5/8/1992

CASWELL FILE

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

03-05-92

5/8/92

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DATA EVALUATION RECORD

I. SUMMARY

MRID No.: 404662-02
PC No.: 080805
RD Record No.: S-402592
EPA ID No.: 080805
Tox Chem. No.: 097
Project No.: 1-2485

Study Type: (84-2) Mutagenicity - Forward mutation in mammalian cell cultures (L5178Y/TK)

Chemical: Prometryn technical

Synonym: G-34-161

Sponsor: Agricultural Division, Ciba Geigy Corporation, Post Office Box 18300, Greensboro, NC 27419

Testing Facility: Ciba Geigy Limited, Agricultural Division, Basel, Switzerland

Title of Report: Mouse Lymphoma Mutagenicity Test

Author: P. Beilstein

Study Number: 831384

Date Issued: December 6, 1985

Conclusions:

Reportedly negative for inducing forward mutation at the thymidine kinase locus ($TK^{+/-}$ to $TK^{-/-}$) of mouse lymphoma (L5178Y) cells exposed with/without activation up to a stated nontoxic HDT, 100 ug/mL.

TB-I Evaluation

UNACCEPTABLE, as not tested up to cytotoxicity or precipitation (solubility), or absent these, to 1000 ug/mL.

II. DETAILED REVIEW

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A. Test Material - G 34 161 techn.

Description: [Not stated]
Batch (Lot): 1045
Purity (%): 100
Solvent/carrier/diluent: Ethanol

B. Test Organism: Established mammalian cell line

Species: Mouse (lymphoma)
Strain: L5178Y ($TK^{+/-}$)
Source: Dr. D. Clive, Burroughs-Wellcome, RTP (NC)

C. Study Design (Protocol) - This study was designed to determine the mutagenic potential of prometryn when administered to mouse lymphoma cell cultures, and measuring forward mutation at the thymidine kinase locus ($TK^{+/-}$ to $TK^{-/-}$), according to recognized (referenced) procedures .

Statements of Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were both provided.

D. Procedures/Methods of Analysis - Following preliminary toxicity (dose-selection) testing (seven concentrations of test article ranging from 1.563 to 100 ug/mL), multiple cultures of $TK^{+/-}$ cells ("cleansed" of spontaneous $TK^{-/-}$ mutants by pretreatment with THMG, a combination of thymidine, hypoxanthine, methotrexate, and glycine) were exposed for 4 hr to six preselected dose levels of prometryn, both in the absence and presence of a mammalian metabolic activation system consisting of the microsomal preparation (S9) of liver homogenates from male RA1 rats pretreated with Aroclor 1254, plus NADP(N)-generating co-factors. Concurrently, parallel cultures treated with the solvent (0.1% ethanol) as negative control, plus the mutagens, ethylmethane sulfonate (EMS, 0.75 ug/mL) and dimethylnitrosamine (DMN, 4.0 ug/mL), as positive controls for, respectively, non-activated and S9-supplemented test series.

Following treatment, all cultures were washed, and re-incubated for 3 days (for expression of mutants), during which time cell counts were recorded daily. At the end of this expression period, cells were cloned (in medium containing bromdeoxyuridine, BUdR, 50 ug/mL, to select for $TK^{-/-}$ mutants and against any surviving wild-type cells) for 16 days. After such selection, cell colonies

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were counted (by Fisher's "Count-All," Model 600), and mutation frequency (MF) expressed as the number of induced $TK^{-/-}$ mutants per 10^6 surviving cells.

A test substance is considered positive in this assay if colony counts exceed solvent control values by a factor of 2.5 or more at any concentration.

E. Results: In preliminary dose-selection (cytotoxicity) testing up to 100 ug/mL, no toxicity was recorded without activation, and only slightly reduced relative cell growth (70-80% of solvent control) in the presence of S9 (Report Table 1, attached to this DER). Thus 100 ug/mL was selected as the HDT for the (main) mutagenicity series, accompanied by six lower doses.

In the [single] main assay without activation (-S9), no increased MFs were found at any concentration up to the HDT (Report Tables 2 and 3, as summarized in Table 4, attached to this DER). In the presence of S9, no induced mutagenicity was recorded at any of the doses tested, according to the 2.5 times-factor set up as critical for a positive test response (Report Tables 5 and 6, as summarized in Table 7, also attached here). By contrast, both positive controls responded to their respective mutagens, 3.5 to 20 times solvent control values.

Hence, the investigators concluded that prometryn did not induce ". . . a mutant factor greater than 2.5 . . . at any of the concentrations tested," i.e., may be considered negative for mutagenicity at the TK locus in L5178Y cells under conditions of this test.

F. TB Evaluation: NOT acceptable. Nowhere in this report is there any justification for the selection of 100 ug/mL--an obviously (relatively) non-toxic concentration--as the highest dose to be tested, namely, absent information and/or data on the nature (physical description) or solubility characteristics of the test article (why ethanol as solvent, and not for example, DMSO). The limit dose for satisfying FIFRA data requirements in this type of assay in the absence of cytotoxicity or solubility is 1000 ug/mL.

Attachment (Data Tables)

RIN # 0615-00

MRID # 40466202 (DERs)

Page _____ is not included in this copy.

Pages 4 through 6 are not included.

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