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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Mancozeb - Company Response to TB Assessment of Certain Studies in the Toxicology Chapter of the Mancozeb Registration Standard
EPA Registration No. 707-78

TB Proj. No.: 7-1004
Caswell No.: 913A

FROM: Irving Mauer, Ph.D. 12/03/87
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Registrant: Rohm & Haas Company
Philadelphia, PA

Request

Appraise registrant's response, submitted under cover letter of July 6, 1987, to assessments by TB of certain studies reviewed for the Toxicology Chapter of the Mancozeb Registration Standard (dated October 26, 1986), and judged inadequate and/or unacceptable. These studies are as follows:

1. McLeod, P.L. and Doolittle, D.J. Dithane M-45 Mammalian Cell Transformation Test for Promotion. (Unpublished Study No. 84P-297, prepared and submitted by Rohm & Haas Company, Spring House, PA; dated May 29, 1985.. Accession No. 259044.

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2. McLeod, P.L. and Doolittle, D.J. Ethylenethiourea Mammalian Cell Transformation Test for Promotion. (Unpublished Study No. 84R-298, prepared and submitted by Rohm & Haas Company, Spring House, PA; dated May 29, 1985.) Accession No. 259044.

3. Haines, L.D. Dithane M-45 Percutaneous Absorption in Rats. (Rohm & Haas Technical Report No. 84F-80-9, May 8, 1980.) Accession No. 250063.

The rationale provided by the registrant for declaring these studies satisfactory is as follows (directly quoted from the July 6, 1987 submission):

Studies 1/2

"RATIONALE FOR WHY THE PREVIOUSLY SUBMITTED TRANSFORMATION ASSAYS FOR PROMOTION WITH MANCOZEB AND ETU ARE ACCEPTABLE

"Transformation assays for promotion studies with both ETU and mancozeb have been submitted to EPA in fulfillment of previous Data Call-In requirements. (Accession No. 259044) In the Re-registration Guidance Document, Toxicology Chapter (Caswell No. 913A, EPA Chem No. 014504, TB Project No. 28) EPA asked that new studies be submitted because only one dose was used in the studies we submitted. The protocols of the studies were reviewed and found to be acceptable by EPA before the studies were conducted. It is our opinion that the dose used in these studies was the maximum tolerated concentration and the promotion potential of mancozeb and ETU has been adequately evaluated in this system. It is also the opinion of our toxicologist that the new transformation assays for promotion suggested in the Re-registration Guidance Document offer no advantages over the assay we submitted to EPA earlier. Details of our toxicologist's comments are attached." (P.K. Chan, R&H)

Study 3

"EXPLANATION OF WHY THE PREVIOUSLY SUBMITTED MANCOZEB DERMAL PENETRATION STUDY IS FULLY ACCEPTABLE

"A dermal penetration study with mancozeb in rat was cited in the Toxicology Chapter of the Registration Standard (Caswell No. 913A, EPA Chem No. 014504, TB Project No. 28). However, the active ingredient was identified as 8.3% a.i. in the Toxicology Chapter. This was an error. The correct active ingredient content of the sample should be 83.0% a.i., which was specified in the technical report submitted by Rohm

and Haas (EPA Accession No. 250063). This study was conducted with a technical material produced in a commercial plant instead of a laboratory produced technical sample. This study has been reviewed by EPA and classified as an acceptable study which demonstrates a 1% dermal penetration for mancozeb, as shown in the attachments. Thus, the requirement of a dermal penetration study with technical mancozeb has been fulfilled." (P.K. Chan, R&H)

Study 1 (Promotion Assay with Dithane M-45 on C3H-10T1/2 Cells)

Preliminary range-finding cytotoxicity assays were performed with mancozeb technical (88%) at six doses ranging from 0.01 to 0.50 ug/mL, resulting in decreases in cell survivals at the two top doses (0.25 and 0.50 ug/mL) of 83 and 67 percent, respectively, following 24-hour exposure, and 75 and 42 percent, respectively, following continuous 9-day exposure. All other doses below 0.25 ug/mL (0.07, 0.033, 0.066, and 0.10 ug/mL) had cell survivals not different from the untreated control. A second cytotoxicity test was run with continuous exposure to the same six concentrations of mancozeb in cultures of C3H-10T1/2 cells preinitiated with the carcinogen MNNG. Except for transient, slight inhibition at the HDT, essentially no definitive cytotoxicity was found in this test. Hence, the results of the first range-finding test were used to determine the appropriate dose for the main transformation/promotion assay. The single dose selected was 0.10 ug/mL, a nontoxic concentration not associated with any inhibition of cell growth.

The main promotion assay in which this singular dose was applied continuously for 6 weeks following short-term exposure (4 to 24 hours) of initiating agents was negative, in that the percent Type III Foci (5.3%) was not different from the MNNG-initiated, untreated control plates.

Hence, the authors concluded that Dithane M-45 does not promote morphological transformation in this test system.

We initially judged this study to be unacceptable because only a single, nontoxic dose was used, which we (along with others*) consider insufficient to demonstrate the test material

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*Dr. Craig Boreiko of the Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, NC, a noted expert on initiation/promotion assays, recommends more than one dose level (5 dose levels are routinely evaluated in this laboratory), because promoters frequently induce erratic and non-dose-related effects.

does not have promoter activity (i.e., promoting neoplastic transformation). Additionally, it is toxicologically incorrect for the registrant to define the "maximum tolerated concentration" as that dose not exhibiting any cytotoxicity.

Further, in the direct transformation assay run 6 months previously (Study No. 84R-055, dated November 19, 1984), a series of five concentrations ranging from 0.05 to 0.50 ug/mL was employed in the same cell line, which resulted in relative (%) cell survivals ranging from 96 (at 0.05 ug/mL) to 14 percent (at 0.50 ug/mL). Since no Type III foci were found following exposure of C3H-10T1/2 cells to any dose of the test material up to levels of severe toxicity, this assay was judged negative and ACCEPTABLE.

