHEMICALS: Mancozeb; Dithane M-45; coordination product of zinc and manganese ethylene bis-dithiocarbamate, and ethylenethiourea (ETU), a principal metabolic derivative.

STUDY/ACTION TYPE: Overview--Registration Action.

MUTAGENICITY OVERVIEW ON THE PESTICIDE MANCOZEB

Introduction: Under FIFRA Guideline Subdivision F: Pesticide Assessment Guidelines: Hazard Evaluation - Human and Domestic Animals, dated 11-30-82, an overview (Section 80-1) is required for the various subdivisions of toxicology. "This subdivision details the toxicity data recommended to support the registration of pesticide products," and should meet the requirements of good laboratory practice (40 CFR Part 160), if applicable.

For each test substance, bioassays must be performed to assess the "potential to affect the qualitative or quantitative integrity of human genetic material." A battery of tests to assess mutagenicity is therefore required with the objectives of:

1. Detecting, with great sensitivity, the capacity of a test material to alter cellular genetic material.
2. Determining the relevance of genetic alterations to mammals.
3. Incorporating positive genetic findings into the risk assessments for heritable effects, carcinogenicity, and other possible health hazards.

There are three categories of genetic effects that must be addressed by the test battery:

1. Gene mutations.
2. Structural chromosomal aberrations.
3. Other mutagenic mechanisms (e.g., direct DNA damage, microtubule/spindle fiber inhibition) as deemed appropriate for the test material.

Mutagenicity data as required by 40 CFR Section 158.135 are submitted to support the registration of each manufacturing-use product and of certain end-use products. The assays are performed with the technical grade of each active ingredient in the product. The product should be tested in nonactivated and metabolically activated in vitro assays, and should also be assayed using in vivo mammalian systems with all appropriate positive and negative controls.

SUMMARY OF STUDY EVALUATIONS:

Category 1: Gene Mutation. There were five unpublished studies submitted by the registrant and one published report submitted by the reviewer which were in this category. They are summarized in Table 1 and discussed in this section according to assay type.

Salmonella typhimurium/microsome (Ames): Report Nos. 84R0059 and 84R0060 stated that Dithane M-45 did not induce mutations in any tester strains with or without S9 activation in a dose range of 2.5 to 250 µg/plate. The assays using S9 were conducted properly and therefore considered acceptable; however, the nonactivated assays performed in these studies did not include direct-acting positive controls and were not considered acceptable.

Host-mediated assay in mice (Salmonella typhimurium TA1530): Report No. 84RC-258, using S. typhimurium TA1530 as the detector in a host-mediated assay in mice, showed that Dithane M-45 was not mutagenic when the host was dosed at 0.5 to 5 mg/kg. However, the doses were inadequate; therefore, the assay was considered unacceptable. Report No. 84RC-48 described the use of a dose range of 0.5 to 5 g/kg in an otherwise identical host-mediated assay. Dithane M-45 was also negative for a mutagenic response and the assay was considered acceptable.

Mammalian cell mutagenicity--CHO/HGPRT: Report No. 84R-207 stated that Dithane M-45 was not mutagenic in the CHO/HGPRT in vitro assay either at 0.5 to 15 µg/mL in the nonactivated assay or at 0.25 to 45 µg/mL in the S9-activated assay. The S9-activated assay was properly conducted and therefore acceptable; however, the nonactivated assay was unacceptable because the positive control, ethylmethane sulfonate, had to be used at a level that was approximately 7-fold higher than the highest non-activated dose, 15 µg/mL of test material.

Published data on Bacillus subtilis and Salmonella typhimurium (liquid preincubation) assays: Using a dose range of 1 to 25 µg/plate in the liquid preincubation assay with B. subtilis and S. typhimurium, Shiau et al. (1980)¹ reported that Dithane Technical (zinc ethylenebisdithiocarbamate) was mutagenic in both B. subtilis TKJ6321 and S. typhimurium TA1535. Although mutagenesis in the Salmonella mutant was not as strong as in B. subtilis, it could be demonstrated at nonactivated concentrations of 1, 5, and 10 µg/plate. In B. subtilis TKJ6321, a dose-responsive increase was observed in the nonactivated system at this same dose range, with a decline due to cytotoxicity at 25 µg/plate. At the highest concentration, approximately 45,000 His⁺ revertants/10⁸ cells was reported, and the calculated potency was 38.4 revertants/nanomole. In the presence of S9, Dithane was reported to show a "marked reduction" in the mutagenic response.

