DATA EVALUATION RECORD

8/9/2000

MESOTRIONE (ZA 1296)

Study Type: §84-2; L5178Y TK +/- Mouse Lymphoma Cell/Mammalian Activation Forward Mutation Assay

Work Assignment No. 2-01-52 HH (MRID 44373525)

Prepared for

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Disclaimer

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HH

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Registration Action Branch 1, HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma cells
OPPTS Number: 870.5300 OPP Guideline Number: §84-2

DP BARCODE: D259369 SUBMISSION CODE: S541375
P.C. CODE: 122990 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): ZA 1296 (mesotrione, 98.1% a.i)

SYNONYMS: 2-[4-(Methylsulphonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione

CITATION: Clay, P. (1994). ZA 1296: Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells. Zeneca Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/P/4183. Laboratory Study No. VV0098. February 28, 1994. MRID 44373525. Unpublished.

SPONSOR: Zeneca Inc., Agricultural Products, Wilmington, DE

EXECUTIVE SUMMARY: In a mammalian cell forward gene mutation assay at the thymidine kinase locus (MRID 44373525), L5178Y TK +/- mouse lymphoma cells cultured in vitro were exposed in repeat (independent) assays to ZA 1296 (98.1% a.i.) in dimethylsulfoxide (DMSO) at concentrations ranging from 125 to 1000 µg/mL with or without S9 activation provided by phenobarbital/beta naphthoflavone-mix. ZA 1296 was tested up to a dose slightly in excess of the limit of solubility. The highest concentrations evaluated for mutagenicity were based on a cell survival of ≥10% compared to the vehicle control. There were no treatment-related increases in mutant frequency in the presence or absence of S9 activation. The positive controls gave the appropriate responses in both trials. **ZA 1296 was negative for inducing forward mutations at the TK locus in mouse L5178Y cells with or without S9 activation.**

This study is classified as **acceptable (§84-2)**, and satisfies the FIFRA Test Guideline requirements for *in vitro* mammalian forward gene mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

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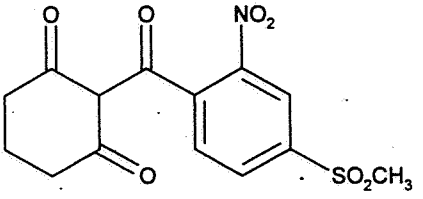
I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: ZA 1296
   Description: Light beige solid
   Lot/Batch #: P6
   Purity: 98.1% a.i.
   Stability of compound: Not specified. It was stated that the sample was used within the stated expiration date, based on information from the Sponsor.
   CAS #: 104206-82-8
   Structure (Not present in report; presumably copied from independent source):



   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: Serial dilutions with DMSO were made from a stock solution.

2. Control Materials:
   Negative (vehicle): DMSO
   Vehicle/final concentration: DMSO/10 µL/mL in culture medium

   Positive:
   Nonactivation: Ethyl methanesulphonate (EMS) in DMSO was used at a concentration of 750 µg/mL.
   Activation: N-nitrosodimethylamine (NDMA) in DMSO was used at a concentration of 600 µg/mL.

3. Activation: S9 derived from male Alderley Park (Alpk:APfSD) albino rats:

____ Aroclor 1254 ____ phenobarbital ____ none __x__ phenobarbital/ ß-naphthoflavone	__x__ induced ___ non-induced	__x__ rat ___ mouse ___ hamster ___ other	__x__ liver ____ lung ____ other

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The S9 fraction was prepared from the livers of Alderley Park (Alpk:APfSD) albino rats. The S9-cofactor solution consisted of NADP (0.75 mM) and glucose-6-phosphate (12.0 mM) in RPMI 1640 Medium (adjusted to pH 7.5). The final concentration of S9 in culture was 5%.

4. Test Cells: Mammalian cells in culture
   _x_ mouse lymphoma L5178Y cells
   ___ Chinese hamster ovary (CHO) cells
   ___ Chinese hamster lung (V79) fibroblasts
   ___ other (list):

   Properly maintained? **Yes**
   Periodically checked for mycoplasma contamination? **Yes**
   Periodically checked for karyotype stability? **Not specified**
   Periodically "cleansed" against high spontaneous background? **Not specified**

   Media: RPMI 1640 medium with Hepes supplemented with 4 mM L-glutamine, 200 U/mL penicillin and streptomycin, and 10% horse serum was used as growth medium and during the expression phase. During treatment, the horse serum component was reduced to 5%. Serum concentration was increased to 20% whenever cells were dispensed into microwells. Selection medium was growth medium containing 4 µg/mL 5-trifluorothymidine (TFT).

5. Locus Examined:
   _x_ thymidine kinase (TK)
   Selection agent: ___ bromodeoxyuridine (BrdU)
   (give concentration) ___ fluordeoxyuridine (FdU)
   4 µg/mL trifluorothymidine (TFT)

   ___ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
   Selection agent: ___ 8-azaguanine (8-AG)
   ___ 6-thioguanine (6-TG)

   ___ Na+/K+ ATPase
   Selection agent: ___ ouabain

   ___ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used

   a. Preliminary Cytotoxicity Assay:
   Nonactivated and activated conditions: A preliminary cytotoxicity assay was conducted with and without S9 activation using a range of concentrations (unspecified) of ZA 1296. Vehicle controls were included.

