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GLYCOLIC ACID (70.58% a.i.) (§ 84-2) MAMMALIAN CELLS IN CULTURE/GENE MUTATION

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DATA EVALUATION RECORD

STUDY TYPE: Mammalian Cells in Culture Gene Mutation Assay in L5178Y TK$^{+/-}$ Mouse Lymphoma Cells; OPPTS 870.5300; (§ 84-2)

DP BARCODE: D261705
P.C. CODE: 000101

SUBMISSION CODE: S571941
EPA ID No.: 071654-R

TEST MATERIAL (PURITY): Glycolic acid (70.58% a.i.)

SYNONYMS: Hydroxyethanoic Acid 70% Solution;
Acetic acid, Hydroxy- 70% Solution

CITATION: Cifone, M.A. (1998) Glycolic acid 70% solution: L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay with a confirmatory assay. Covance Laboratories Inc.(Covance), 9200 Leesburg Pike, Vienna, Virginia 22182. Laboratory Project ID: DuPont-1616, Covance Study No.: 19634-0-431OECD, Medical Research Project No.: 12151, October 15, 1998. MRID 44975306. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, Delaware 19898

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the TK locus (MRID 44975306), L5178Y TK$^{+/-}$ mouse lymphoma cells cultured *in vitro* were exposed to Glycolic acid (Batch No. not provided, 70.58% a.i.) at concentrations of 39.3, 78.5, 157, 313, 625, 1250, 2500 and 5000 µg/mL in the absence of mammalian metabolic activation (S9-mix) and at concentrations of 250, 500, 1000, 2000, 2500, 3000, 4000 and 5000 µg/mL in the presence of S9-mix. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Glycolic acid was tested up to a limit concentration of 5000 µg/mL. No cytotoxicity was seen in a preliminary test with ten concentrations of Glycolic acid ranging from 9.85 to 5000 µg/mL, with and without S9-mix. Minimal cytotoxicity was seen both with and without S9-mix in two mutagenicity assays. The average

relative growth of the solvent controls was approximately 99% with or without S9-mix in both assays. The average relative growth of Glycolic acid treated cultures was approximately 50% at 5000 µg/mL with or without S9-mix in both assays.

In the initial mutation assay, both with or without S9-mix, a positive response [two-fold or greater increase in mutant frequency compared to the solvent control value] was seen only at 5000 µg/mL. The mutant frequency showed a 4.6X increase with S9-mix and a 2.9X increase without S9-mix compared to the controls. The positive response seen in the absence of S9-mix in the initial assay was not reproduced in the confirmatory assay. However, the positive response seen with S9-mix in the initial assay was also seen in the confirmatory assay. A positive dose-response increase compared to the control was obtained in the 4 dose range of 2500 µg/mL (32.9 mM)[2.02X] through 5000 µg/mL (65.8 mM) [4.59X]. The mutant colonies were predominantly small colonies, indicating a clastogenic mechanism of action. Positive and solvent controls gave the appropriate response.

Although Glycolic acid was mutagenic in the presence of S9-mix as tested in this study, mutagenic activity was only seen at concentrations 3 to 6X above the maximum testing concentration recommended by the EPA guidelines for this assay (10 mM). **For regulatory purposes, therefore, Glycolic acid, was not considered to be a mutagen.**

This study is classified as **Acceptable** and satisfies the requirement for FIFRA Test Guideline, OPPTS 870.5300 (§ 84-2) for *in vitro* mammalian forward gene mutation data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glycolic acid 70% a.i. solution

Description: pale-yellow liquid
Lot/Batch #: not provided
Purity: 70.58% a.i.
Stability of compound: stable
CAS #: 79-14-1
Structure: not provided, molecular formula - $HO-CH_2-COOH$.
Solvent used: Fischer's medium for mutation assay, water for cytotoxicity assay

2

Other comments: Known impurities - 0.42% formic acid, 0.47% methoxyacetic acid, 0.70% diglycolic acid

2. Control materials

Negative: none
Solvent/final concentration: 10% Fischer's medium
Positive (concentrations/solvent):
Nonactivation: Methyl methanesulfonate / 6.5 and 13 µg/mL / not specified
Activation: 3-Methylcholanthrene / 2.0 and 4.0 µg/mL / not specified

3. Activation: S9 derived from male Sprague-Dawley rats

_x_ Aroclor 1254 _x_ induced _x_ rat _x_ liver

S9 mix composition:

S9 homogenate (unspecified commercial source)	10 µL/mL
NADP, sodium salt	3 mM
Isocitrate	15 mM

4. Test cells: mammalian cells in culture

_x_ mouse lymphoma L5178Y cells
___ Chinese hamster ovary (CHO) cells
___ V79 cells (Chinese hamster lung fibroblasts)
___ other (list):

Properly maintained? Y
Periodically checked for Mycoplasma contamination? Y
Periodically checked for karyotype stability? Y
Periodically "cleansed" against high spontaneous background? Y

Media: Culture medium was RPMI 1640 supplemented with horse serum (10% by volume), Pluronic® F68, L-glutamine, sodium pyruvate, penicillin and streptomycin. Treatment medium was Fischer's medium with the same supplements as culture medium except the horse serum was reduced to 5% by volume. Cloning medium was RPMI 1640 growth medium with up to 20% horse serum, without Pluronic® F68 and with the addition of 0.24% BBL® agar. Selection medium was cloning medium containing 3 µg/mL of TFT.

