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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 083301, Grotan, Unscheduled DNA Synthesis Study

Tox. Chem. No.: 481C
DP Barcode #: D197352
Record No. : S454567

FROM: Melba S. Morrow, D.V.M. MSM 5/12/94
Review Section II, Toxicology Branch I
Health Effects Division (H7509C)

TO: Bonnie Adler/ Kathryn Davis, PM 52
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THRU: Joycelyn E. Stewart, Ph.D. KBJ 5/22/94
Head, Section II
Toxicology Branch I
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EXECUTIVE SUMMARY:

Although Grotan was reportedly negative for unscheduled DNA synthesis (UDS) in this study, equivocally positive trends in both mean net nuclear silver grain counts and in the percentage of cells in repair were obtained (MRID 430200-01). It is recommended that a repeat assay be conducted in order to resolve this equivocation. In repeating the assay, an attempt should be made to titrate dosages in a finer sequence in order to recover cultures treated at a higher concentration for evaluation of UDS. In addition, it is recommended that hepatocytes from female rats also be tested and the purity of the test material be provided.

The study is unacceptable. Copies of the DER are provided for your reference.

Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (7509C)
Secondary Reviewer: Karl D. Baetcke Ph.D., Chief
Toxicology Branch-I, HED (7509C)

05-12-94

5/23/94

DATA EVALUATION RECORD

MRID No.: 430200-01
PC No.: 083301
RD Record No.: S454567
EPA ID No.: 083301
Tox Chem. No.: 481C
Project No.: D197358

I. SUMMARY

STUDY TYPE: (84-4) Other genotoxicity---DNA damage/repair in vitro (HPC/UDS)

CHEMICAL: Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine.

SYNONYMS: GROTAN®

SPONSOR: Triazine Joint Venture, Buffalo Grove, IL

TESTING FACILITY: Microbiological, Rockville, MD

TITLE OF REPORT: Unscheduled DNA Synthesis in Rat Primary Hepatocytes.

AUTHOR(S): R. H. C. San and H. A. Raabe

STUDY NUMBER: TC836.380

DATE ISSUED: April 23, 1993

EXECUTIVE SUMMARY : Although reportedly negative for UDS in this replacement study (for a prior assay, TC102.380,7/20/88---MRID 41262301, judged UNACCEPTABLE, since it lacked confirmation for a positive result at the HDT---HED Doc. No. 008045), a positive (dose-dependent) trend was evident in cultures treated at 0.05 through 0.2 ug/ml, but which did not reach the criterion for significance proposed by the investigators, namely, increase of 5+ counts over control.

TB-I EVALUATION: TB concludes this study is equivocally (presumptive) positive, but UNACCEPTABLE until repeated at higher concentrations (See TB Evaluation.) In addition, purity of test article should be provided.

II. DETAILED REVIEW

A. TEST MATERIAL: Hexahydro-1,3,5-tris (2 hydroxy-ethyl/-s-triazine

Description: Straw-colored liquid
Batches (Lots): [Not provided]
Purity (%): [Not provided]
Solvent/carrier/diluent: Serum-free TC Medium WME

B. TEST ORGANISM: Primary cell cultures (hepatocytes)

Species: (From) Fischer-344 rats (normal adult males)
Source: Harlan Sprague-Dawley, Frederick, MD

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the genotoxic potential of the test article, when administered in vitro to rat hepatocyte culture, and determining unscheduled DNA synthesis radioactively, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following preliminary cytotoxicity testing (by lactate dehydrogenase, LDH, release determinations), triplicate coverslip cultures (per dose) of rat hepatocytes were exposed for 18-20 hours to a series of nine concentrations of test article (ranging from 0.01 to 0.30 ug/ml), together with a constant dose of tritiated thymidine (^{3}HTdr, 10uCi/ml). Other cultures were treated with 7, 12-dimethylebenz(a)anthracene (DMBA, 3 and 10 ug/ml), to serve as positive controls. After such treatment, the coverslip cultures were treated with sodium citrate (1%, to swell cells), fixed in methanol-glacial acetic acid, and mounted cell-side out onto standard glass microscope slides. After drying, slides were coated (under darkroom conditions) with photographic emulsion (NTB-2), and stored under refrigeration in light-tight boxes. After seven days storage, the silver grains on the slides were developed with standard photographic solutions (D19, Kodak Fixer), and stained with H&E buffered by sodium acetate.

Coded slides were read by an automated colony counter; nuclear and cytoplasmic silver grains were counted in 50 cells per slide, and net nuclear counts (NNC) calculated (crude nuclear count less mean of three cytoplasmic counts), and averaged for each treatment. In addition, the percent cells in repair was recorded for each dose level. Means, standard deviations and percent survival were computed using a Lotus 1-2-3 Program on an IBM-PC. The grain count results represent unscheduled DNA synthesis.

Conventional criteria for assessing both validity of the assay as well as genotoxic responses were stated to have been followed.

E. RESULTS: The preliminary cytotoxicity test demonstrated that the test article produced dose-related relative toxicities (based on LDH release) from >80% at 1.0 ug/ml down to 1% at 0.1 ug/ml (Report Table 1). Hence the HDT selected for the UDS assay itself was 0.3 ug/ml (at which relative toxicity was 79%).

Examination of the fixed and stained cells which had been treated at doses of 0.30, 0.25, 0.20 and 0.15 ug/ml revealed that these could not be evaluated for UDS "because of excessive cytotoxicity"[1], resulting in relative cell survivals less than 35% (Report Table 2). Although none of the remaining (lower) test article doses (0.01 to 0.10 ug/ml) caused a significant increase in mean NNC, according to the authors' criteria established for declaring a positive result (i.e., an increase of at least five counts over solvent control), there was a dose-related increased trend in both mean NNC, as well as percentage of cells showing DNA repair (Report Table 3---attached here). On the other hand; DMBA-treated cultures showed a definitively significant mean NNC (>5 counts) in 99% of cells in repair.

Hence, the authors concluded that the test article was not considered positive according to their criterion for a genotoxic response.

F. TB EVALUATION: Since equivocally "positive" (dose-related) trends in both mean net nuclear silver grain counts; as well as in percentage of cells in repair have been obtained in this singular assay (although not attaining this lab's criterion), a repeat assay is

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[1]However, such "cytotoxicity" was insufficiently characterized by the investigators.

needed to resolve this equivocation. It is recommended that an attempt be made to titrate dosages in a finer sequence in order to recover (viable) cultures treated at higher concentrations for evaluation of UDS. Further, it is recommended that hepatocytes from a female rat be also tested.

ATTACHMENT: Data Tables

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Disk 11:430200.01:MAUER:MB

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Page_____ is not included in this copy.

Pages 7 through 9 are not included.

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