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Overall, the unpublished reports showed that Dithane M-45 was not mutagenic either in vitro using bacteria and mammalian cells with or without S9 activation, or in the host mediated assay. However, none of the assays were acceptable for the in vitro nonactivated system. Since published data using the liquid preincubation nonactivated system for both B. subtilis and S. typhimurium were positive, and in fact showed that Dithane was a very potent mutagen, inducing 38.4 revertants/nanomole, the test material should be considered mutagenic. It is classified as a base-pair mutagen on the basis of its positive activity for S. typhimurium strain TA1535 and B. subtilis strain TKJ6321. If all the unpublished and published data are considered, there are no data gaps for Category 1.

Category 2: Structural Chromosomal Aberrations.

Sister chromatid exchange: Study No. 84RC-60 reported that Dithane M-45 caused an increase in sister chromatid exchange in CHO-WB1 cells which was dose responsive in two assays in the range of 7.5 to 17.5 µg/mL in the nonactivated system (positive response beginning at 7.5 µg/mL), and inconclusive but presumed positive in the range of 10 to 17.5 µg/mL in the S9-activated assay (positive response beginning at 10 µg/mL). The assays were conducted properly and, therefore, acceptable.

In vivo cytogenic study in rats: The report No. 84R-246 stated that Dithane M-45 did not cause a significant increase in chromosomal aberrations in bone marrow cells of male rats exposed to 4.4 g/kg (maximum tolerated dose) sampled over the entire mitotic cycle. This data was acceptable; however, the study did not include female animals and was, therefore, deficient.

Except for the data gap on chromosomal aberrations in female animals, there is sufficient data to fulfill the requirements in Category 2. Since a positive response was obtained in the SCE assay in the nonactivated system and a presumptive positive in the S9-activated system, we presume that the test material causes structural chromosomal aberrations and that the frequency of inducing these aberrations could be reduced in either the presence of an exogenous or endogenous mammalian metabolic system.

Category 3: Other Mutagenic Mechanisms.

Unscheduled DNA synthesis in rat hepatocytes: The report No. 84R-280 stated that Dithane M-45 did not induce unscheduled DNA synthesis in isolated Fischer rat hepatocytes. However, we conclude that the net nuclear grain counts at 1.0, 2.5, and 5.0 µg/mL, although complicated by a higher than usual cytoplasmic grain count for the solvent and a decrease at 10 µg/mL, were sufficient to presume a positive but inconclusive UDS response.

DNA-damage in Bacillus subtilis: Shiau et al. (1980)¹ published data showing that Dithane technical caused DNA damage in several repair-deficient strains of B. subtilis at 50 µg/plate in the nonactivated system, but that S9 metabolism reduced the effect so that 300 µg/plate was required for a positive response. This data is supported by the

thesis work of Lee² (1980) who demonstrated DNA damage in B. subtilis strains hcr-9, fh2006-7, and mc-1 in the nonactivated system at all doses between 50 and 300 µg/plate. In addition, a positive DNA-damaging effect in a liquid incubation assay was demonstrated at all doses from 10 to 50 µg/mL. In an attempt to define the mechanism of Dithane action, Lee also showed that the DNA derived from B. subtilis cultures treated with 10 µg/mL of Dithane was damaged, and therefore had reduced DNA transformation activity in the histidine gene; furthermore a 1:1 ratio of Dithane mixed with purified DNA, damaged the DNA and specifically reduced DNA transformation for the histidine locus.

Hume³ (1980) reported that nonactivated Dithane was negative in the phage induction assay (β-galactosidase induction) in Escherichia coli B13 (λ+) when doses ranging from 50 to 300 µg/plate were assayed in a semiquantitative spot test, but it was weakly positive at 50 µg/mL in the liquid (tube) assay.

In vitro transformation assays in C3H/10T1/2 mouse fibroblasts: Report 85R-055 stated that Dithane M-45 at doses ranging from 0.05 to 0.5 µg/mL did not induce transformed foci in C3H/10T1/2 mouse fibroblasts; likewise, ethylenethiourea (ETU) was reported to be negative in this system at doses ranging from 100 to 1,000 µg/mL (Report 84-R-056). These studies were properly conducted and therefore acceptable. However, study Nos. 84R-297 and 84R-298, designed to assess promoter activity in C3H/10T1/2 mouse fibroblasts, were unacceptable because only one dose of Dithane M-45 (0.1 µg/mL) or ETU (333 µg/mL) was used.

SUMMARY TABLE:

A one-liner table has been included in this overview. It identifies the individual studies, specifies the dose range, presents the reviewers' evaluations, places each study in its proper category, and classifies the studies according to their acceptability.

