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

007541

OCT 13 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Thiabendazole - Mutagenicity Study Submitted in Response to Data Call-In Notice (MRID No. 41170103)
EPA ID No. 618-67

TOX Chem No.: 849A
IB Project No.: 9-2052
RD Record No.: 250,451

FROM: Irving Mauer, Ph.D., Geneticist 09/27/89
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Franklin D. Rubis, PM Team 50
Special Review and Reregistration Division (H7508C)

THRU: Karl Baetcke, Ph.D., Chief 10/6/89
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Registrant: Merck, West Point, PA

Request

Review and evaluate the following assay to satisfy data requirements for DNA damage repair: Thiabendazole: In Vitro Alkaline Elution/Rat Hepatocyte Assay, performed at the Merck Sharp & Dohme Research Laboratories, Project TT No. 89-8312, Final Report dated May 19, 1989 (EPA MRID No. 41170103).

TB Conclusion

The study is judged provisionally acceptable, pending submission of historical solvent control data.

ATTACHMENT (DER)

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

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DATA EVALUATION RECORD

I. SUMMARY

MRID (Acc.) No.: 41170103
ID No.: 618-07
RD Record No.: 250,451
Shaughnessy No.: 060101
Caswell No.: 849A
Project No.: 9-2052

Study Type: Mutagenicity - DNA damage/repair in vitro

Chemical: Thiabendazole

Synonyms: TBZ; MK-0360

Sponsor: Merck, West Point, PA

Testing Facility: Merck Sharp & Dohme Research Laboratories
West Point, PA

Title of Report: Thiabendazole: In Vitro Alkaline Elution/Rat Hepatocyte Assay.

Author: George R. Lankas

Study Number: TT#89-8312

Date of Issue: May 19, 1989

TB Conclusions:

Reported negative in alkaline elution assays presumed to measure DNA strand breakage at concentrations up to a precipitating concentration (1.3 mM).

Classification (Core-Grade):

Provisionally acceptable pending submission of historical solvent control data.

II. DETAILED REVIEW

A. Test Material - Thiabendazole (MK-0360)

Description: (Not stated)
Batch (Lot): L-58,216-000,S159
Purity (%): 98.9
Solvent/Carrier/Diluent: Dimethylsulfoxide (DMSO)

B. Test Organisms - Primary hepatocyte cultures prepared from rodent livers

Species: Rat (males only)
Strain: Sprague-Dawley Crl:CD(SD)Br
Weight: Males: 185 g
Source: Charles River

C. Study Design (Protocol) - This study was designed to assess the genotoxic potential (measured as DNA single and double strand breaks) of thiabendazole when administered in vitro to primary rat hepatocytes. A copy of the procedures employed is appended to this DER (from the Appendix of the investigator's FINAL REPORT) as ATTACHMENT A.

A statement affirming compliance with Agency GLPs was provided, as well as a Statement of Quality Assurance measures (inspections/audits).

D. Procedure/Methods of Analysis - The test material was first assayed in a cytotoxicity test employing trypan blue exclusion as a measure of cell viability in cultures exposed for 3 hours at concentrations up to precipitating levels (ca. 1.3 mM) in culture medium (Leibowitz, L-15). Concentrations selected for testing in the main assay were 0.3, 0.7, 1.0, and 1.3 mM, applied for 3 hours to duplicate monolayer cultures of hepatocytes, following which cells were gently scraped from culture dishes and suspended in fresh medium. Cell viability was determined from a small aliquot, and the remainder lysed and fractionated under tetrapropyl ammonium hydroxide, then eluted for fluorometric determinations of DNA according to conventional (published) procedures. Aflatoxin B1 (AFL, 1 uM) in DMSO served as the positive control.

Data from these fractions were transformed into elution slopes, which were then compared to known standards, according to the following criteria for defining

positive results (presumed to measure increased DNA strand breakage):

1. Soluble doses of the test compound must induce a threefold or greater increase than either concurrent or historical* negative control slopes (whichever is greater).

2. This increase in presumed strand breakage should not be associated with significant decreases in cell viability (defined as less than 70% of control values, as determined by trypan blue exclusion), since cytotoxicity per se is reported to induce significant increases in strand breakage.

E. Results - At none of the concentrations tested (0.3 to 1.3 mM) did the test material produce a significant (at least threefold) increase in elution slope relative to concurrent negative control (Report Table 2, appended to this DER as ATTACHMENT B). By contrast, the positive control, AFL, produced a twentyfold increase, indicating that the cells were responding to a known strand-breaking mutagen.

The author concluded that thiabendazole did not induce DNA strand breakage in primary rat hepatocytes exposed to concentrations up to the level of its insolubility in culture medium.

TB EVALUATION

The study appeared to have been conducted according to standardized (published) procedures for this type of genotoxic assay which indicates DNA damage/repair. The assay, however, should have been repeated at least once, preferably with hepatocytes isolated from a female rat of the same strain.

This study is provisionally acceptable providing the statement about the mean elution slope from historical controls is supported by the submission of detailed data from the GLP assays alluded to (i.e., these having been completed in the preceding year).

Attachments

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*Defined by the author as the average of the mean elution slopes for the same control substance (DMSO) in all GLP assays completed within the preceding 12 months. However, no actual data from these studies documenting this assertion were provided in the FINAL REPORT.

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R:55839:Mauer:C.Disk:KENCO:9/26/89:DG:SW:VO:AS

ATTACHMENT A

Materials & Methods

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APPENDIX

Merck Sharp & Dohme Research Laboratories

West Point, PA 19486

Safety Assessment

Genetic Toxicology

Standard Operating Procedure

Volume I

III. Measurements of DNA Strand Breaks in Rat Hepatocytes by Alkaline Elution

In Vitro Assay

Effective: July, 1982

Revised: April 1983
May, 1983
October, 1983
February, 1985
July, 1988

Richard D. Storer 9/15/88

Richard D. Storer, Ph.D. Date
Responsible Investigator

ID57/Template
9/13/88

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Pages 8 through 35 are not included.

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____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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