5. Locus Examined:

__x__ thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
__3 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

6. Test compound concentrations used:

Preliminary cytotoxicity assay:

Nonactivated and activated conditions: 9.85, 19.7, 39.3, 78.5, 157, 313, 625, 1250, 2500 and 5000 µg/mL

Mutagenicity assays:
Initial:
[Nonactivated and activated conditions]: 39.3, 78.5, 157, 313, 625, 1250, 2500, 5000 µg/mL

Confirmatory:
[Nonactivated and activated conditions]: 250, 500, 1000, 2000, 2500, 3000, 4000, 5000 µg/mL

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound, negative/solvent or positive controls for:
__4__ hours (nonactivated) __4__ hours (activated)

b. After washing, cells cultured for __2__ days (expression period) before cell selection:

c. After expression, __1 x 10^6__ cells/dish (__3__ dishes/group) were cultured for __12-13__ days in selection medium to determine numbers of mutants and __200__ cells/dish (__3__ dishes/group) were cultured for __12-13__ days without selective agent to determine cloning efficiency.

4

2. Statistical methods: No statistical analysis was performed.

3. Evaluation criteria: The mutant frequency, expressed as 10^{-6} units (number of mutants per 10^6 viable cells), was determined for each experimental point. The size distribution of mutant colonies (small and large colonies) was also reported for each experimental point. The results were considered positive if the test material induced a dose-dependent increase in mutant frequency to a value at least twice that of the solvent control. The dose-relationship ideally should cover at least three doses but this was not an absolute requirement for a positive determination as choice of dose steps and cytotoxicity could prevent a three-step response. A dose-response was not required if a 4-fold or higher increase in mutant frequency was seen for a single dose at or near the highest testable toxicity. The result must be repeatable.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

Ten concentrations of Glycolic acid ranging from 9.85 to 5000 µg/mL were tested, with and without S9-mix, in the preliminary cytotoxicity assay. Cells were treated for four hours. Glycolic acid was not cytotoxic at any tested concentration, with or without S9-mix; therefore, 5000 µg/mL was selected as the upper concentration for the mutagenicity assay. Results of the cytotoxicity assay are presented in Appendix Table 1 (MRID 44975306, p. 30, attached).

B. MUTAGENICITY ASSAY

Two mutation assays were conducted using one culture per dose, three dishes per culture. In the initial assay, eight concentrations of Glycolic acid ranging from 39.3 to 5000 µg/mL were tested with and without S9-mix and in the confirmatory assay, eight concentrations ranging from 250 to 5000 µg/mL were tested with and without S9-mix. Minimal cytotoxicity was seen both with and without S9-mix in both assays. The average relative growth of the solvent controls, defined as (relative suspension growth x relative cloning efficiency)/100 was approximately 99% with and without S9-mix in both assays. The relative growth of Glycolic acid treated cultures ranged from 100.9% to 57.0% in the initial assay with S9-mix, from 87.4% to 46.0% in the

initial assay without S9-mix, from 99.2% to 52.9% in the confirmatory assay with S9-mix and from 110.7% to 74.2% in the confirmatory assay without S9-mix.

In the initial mutation assay, a positive response was seen at 5000 µg/mL, both with and without S9-mix. The mutant frequency was 334.5 x 10^{-6} with S9-mix compared to the average solvent control value of 72 x 10^{-6}, a 4.6 fold increase and 150.4 x 10^{-6} without S9-mix compared to the average solvent control value of 51.1%, a 2.9 fold increase. Both increases exceeded the criterion of a two-fold increase for a positive response. Mutant frequencies at lower concentrations did not reach a two-fold increase over solvent control values although the mutant frequency at the second highest concentration with S9-mix, 2500 µg/mL, approached the two-fold limit with an increase of 1.9 fold. Solvent and positive control values were within the testing laboratory's historical control ranges.

The positive response seen in the absence of S9-mix in the initial assay was not reproduced in the confirmatory assay where a mutant frequency at 5000 µg/mL of 138.5 x 10^{-6} was seen compared to the average solvent control value of 74.2 x 10^{-6}, a 1.87 fold increase. The positive response seen with S9-mix in the initial assay was reproduced in the confirmatory assay with a four dose positive dose-response from 2500 through 5000 µg/mL. The mutant frequency increased from 194.9 x 10^{-6} at 2500 µg/mL to 442.5 x 10^{-6} at 5000 µg/mL compared to the average solvent control value of 96.4, a 2.02 to 4.59 fold increase. The mutant colonies were predominantly small colonies, indicating a clastogenic mechanism of action. Solvent and positive control values were within the testing laboratory's historical control ranges. Results of the initial mutagenesis assay without activation are presented in Appendix Tables 2 and 3 (MRID 44975306, pp. 31 and 32) and with activation are presented in Appendix Tables 4 and 5 (MRID 44975306, pp. 35 and 36, attached). Results of the confirmatory assay without activation are presented in Appendix Tables 6 and 7 (MRID 44975306, pp. 33 and 34, attached) and with activation in Appendix Tables 8 and 9 (MRID 44975306, pp. 37 and 38, attached).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. Glycolic acid was tested to a limit dose of 5000 µg/mL, proper experimental protocol was followed and the solvent and positive control values

were appropriate. The test material was mutagenic in the presence of S9-mix in both the initial and the confirmatory assays but was not reproducibly mutagenic in the absence of S9-mix. Mutagenic activity was seen at 5000 µg/mL in the initial assay and at concentrations of 2500 µg/mL (32.9 mM) and higher in the confirmatory. The study author points out that mutagenic activity was only seen at concentrations three to six fold above the maximum testing concentration recommended by the EPA guidelines for this assay (5 µl/mL or 10 mM whichever is less) (Federal Register, 62(158): p. 43847).

Glycolic acid was clearly mutagenic as tested in this assay; however, for regulatory purposes the material was not considered to be a mutagen.

This study is classified as **Acceptable**. It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5300 (84-2) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

B. STUDY DEFICIENCIES: No study deficiencies were identified.

7