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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM JUL 22 1983

TO: George LaRocca, PM #15
Registration Division (TS-767)

THRU: Robert B. Jaeger, Section Head 7/22/83
Review Section #1
Toxicology Branch/HED (TS-769)

SUBJECT: Lindane 20954-Q: Review of Protocol for the In Vivo Sister Chromatid Exchange Assay in CFI-Mouse Bone Marrow Cells (RCC Draft Protocol, Itingen/Schweiz). CASWELL #527

The in-vivo sister chromatid exchange assay used for detecting the ability of test compound to enhance the exchange of DNA between two sister chromatids of a duplicating chromosome in mice appears to follow the general guidelines recommended for the sister chromatid exchange assay in mammalian cell(EPA 1982 and OECD 1981). However, the following deficiencies are noted:

1. The described method for the Bromodeoxyuridine (BrdU) tablet implantation was not clear and must be clarified. The technique is intended to release a slow constant concentration of BrdU in test animal throughout the desired time interval. Therefore, the method should assure the BrdU pellets of equivalent hardness and uniform shape before use, and also include the procedure for subcutaneous implantation of BrdU to the laboratory animals.

2. The described procedures used for slide preparation were not adequate to assure the top quality of chromosome spread for sister chromatid exchange analysis.

a. The hypotonic treatment (0.075 M KCl at 37° C) is intended to cause swelling of the cells and spreading of the chromosomes. If the hypotonic treatment is too long or too short, chromosomes will not be spread properly.

b. Cell fixation is normally carried out in three changes of 3:1 methanol: glacial acetic acid, and refrigerated overnight at 4° C. The fixed cells are dispensed on chilled, grease-free, wet microscope slides. Flame ignition of the fixative is used to aid in spreading chromosomes. Differentiation of the sister chromatid is achieved by the fluorescene-plus-giemsa staining technique (Perry et al., 1974). Caution should be taken to handle the 33258 Hoechst stained cells in darkness in order to minimize the photolysis of BrdU containing DNA.

3. Although mentioned in the procedure sheet, the method used for statistical analysis of data was not included in the protocol. Choice of analyses should consider tests appropriate to the experimental design.

4. The interpretation of results was not clear and must be clarified. A test substance which produced neither a statistically significant dose-related increase in the number of sister chromatid exchange nor a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this sytem.

John H.S. Chen

John H.S. Chen, D.V.M.
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