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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM APR 21 1983

TO: Richard Mountfort, PM #23
Registration Division (TS-767)

THRU: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

SUBJECT: Thidiazuron (DROPP) 2139-122: Review of Protocol for the Human Lymphocytes Cytogenetics (Litton Bionetics Protocol No. 449.1). CASWELL 659A

The submitted protocol for the Chromosome Aberrations in Human Lymphocytes with and without in Vitro Metabolic Activation appears to follow the general guidelines recommended for the in vitro mammalian cytogenetics (EPA 1982 and OECD 1981). However, the following deficiencies are noted:

1. Dose Determination:

Details of procedure used for dose determination were not given and should be clarified. Ideally, maximum dose of a test compound should be determined from the preliminary range-finding study for mitotic delay or lose in growth potential in the stimulated human lymphocytes test system (mitogenic stimulation).

2. Treatment of Cultures with the Test Compound:

Human lymphocyte cells in the exponential phase of growth should be tested with the test compound in the presence of an S9 rat liver activation system for two hours at 37 C in the growth medium containing no fetal calf serum. Statements of justification for the selection of one hour exposure time under the activation assay should be provided to support the change in the report.

Because active mutagen may provide mitotic delay and aberrations not only at the first but also at succeeding mitoses in culture (M1 & M2 divisions), the total culture time greater than 48-52 hours should be considered.

3. Lymphocyte Fixation:

The described procedure for preparing chromosome slides were inadequate to obtain a top quality cytological preparation for chromosome analysis. The following deficiencies are noted:

a. The hypotonic treatment is critical to cause swelling of the cells and spreading of the chromosomes. The length of time that the cells are held in the hypotonic solution depends on the size of cell pellets.

b. Cell fixation is almost universally carried out in 3:1 vol:vol mixture of absolute methanol and glycial acetic acid. The fixation solution should be made fresh each day. Cells are suspended in the fixative and allowed to sit for 20 to 30 minutes at room temperature followed by three washes with fresh fixative. The centrifuge tubes containig the fixed cells are regrigerated for a minimum of 12 hours at 4 C before slide preparation is begun.

c. Chromosome slide preparation is carried out by suspending the refrigerated cells in 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, and stained in 10% Giemsa at pH 6.8.

John H. Chen
Review Section #1
Toxicology Branch/HED (TS-769)

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