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b. Mutation assays:
Nonactivated and activated conditions: 125, 250, 500, and 1000 µg/mL

In each assay, duplicate cultures were used for each dose level and condition, including the vehicle and positive controls.

B. TEST PERFORMANCE

1. Cell Treatment:

a. Cells were exposed to test compound, negative/vehicle or positive controls for: 4 hours (nonactivated and activated). To assess survival, a sample of each culture was diluted (8 cells/mL) immediately post treatment, dispensed into two 96-well micro well plates (1.6 cells/well), and incubated for 10-12 days.

b. After treatment and washing, the cells were cultured for 3 days (expression period) before cell selection. Cell densities were determined daily and were adjusted to appropriate densities to maintain optimal growth rates.

c. After expression, 1×10^4 cells/mL from each post-expression culture were added to four 96-well micro well plates (2000 cells/well) containing selection (TFT) medium and incubated for 10-12 days to determine numbers of mutants. Cell samples from each post-expression culture were grown in two 96-well micro well plates (1.6 cells/well) without TFT for 10-12 days to evaluate cloning efficiency.

Cloning efficiency was evaluated by scoring the number of wells containing no colonies. Mutation plates were scored for large (average diameter >25% well diameter with less densely packed cells, especially around edges of colony), small (average diameter <25% well diameter, usually ~15% well diameter, with dense colonal morphology), or no colonies. A well with more than one small colony was scored as a small colony. A well with more than one large colony or a large and small colony was scored as a large colony.

The following calculations were made:

$$P(0) = \frac{\text{number of negative wells}}{\text{total wells plated}}$$

$$\text{Plating Efficiency (PE)} = \frac{-\ln P(0)}{\text{number of cells per well}}$$

$$\text{0-hour survival} = \frac{\text{PE for a specific treatment}}{} \times 100$$

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PE total for negative control plates

$$\text{Mutant Frequency (MF)} = \frac{\text{PE in selective medium (mutation)}}{\text{PE in non-selective medium (viability)}}$$

2. Statistical Methods: Statistical analysis was not performed.

3. Evaluation Criteria:

   a. Assay validity: The assay was considered valid if both of the following criteria were met: (i) the spontaneous mutant frequencies in the presence and absence of S9 were within 0.8-6.0 x 10^{-4} mutants per survivor, and (ii) the positive controls induced unequivocal positive responses.

   b. Positive result: The test article was considered mutagenic if both of the following criteria were met: (i) a reproducible, dose-related increase in mutant frequency was observed over a range of doses, not only at dose levels eliciting excessive toxicity (i.e. <10% survival), and (ii) an associated absolute increase in mutant number was observed above the negative control values.

II. REPORTED RESULTS

Analysis of the dose formulations for stability, homogeneity, and achieved concentrations was not performed.

A. Preliminary Cytotoxicity Assay: A preliminary cytotoxicity assay was conducted with and without S9 activation using a range of concentrations (unspecified) of ZA 1296. Minimum precipitation of the test article was observed at 1000 µg/mL. Based on the results of the preliminary assay, 1000 µg/mL was selected as the highest concentration with and without S9 activation for both mutation assays.

B. Mutagenicity Assay: In two trials, ZA 1296 was tested with and without S9 activation at four concentrations ranging from 125 to 1000 µg/mL. Mean % relative survival in the two trials without S9 ranged from 93 to 109% and with S9, from 97 to 128%. In the presence or absence of S9 activation, there were no reproducible, dose-related increases in mutation frequency over negative controls. Mean mutation frequencies in the two trials ranged from 0.8 to 2.1 x 10^{-4} compared to 1.4 to 1.9 x 10^{-4} in the vehicle controls (-S9) and from 1.0 to 2.0 x 10^{-4} compared to 1.2 to 1.3 x 10^{-4} in the vehicle controls (+S9). The positive controls, EMS and NDMA, induced substantial increases in mutant frequency in both trials. The distribution of colony sizes in both trials (±S9) was similar to the vehicle controls. Summaries of the mutagenicity assay results (study report Tables 1 and 2, pages 17 and 18) are presented as an attachment to this DER.

III. DISCUSSION/CONCLUSIONS:

A. Investigator's Conclusions: The investigator concluded that under the conditions of this mouse lymphoma forward mutation assay, ZA 1296 was not considered to be mutagenic in the presence or absence of S9 activation.

B. Reviewer's Discussion: ZA 1296 was tested for mutagenicity at concentrations ranging from 125 to 1000 µg/mL with or without S9 activation. The test substance was tested up to a dose just in excess of the limit of solubility under both conditions. Relative survival was unaffected by treatment. The reviewers agree with the investigator's conclusion that under the conditions of this study, ZA 1296 was negative for mutagenicity with or without S9 activation. The positive controls gave the appropriate responses.

C. Study Deficiencies: The following deficiencies were noted, but would not alter the conclusions of this study: (i) the dose preparations were not analyzed for stability, homogeneity, or achieved concentrations; however, ZA 1296 was tested at concentrations up to the limit of solubility in culture medium with and without S9 activation (125-1000 µg/mL), and (ii) it was not reported whether the cells were periodically checked for karyotype stability or cleansed against high spontaneous background.

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APPENDIX

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