CONCLUSION:

If all studies submitted by the registrant and published articles are considered, testing in all three genetic effect categories are fulfilled. Although there were many studies submitted in which negative responses were obtained, there is sufficient evidence which shows that Dithane is mutagenic. Since this test material was shown to induce base-pair mutations in B. subtilis and S. typhimurium,¹ induce sister chromatid exchange (study No. 84RC-60), presumably induce unscheduled DNA synthesis (study No. 84R-280), induce DNA damage in B. subtilis,^{1,2} induce β-galactosidase synthesis in the E. coli B13 (λ+) system,³ and directly interact with DNA of B. subtilis,² it must be considered to have a significant genotoxic potential. The negative responses in some of the in vitro assays could have been contributed, at least in part, by the problems of solubility; the test material is very insoluble in water, therefore, using DMSO as the solubilizer was required.^{1,2,3} In

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addition, it appears that the S. typhimurium/microsome assays (study Nos. 84R0059 and 84R0060) lacked sensitivity and perhaps even specificity for detecting a gene mutation response; however, liquid preincubation and the use of DMSO as a solubilizer appeared to improve the sensitivity of the assay.¹ The inconclusive result in UDS assays could also be attributed to a failure in solubilizing the test material. When the transformation assays were performed, there was no indication that either Dithane or ETU induced or promoted transformed foci; these results are not surprising considering that the test materials may not have reached the target site under the conditions of the assays. In addition, transformation is considered to be a multistage phenomenon and the requirements to cause cell transformation would likely be less probable than induction of a point mutation, DNA interaction, or a clastogenic effect.

Literature Cited

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¹Shiau, S. Y., R. A. Huff, B. C. Wells, and I. C. Felkner. Mutation Research 71(1980): 169-179.

²Lee, M. C. DNA Transformation and Mutagenesis Studies on Dithane with Bacillus subtilis Repair-Deficient Strains. M. S. Thesis at Texas Tech University, supported by EPA Contract CR806904-01, 1980, pp. 1-21.

³Hume, S. H. Screening Pesticides with a Colorimetric Phage Induction Assay. M. S. Thesis at Texas Tech University, supported by EPA Contract CR806904-01, 1980.

Study / Lab / Study Date	Material*	No.	Dose	Conclusions (Evaluations)	Category	Classification
1. Mutagenicity Reverse Mutation in <i>Salmonella</i> /Rohm and Haas Co./84R0059/6-21-84	Dithane M-45, TD No. 83-224, lot No. 0842, 88% a.i.	259044	2.5-250 µg/plate	Not mutagenic for S9-activated; data inadequate for nonactivated but negative	1	S9--acceptable; nonactivated--unacceptable
2. Mutagenicity--Reverse Mutation in <i>Salmonella</i> /Rohm and Haas Co./84R0060/6-21-84	Dithane M-45, TD No. 83-224, lot No. 0842, 88% a.i.	259044	2.5-250 µg/plate	Not mutagenic for S9-activated; data inadequate for nonactivated but negative	1	S9--acceptable; nonactivated--unacceptable
3. Mutagenicity--Host-mediated Assay- <i>Salmonella</i> in mice/Hazleton Labs./84RC-25B/9-26-84	Dithane M-45, 88% a.i.	259044	0.5-5.0 mg/kg	Inadequate dosage; negative result	1	Unacceptable
4. Mutagenicity--Host-mediated Assay- <i>Salmonella</i> in mice/Hazleton Labs./84RC-4B/7-1-85	Dithane M-45, 88% a.i.	259044	0.5-5.0 g/kg	Not mutagenic	1	Acceptable
5. Mutagenicity--CHO/HGPRT/Rohm and Haas Co./84R-201/2-11-85	Dithane M-45, TD83-224, lot No. 0842, 88% a.i.	259044	0.25-45 µg/mL	Not mutagenic for S9-activated; data inadequate for nonactivated	1	S9--acceptable; nonactivated--unacceptable
6. Mutagenicity--Sister Chromatid Exchange in CHO/Litton Bionetics, Inc./84RC-60/3-85	Dithane M-45, TD83-224, lot No. 0842, 88% a.i.	259044	7.5-17.5 µg/mL (2 assays)	Mutagenic in nonactivated assay (7.5 µg/mL); presumed mutagenic (10 µg/mL) in S9-activated assay	2	Acceptable

*Dithane M-45 which is the Rohm and Haas technical coordination product of zinc and manganese ethylene bis-dithiocarbamate.

