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

006910

OCT 19 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Maneb Data Call-In; Submission of Supplementary Data on Maneb Mutagenicity Studies

TO: Frank Rubis/G. Werdig
Product Manager (50)
Registration Division (TS-767C)

FROM: Linda L. Taylor, Ph.D. 9/30/88
Toxicology Branch II, Section II
Hazard Effects Division (TS-769C)

THROUGH: Marcia van Gemert, Ph.D. 9/30/88
Acting Section Head, TB II
Toxicology Branch II, HED (TS-769C)

and

William Burnam, Ph.D.
Acting Chief, Toxicology Branch II, HED (TS-769C)

Chemical: Maneb
Caswell No.: 539
Record No.: 231053
Project No.: 8-1145
Registrant: Pennwalt Corporation
Action Requested: None specified. Submission is supplemental data that was requested for two mutagenicity tests - "In Vitro Unscheduled DNA Synthesis Assay in Rat Hepatocytes: the Effect of Technical Grade Maneb" and "CHO/HGPRT In Vitro Mammalian Cell Mutation Assay on Technical Grade Maneb".

Comment: In response to comments made in EPA's review dated July 30, 1987, Pennwalt Corporation submitted additional data and clarification in order to upgrade two mutagenicity studies.

1. MRID = 40091303 - CHO/HGPRT Mutagenesis Assay

   The purity of the test material was incorrectly listed in the final report. The correct percentage of Technical grade Maneb is 88.1%.

2. MRID = 40163901 - UDS Synthesis Assay

   The individual raw data have been provided and have been evaluated (see memo dated 9/27/88 K. Dearfield to L.L. Taylor, copy attached). Under the conditions of the study, it appears that maneb did not induce UDS.

Note: As discussed in the attached memo, a similar study (on contract to EPA) was performed on two samples of maneb (Dithane M-22 and Manzate-D) in cultured human fibroblasts (WI-38 cells). The results indicated that Manzate-D induced a small, concentration-dependent, statistically significant increase in UDS over a narrow concentration range without metabolic activation. It was concluded that, since the response in this study was, at best, a very weak one, and it may have been influenced by the test material formulation, maneb appears to present a minimal concern in the UDS assay at this time. If further evidence suggests a larger concern, it may be necessary to pursue this aspect of genotoxicity (DNA damage and repair) for maneb in the future.

CONCLUSION

Both of the Pennwalt mutagenicity studies are now acceptable.

cc: Valerie M. Bael
Special Review Branch
Registration Division (TS-767C)

Susan Lewis
Registration Standard Project Support Team

Lois Rossi
Product Manager (21)
Registration Division (TS-767C)

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

SEP 27 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Unscheduled DNA Synthesis Assay Re-evaluation for Maneb, Caswell #539, [12427-28-2]

FROM: Kerry L. Dearfield, Ph.D.
Geneticist
Toxicology Branch II
Health Effects Division (TS-769C)

TO: Linda Taylor, Ph.D.
Toxicology Branch II
Health Effects Division (TS-769C)

THRU: William Burnam
Acting Chief
Toxicology Branch II
Health Effects Division (TS-769C)

A previous review on a submitted unscheduled DNA synthesis (UDS) assay on maneb had concluded that although maneb did not appear to induce UDS in primary rat hepatocytes, individual data were required to allow for a more complete evaluation. The assay was therefore classified as unacceptable. Other comments in the original review suggested that the background counts for the negative controls appeared unacceptably high and that at least one of the positive control slides was not clearly shown to have met appropriate criteria for a significant induction of UDS. The submitting company has since submitted the individual data to address these concerns.

Primary rat hepatocytes were exposed two hours after seeding to technical maneb. Exposure lasted 18 hours. There were six samples per concentration with the concentrations ranging from 0.05 to 500 ug/ml. Extreme toxicity was evident at 500 ug/ml (very few cells, little or no cytoplasm, and debris); therefore, the top concentration examined was 100 ug/ml, where some toxicity was seen. Two slides per concentration were counted with 50 cells/slide scored. Examination of the individual counts indicate that there is no apparent increase in UDS among treated cultures. While average background counts for the medium control are high, they are not into an unacceptable range. The testing laboratory should ensure that these counts are brought down in future submissions (consistent elevated counts would indicate a problem in the testing laboratory). The one positive control

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replicate which did not reach >5 net grains/nucleus is a result of the counting technique used by the testing laboratory. They did not count cytoplasmic counts when the nucleus had >10 grains; therefore, these numbers are minimum numbers. It appears that a better accounting should have been performed, but most likely these results are minimally adequate for assessment. Overall, it appears that maneb did not induce UDS under the test conditions in this report. The review can be upgraded to acceptable.

It should be noted that the EPA, through a contract to a testing laboratory, has also tested maneb in an unscheduled DNA synthesis assay (Simmon et al., 1979). Two samples of maneb, Dithane M-22 from Rohm and Haas and Manzate-D from duPont, were tested for UDS in cultured human fibroblasts (WI-38 cells). Contact-inhibited WI-38 cells, pretreated with hydroxyurea, were exposed to test compound for 3 hours without metabolic activation and for 1 hour with metabolic activation. UDS was determined by the liquid scintillation counting method expressed as disintegrations per minute (dpm) of incorporated tritiated thymidine per unit of DNA as compared to that in solvent controls. Neither sample induced UDS in the presence of metabolic activation. Only Manzate-D induced a small concentration-dependent, statistically significant increase in UDS over a very narrow concentration range (2.22 - 7.5 ug/ml) without metabolic activation; the top response was just above a doubling of the background levels. Dithane M-22 however did not produce an increase in UDS without metabolic activation. An important variable may be the formulation of each of these test samples from the different sources.

Overall, while maneb does not appear to induce UDS in primary rat hepatocytes as seen in the submitted study, maneb may have some DNA damaging capability as evidenced by the contracted EPA study. As the response in the positive assay was at best a very weak one, and the positive result may be influenced by test compound formulation, it appears that maneb presents a minimal concern in the UDS assay at this time. If further evidence suggests a larger concern, it may be necessary to pursue this aspect of genotoxicity (DNA damage and repair) for maneb in the future.

Reference

Simmon V, Riccio E, Robinson D, Mitchell A (1979) In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of fifteen pesticides. Final Report--Phase III, Contract No. 68-01-2458, U.S. Environmental Protection Agency.