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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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3/28/83

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Henry Jacoby, PM #21
Registration Division (TS-769)

THRU: David Ritter, Acting Section Head
Review Section #1
Toxicology Branch/HED (TS-769) *DLR 3-28-83*

SUBJECT: Thiabendazole (Merteck-340F) - New Mutagenicity Study
and Supplemental Data. CASWELL NO. 849A

Action Requested:

1. Review and evaluation of the in vitro cytogenetic analysis of human WI-38 cells treated with thiabendazole and the registrant's reply to the questions raised in mutagenicity testing by the TB previous review (August 16, 1982).

Recommendation:

The registrant should be apprised of the deficiencies reported for the following mutagenicity studies.

- A. In Vitro Cytogenetic Analysis of WI-38 Cells
SRI Project LSC-5438 Accession No. 247112
- B. Cytogenetic Assay in Rat Bone Marrow Cells
IET Japan Accession No. 247112
- C. Ames Salmonella typhimurium Reverse Mutation Test
USDA Western Regional Res. Center Accession No. 247112
- D. Micronucleus Test in Mouse
MITR TT#768038 Accession No. 247112
- E. Dominant Lethal Test in Mice
MITR TT#76-703-0

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I. Review and Assessment of New Mutagenicity Study: In Vitro Cytogenetic Analysis of WI-38 Cells Treated with Thiabendazole.

Stanford Research Institute, LSC-5436, March 1977

Procedure

Human diploid fibroblasts, WI-38 cells in the log phase of growth, were propagated in basal minimum essential medium with 10% fetal calf serum, 10 units/ml Penicillin, and 100 ug/ml Streptomycin. The WI-38 cells were exposed to five concentrations of Thiabendazole (0.1, 1, 10, 100, 1000 ug/ml) for 3 hours. At the end of the 3-hour exposure, the treated cultures were washed twice with warmed PBS, and fresh culture medium was added for additional incubation of 24 hours. Three hours before harvesting the cells, colchicin (10^{-4} mg/ml) was added into the culture vessel. The mitotic cells were harvested by a brief treatment with trypsin solution, fixed with methanol: acetic acid (3:1), stood at room temperature for 10 minutes, and then centrifuged. The centrifuged cells were washed three times and resuspended with fresh fixative. The air dried slides were prepared for each sample and stained in 2% Aceto-Orcein for observation of the mitotic cells under a phase contrast microscope. The stained slides were coded and scored for both mitotic index and chromosomal aberrations. Mitotic indices were calculated based on 100 cells per slide. At least 50 metaphase cells per sample were analyzed for the evidence of chromosomal aberrations. The negative (solvent only) control and positive control (4NQO- 10^{-6} M) were run concurrently.

Results

1. The highest concentration of the test compound, 1000 ug/ml was selected for the study from the preliminary cytotoxicity study of WI-38 cells.

2. No chromosomal aberrations were observed at the dose levels tested (0.1 through 1000 ug/ml). The negative and positive controls used in the evaluation of the validity of the WI-38 cell assay system gave the expected negative and positive responses respectively.

Evaluation

The evaluation of mutagenic activity of the test compound, Thiabendazole, in the in vitro cytogenetic assay of WI-38 cells cannot be accomplished without supplemental information accompanying the report.

The following inadequacies in the reporting of this study were noted:

1. The described procedures for the preparation of viable WI-38 cells were not clear and must be clarified:
 - a. The source of human diploid fibroblasts WI-38 cell line was not identified. The passage number and cell density of the WI-38 cells at the time of treatment were not given in the report. Logically, prior to use in assay, cells should be standardized to a desired density of viable cells per milliliter and then, seeded quantitatively into each culture vessel.
 - b. CO₂ concentration, temperature, and humidity of the incubator are critical factors in maintaining the proper cell growth and should be included in the report.
2. The described procedures for slide preparation were inadequate to assure the top quality of chromosome spread for aberration analysis.
 - a. The hypotonic treatment procedure was missing in the described method. The hypotonic treatment is intended to cause swelling of the cells and spreading the chromosome. The most commonly used hypotonic treatment is to suspend the cells in 0.07M KCl hypotonic solution at 37 C.
 - b. Cell fixation is almost universally carried out in 3:1 Vol:Vol mixture of absolute methanol and glacial acetic acid. The fixed cells are generally refrigerated for a minimum of 12 hours at 4 C before slide preparation is begun.
 - c. Chromosome slide preparation is generally carried out by resuspending the refrigerated cells in a small volume of fresh fixative (0.5 ml). The fixed cells are then, dropped on labelled slides that have been chilled in ice water through a distance of at least 1-2 feet. Slides are flame-dried, drained for 1-2 minutes, and stained in Giensa (10%) at pH 6.8.

3. The cytogenetic assay was not conducted with metabolic activation. Ideally, cells should be exposed to the test compound both in the presence and absence of an appropriate metabolic activation system. Example of such systems include cofactors supplemented postmitochondrial fractions prepared from the livers of mammals treated with enzyme inducers and primary cultures of mammalian hepatocytes.

4. The appropriate statistical method for the evaluation of chromosomal aberrations was not included in the test design of the in vitro cytogenetic assay.

5. The interpretation of results was not clear and must be clarified. A test compound which produces neither a statistically significant dose-related increase in the number of structural chromosomal aberrations nor a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

II. Review of the Responses to the Questions Raised by the TB Previous Review (August 16, 1982).

Merck Sharp & Dohme Research Laboratories, R.R. Buck & R. Robertson December 30, 1982.

1. Cytogenetic Assay in Rats

The clinical findings and body weight changes from both range-findings and the cytogenetic study of Thiabendazole in rats appear to be adequate. However, there were no explanations to the measurement of any effects on mitosis or cell division which may be influenced by the treatment of the test compound. Because the cell cycle kinetics (G₁, S, & G₂ phases of cell cycle) can be influenced by the treatment of chemical agent, adequate sampling times (Three sampling times spaced within the range of 6 to 48 hours) should be considered in this study.

2. Dominal Lethal Study in Mice (Tox. Div., IET)

The explanations submitted for the repeated oral administration at 600 mg/kg/day (5 successive days) in this study (Epstein's Method 1971) appears to be adequate.

3. Ames Test

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The following inadequacies in the submitted protocol for the Ames Salmonella/Mammalian Microsomal Mutagenicity Test were noted:

a. The bacterial cell suspensions of tester strains at the late exponential phase of growth must be standardized prior to testing.

b. Individual numerical data for checking the tester strain genotypes are required. The specific procedure used in this study to confirm the histidine requirement, deep-rough character, ultraviolet sensitivity as well as the presence of R factor conferring ampicillin resistance of Salmonella tester strains should be included.

4. Micronucleus Test

The response to the questions raised by the TB previous review in this study was inadequate. In order to insure that the oral schedule of Thiabendazole employed was sufficient in this test. The maximum tolerated dose must produce some indication of toxicity such as that evidenced by a change in the ratio of polychromatic to normochromatic erythrocytes. Therefore, the explanation is unacceptable.

5. Dominal Lethal Study in Mice (TT#76-703-0)

No positive control reference substance was provided for the dominal lethal study in mice as previously indicated in TB review. Any compound known to induce dominal lethal in the species being tested is acceptable.

6. In Vitro Cytogenetics Study of Thiabendazole in WI-38 Cells.

See Review I.

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