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7. Mutagenicity--In vivo Cytogenetics in Rats/Rohm and Haas Co./ 84R-246/12 21-84	Dithane M-45,* TD No. 83-224, lot No. 0842, 88% a.i.	4.4 g/kg	259044	Not mutagenic in male rats; not tested in female rats	2	Acceptable in male rats; unacceptable in female rats
8. Mutagenicity--In vitro Unscheduled DNA Synthesis/Rohm and Haas Co./ 84R-280/5-29-85	Dithane M-45,* TD 83-224 lot No. 0842, 88% a.i.	0.025-10.0 µg/ml	259044	Presumptive positive at 1.0, 2.5, and 5.0 µg/ml	3	Inconclusive
9. Mutagenicity In vitro Transfor- mation in C3H/101 1/2 Mouse Fibroblasts/Rohm and Haas Co./ 85R-055/11-19-84	Dithane M-45,* TD 83-224 lot No. 0842, 88% a.i.	0.05 0.5 µg/ml	259044	Negative at all doses	3	Acceptable
10. Mutagenicity--In vitro Transfor- mation in C3H/101 1/2 Mouse Fibroblasts/Rohm and Haas Co./ 84R-056/11-19-84	Ethylenethio- urea, TD 83-223, lot No. 088-36, 99.8% pure	100-1000 µg/ml	259044	Negative at all doses	3	Acceptable
11. Mutagenicity--In vitro Transfor- mation Assay for Promotion in C3H/101 1/2 Mouse Fibroblasts/ Rohm and Haas Co./84R-297/ 5-29-85	Dithane M-45* TD 83-224, lot No. 0842, 88% a.i.	0.1 µg/ml	259044	Negative, at insufficient dose range	3	Unacceptable

*Dithane M-45 which is the Rohm and Haas technical coordination product of zinc and manganese ethylene bis-dithiocarbamate.

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TABLE 1. Line 1 liner summary table of Mutagenicity Studies with Monozob (Dithane M 45) and Ethylenethiourea (continued)

Study / Lab / Study Date	Material	Accession No.	Dose	Conclusion: (evaluations)	Genetic Effects Category	Classification
12. Mutagenicity - In vivo Trans-formation Assay for Promotion In C3H/101 1/2 Mouse Fibroblasts/ Rohm and Haas Co./84R 298/ 5 29 85	Ethylenethiourea, ID 83-223, lot No. 088-36, 99.8% pure	259044	333 µg/ml	Negat.ve, but insufficient dose range	3	Unacceptable
13. Mutagenicity and DNA damaging Activity for Several Pesticides Tested with <i>Bacillus subtilis</i> mutants. Mutation Reg. 71(1980): 169 1/9/Texas Tech Univ. EPA Contract No. 68-01 3963	Dithane (technical) zinc and manganese ethylene-bisdithio-carbamate	N/A - Published data provided by reviewer/sponsor	(a) 50-300 µg/plate (b) 1-25 µg/plate (Liquid incubation using DMSO as solvent)	(a) Positive DNA damage in <i>B. subtilis</i> at 50 µg/plate--non-activated and 300 µg/plate S9-activated (b) Positive mutagen in <i>B. subtilis</i> (b) 1 IKJ 6321. Potency = 38.4 mutants/nanomole, nonactivated. Positive mutagen in <i>S. typhimurium</i> TA1535 at 2 µg/plate, nonactivated	(a) 3 (b) 1	(a) Acceptable (b) Acceptable
14. DNA damaging and Direct effects on DNA in <i>Bacillus subtilis</i> / Thesis, Texas Tech Univ. EPA Contract CR806904-01; NCI Grant 1-ROI-CA21020 02A1/8-80	Dithane (technical) zinc and manganese ethylene-bisdithio-carbamate	N/A - Published data provided by reviewer/sponsor	(a) 50-300 µg/plate (b) 10-50 µg/ml (liquid) (c) 10 µg/ml (d) 100 µg/ 100 µg DNA	(a) Positive DNA damage in <i>B. subtilis</i> strain HCP-3, fh2006-1, and MC-1 at all levels, nonactivated (b) Positive at 10 µg/ml in <i>B. subtilis</i> hcp-3, fh2006-1, and MC-1, nonactivated (c) DNA in whole cells damaged for gene transformation (d) Purified DNA damaged for gene transformation	(a) 3 (b) 3 (c) 3 (d) 3	(a) Acceptable (b) Acceptable (c) Acceptable (d) Acceptable

TABLE I One liner Summary table of Mutagenicity Studies with Manganese (Dithane M 45) and Ethylenethiourea (continued)

Study / Lab / Study Date	Material	Accession No.	Dose	Conclusions (Evaluations)	Genetic Effects Category	Classification
15. Mutagenicity Phage Induct Test for DNA Damage (Escherichia coli strain 813(A)β-galactosidase assay). Thesis, Texas Tech Univ. EPA Contract CR806904 01/5-81	Dithane (technical) zinc and manganese ethylene bisdithio-carbamate	N/A Thesis data provided by reviewer/ sponsor	50 300 µg/plate 1 50 µg/ml (liquid assay)	Negative in plate assay Weakly positive in tube assay at 50 µg/ml	3 3	Acceptable Inconclusive presumptive positive

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