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

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6/3/83

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Caswell 849A

SUBJECT: Additional Data in Support of Mutagenicity Studies with Thiabendazole, (TBZ) Active Ingredient of "Mertect 340-F". Accession No. 249845. EPA Reg. No. 618-75/Action Code 571

TO: Henry M. Jacoby/Eugene Wilson
Fungicide-Herbicide Branch
Registration Division (TS-767-C)

APPLICANT: Merck, Sharp and Dohme (MSD), Rahway, N.J.

ACTIONS REQUESTED: A. Review the following additional data and rebuttal comments submitted March 22, 1983, in response to TB evaluation of those mutagenicity studies previously submitted under Accession No. 247112 judged UNACCEPTABLE (See previous review, dated August 16, 1982):

1. Appendices 1-5: Clinical data from both oral range-finding and oral (in vivo) cytogenetic studies of TBZ in rats, performed at the Institute of Environmental Toxicology (IET, Tokyo, Japan).

2. Appendices 6-8: Results from range-finding mouse study (LD_{50}), as well as clinical observations and bodyweights on mice treated with TBZ in the dominant lethal study performed at the IET.

3. Appendix 9: Complete protocol for the report: "Mutagenicity Testing on Thiabendazole (TBZ) in Microbial Systems," performed at the IET July, 1976.

4. Rebuttal Comments regarding our evaluation of the mouse micronucleus test performed at MSD.

5. Rebuttal comments in response to our review of the "subacute dominant lethal" study performed in mice at MSD.

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B. Review and evaluate Appendix 10: An in vitro cytogenetic study of TBZ in human WI-38 cells, performed at the Stanford Research Institute (inadvertently not included in previous submission).

A. TB APPRAISAL OF RESPONSES AND ADDITIONAL DATA.

Item 1: Applicant's additional data (Appendices 1-5) satisfy the deficiencies previously cited in TB's review of the IET rat cytogenetic study; and this study is now judged ACCEPTABLE.

Item 2: The additional range-finding results for the IET mouse dominant lethal study, yielding an LD_{50} = 3100 mg/kg (1800-5300 mg/kg) according to the standard Litchfield and Wilcoxon Method (Appendix 6), would support oral dose selection for an acute dominant lethal test of TBZ; test compound, however, would still have to be administered at doses up to the MTD. Further, reference to literature (sig: Epstein and Robertson, 1971) "recommendations" (test substance "should be administered at approximately 1/5 of the LD_{50} for five successive days") do not substitute for appropriate dosages sufficient to assure adequate transport of "active" substance to target tissues (in this case, testes), which apparently was not achieved in this study, as evidenced by the (acknowledged) lack of clinical toxicity (Appendix 7), or body weight changes (Appendix 8), with the 5-day 600 mg/kg dose-schedule employed. Hence, this IET study remains UNACCEPTABLE as an adequate assay to assess the potential of TBZ to induce dominant lethal mutations in C3H/HeCR mice.

Item 3: The study under Accession # 247112 entitled: "Mutagenicity Tests of Thiabendazole in the Ames Salmonella typhimurium Reverse Mutation Test", performed by James McGregor, USDA Western Regional Research Center, (Albany, CA) and dated May 28, 1976, was judged UNACCEPTABLE, because only a cover letter and a single tabulation of values, but no protocol, were submitted. The present re-submission of "detailed materials and methods and result section" provided in an entirely different report, namely the IET's: "Mutagenicity Testing of Thiabendazole (TBZ) in Microbial Systems" (Attached as Appendix 9) is not an acceptable substitute, and hence does not change the original evaluation (=UNACCEPTABLE).

Item 4: The applicant's rebuttal comments with reference to TB's evaluation of MSD's mouse micronucleus test are not accepted (hence this study remains UNACCEPTABLE), because:

(i) The applicant acknowledges "no toxicity was apparent in this study";

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(ii) Inadequate protocols (dosage, etc) employed to test TBZ in one species (Wistar rats, not mice) for direct bone-marrow cytogenetic damage (="clastogenic activity") do not substitute for inadequate procedures (at the least, apparently insufficient dosage) in CD-1 mice to assay for a less sensitive end-point (micronuclei induction).

Item 5: Applicant's rebuttal comments regarding TB's evaluation of the MSD subacute dominant lethal study in CF_1S mice are not accepted, since no additional data have been submitted to support the "suggestion" of adequate TBZ dosage to these mice in this study (by citing "other toxicity studies"), nor indeed to justify (or explain) the lack of a positive control. Hence this study remains UNACCEPTABLE.

B. TB SUMMARY OF IN VITRO CYTOGENETIC STUDY (DER ATTACHED):

Provisionally acceptable, subject to submitting additional data (see DER).

Irving Mauer, Ph.D., Geneticist
Toxicology Branch
Hazard Evaluation Division (TS-769)

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Attachment (DER)

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Thiabendazole (TBZ) Caswell 849A

TOXICOLOGY BRANCH
(DER) DATA EVALUATION RECORD

Study Type: Mutagenicity: in vitro cytogenetics in WI-38 cells

Accession Number: 249845

MRID Number: (Not assigned)

Sponsor: Merck, Sharp and Dohme, Rahway, NJ

Contracting Lab: Stanford Research Institute, Menlo Park, CA
(Study #LSC-5436 March, 1977)

Test Material: [Unstated] Presumably technical, but of unstated purity.

Protocol: Log-phase replicate cultures of the human diploid fibroblast cell line, WI-38 were exposed to TBZ for 3 hr in both cytotoxicity (5 log-dilutions, 0.1 through 1000 ug/ml) and cytogenetic assays (same concentrations), as well as to a positive control, 4-nitroquinoline oxide (4NQO, 1 and 3 X 10^{-6} M), and prepared for metaphase analysis according to standardized procedures. Stained, coded microscope slide preparations were scored ("blind") for mitotic indices (1000 cells per dose), and 50 metaphases per slide analyzed for chromosomal aberrations (numerical as well as structural), as well as for unusual morphology ("stickiness," pulverization, etc.). The solvent (and negative control) was 1% dimethylsulfoxide (DMSO). Only one test was performed.

Results: The highest concentration of the test compound (1000 ug/ml) fell out of solution when added to the cell culture medium; at this dose, over 96% of cells initially planted survived the three hour treatment.

Mitotic indices were depressed in the presence of all levels of TBZ (3.9% in DMSO control, decreasing to 0.6% at HDT) as well as 4NQO (1.4 % and 0.2%). Compared to 12% and 48% chromosome damage (breaks, markers) induced by the positive control, no structural aberrations were found in any TBZ-treated culture, up to the limit of solubility. A few TBZ-treated cells displayed "gaps" (1, 3 and 2 at 0.1, 1.0 and 100 X 10^{-6} M), comparable to 4NQO-treatment (2% and 6%).

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Conclusions: TBZ was not clastogenic in this test with (human) WI-38 cells.

TB EVALUATION: This study is provisionally acceptable, subject to the following being submitted:

1. Repeat study results, with any other known solvent(s) for TBZ, to achieve at least 50% cytotoxicity.

2. Numerical (chromosome) count data, as indicated in the protocol, but not separately scored in the summary tabulation (Report Table 9).

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