Company Response

The registrant offers the "opinion of Rohm & Haas Company Toxicology Department this [one dose] was the maximum tolerated concentration and the promotion potential was adequately evaluated." Further, since the registrant maintains that the protocols were accepted by EPA before the studies were initiated, a repeat study should not be required.

EPA Appraisal

It is evident that the single dose employed in the promotion assay is not the maximum tolerated concentration, being acknowledged as nontoxic by the authors themselves. However, it is a dose just below those doses inducing some cell toxicity. As indicated in the protocol submitted for this study, transformation results were not corrected by the customary procedure for cell survival (toxicity) because it is not known how the parameters of toxicity and dose alter the probability of transformation in each plate. Hence, since (1) there are currently no established guidelines for such initiation/promotion assays, and (2) we have already accepted a protocol for such a study, limited as it is by the lack of the customary criteria for in vitro assays, the study can be acceptable as a negative, given the limitations noted.

Study 2 (Promotion Assay with Ethylenethiourea (ETU) on C3H-10T1/2 Cells)

As with the Dithane M-45 study, range-finding cytotoxicity tests were conducted with multiple doses of ETU (100, 333, and 1000 ug/mL), but no growth inhibition was observed at any dose, either following 24-hour or 9-day exposures. A second cytotoxicity test using an initiating agent (0.5 ug/mL MNNG)

resulted in a 60 percent cell inhibition at the HDT (1000 ug/mL) at day 4, and 22 percent less than control at day 7.

The mid-dose (333 ug/mL) showed 30 percent inhibition at day 4, but recovered by day 7, exceeding the solvent control from then on. Based on these cytotoxicity assays, 333 ug/mL was selected as the single dose for the main transformation/promotion assay.

In this main assay, no Type III foci were observed in ETU plates after 5 weeks continuous application following short-term exposure (4 to 24 hours) of initiating agent(s). Thus the authors concluded that ETU does not promote morphological transformation in this test system.

We also initially judged this assay to be unacceptable because only a single dose was employed, which may be insufficient for establishing any promoter action. However, at least in this assay, a dose giving transient toxicity was employed. Again we accepted the protocol submitted employing a single dose, although we also note the registrant's disclaimer that transformation results with ETU were also not corrected by the customary procedure for cell survival (toxicity) because it is unknown how the parameters of toxicity and dose alter the probability of transformation in each plate. Given the limitations noted, this study can also be upgraded to acceptable, as a negative.

Study 3 (Mancozeb Dermal Penetration Study)

In the Toxicology Chapter of the Mancozeb Registration Standard, a dermal penetration study, submitted in response to the Data Call-In (DCI) Notice of January 17, 1983, was also reviewed. The study involved the application of 10 mg of "commercial Dithane M-45," purportedly containing 8.3% ai (and [redacted] ETU), to a 20 cm^2 shaved area of the backs of 9 and 12 adult female Sprague-Dawley rats in two separate experiments. The material was secured under bandages for a maximum of 6 hours. Residues of the EBDC and ETU were determined in both skin contact areas and in urine and feces collected over the 6-hour exposure, as well as 18 hours later (following termination of treatment). The values for skin (plus bandage were 0.23 percent for 6 hours and 0.89 percent for 24 hours. The value from 24-hour excretion data was 1.01 percent. These values are in general agreement with each other, and permit a general value of 1 percent dermal absorption to be used for this study. However, this study was considered Core-Supplementary because only one dose was employed (and it was unstated why this particular dose was selected). The use of only a single dose does not permit the maximum absorption rate to be determined. Further a study using the technical

grade of mancozeb (and not the 8.3% formulation as given) was declared to be required to satisfy regulatory requirements.

Company Response

As indicated in the company's July 6, 1987 submission, the sample used in this study was actually an 83.0 percent technical produced in a commercial plant instead of a laboratory-produced technical sample (and not the 8.3% ai identified in the Toxicology Chapter). This is actually stated in the conclusion of the first review of this study (Zendzian to Sandusky, May 7, 1984), which states:

> Approximately one percent of EBDC in a 10 mg dose of Dithane M45, containing 8.3 mg of the active ingredient (EBDC), is absorbed through the skin of female rats following a 6-hour application.

In the next paragraph of this review ("Materials"), the test article is stated as "Commercial M-45 Lot #4268, active ingredient 8.30% . . ." i.e., an inadvertent transposition of the decimal; the ai should have been stated as 83.0 percent.

This study was initially judged "Acceptable" (review by Zendzian, May 7, 1984, attached to memorandum: Zendzian to Sandusky, May 7, 1984), although the reviewer suggested that it was impossible to determine if a maximum absorption rate had been reached in this study since only one dose was used. In reexamining the report of this study (34F-80-9), we find no justification for the choice of the single 10 mg dose, which is extremely low in comparison to acute dermal LD_{50} values (2000 to 5000 mg/kg). The authors of the study stated the 10 mg dose (= approximately 50 mg/kg based on the animal's weight, provided in the appendices to the Report) was meant to be an "excessive dose," or "saturating level," for maximum skin penetration. However, no evidence is provided in the report for this assertion.

The 3(a)(2)(B) DCI Notice of January 17, 1983 specified a protocol for a dermal absorption study using four doses (not a single dose) and at least 20 young adult male rats per dose level (not 9 or 12 females). No valid justification is provided for using females, although the report states this was to "allow an estimation of teratogenic safety factors." However, only nonpregnant animals were used in this study, hence it is difficult to imagine any correlation to teratogenic (or embryonic) safety factors.