(11-20-01)

DATA EVALUATION RECORD

KBR3023

STUDY TYPE: MAMMALIAN CELLS IN CULTURE GENE MUTATION ASSAY IN CHINESE HAMSTER V79 CELLS; [OPPTS 870.5300 (§84-2)]
MRID 45231801

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster V79 cells; [OPPTS 870.5300 (§84-2)]

DP BARCODE: D269953
P.C. CODE: 070705

SUBMISSION CODE: S586957
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): KBR 3023 (KBR3023, 98.7% a.i.)

SYNONYMS: 1-Piperidineycarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester

CITATION: Herbold, B. (1999) KBR 3023 V79/HPRT-test *in vitro* for the detection of induced forward mutation. Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Laboratory Project ID: Bayer AG Report No. 29290; Bayer AG Study No. T 8068419, October 2, 1999. MRID 45231801. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the HPRT locus (MRID 45231801), Chinese hamster V79 cells cultured *in vitro* were exposed to KBR 3023, (Batch No. 898711001, 98.7% a.i.) in ethanol in two independent assays. In the presence of mammalian metabolic activation (S9-mix), cells were exposed at concentrations of 400, 600, 800, 1000, 1200, 1300, 1400, 1500 and 1600 µg/mL in both assays and in the absence of S9-mix, at concentrations of 400, 600, 800, 1000, 1200, 1400 and 1600 µg/mL in the first assay and at concentrations of 600, 800, 1000, 1200, 1400, 1500 and 1600 µg/mL in the second assay. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

KBR 3023 was tested up to cytotoxic concentrations with excess cytotoxicity seen at 1600 µg/mL (no culture at this concentration was cloned for mutagenicity evaluation). The solvent control values without S9-mix (0.6 - 1.5 mutants per 10^6 surviving cells) and with S9-mix (0.0 - 3.3 mutants per 10^6 surviving cells) were within the testing laboratory's historical ranges. There were no statistically significant increases in the mutant frequency over these control values at any KBR 3023 concentration in either assay, with or without S9-mix. The positive control values were significantly increased over the respective solvent control values. **There was no evidence of induced mutant colonies over background.**

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

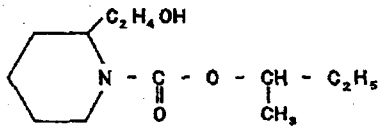
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: KBR 3023

   Description: colorless liquid
   Lot/Batch #: 898711001
   Purity: 98.7% a.i.
   Stability of compound: stable
   CAS No.: 119515-38-7
   Structure:



   Solvent used: ethanol
   Other comments: none

2. Control materials

   Negative: culture medium
   Solvent/final concentration: ethanol / ≤ 1%
   Positive(concentrations/solvent):
   Nonactivation: ethyl methanesulfonate / 900 µg/mL / not specified..
   Activation: DMBA / 20 µg/mL / DMSO

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

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	_ non-induced	_ mouse	_ lung
_ none		_ hamster	_ other
_ other		_ other	

   If other, describe below

   S9 mix composition: (S9-fraction purchased from ICN Biomedicals Inc.)

$MgCl_2$ x $6H_2O$	8 mM

KCl	33 mM
Glucose-6-phosphate	5 mM
NADP	1 mM
S9-fraction	40% (v/v)
Sodium phosphate buffer (pH 7.4)	60% (v/v)

4. Test cells: mammalian cells in culture

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

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

Media: Cells were cultured in hypoxanthine-free Eagle's Minimal Essential Medium (MEM) supplemented with nonessential amino acids, L-glutamine (2 mM), MEM-vitamins, $NaHCO_3$, penicillin (100 units/mL), streptomycin (100 µg/mL) and heat-inactivated fetal calf serum (10% final concentration). The serum level was reduced to 2% final concentration during treatment with KBR 3023. Selection medium was hypoxanthine-free MEM containing 10 µg/mL 6-thioguanine.

5. Locus examined

__ thymidine kinase (TK)
Selection agent: ___ bromodeoxyuridine (BrdU)
(give concentr.) ___ fluorodeoxyuridine (FdU)
___ trifluorothymidine (TFT)

_x_ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: ___ 8-azaguanine (8-AG)
(give concentr. 10 µg/mL 6-thioguanine (6-TG)

__ Na^+/K^+ ATPase
Selection agent: ___ ouabain
(give concentration)

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

6. Test compound concentrations used:

Preliminary cytotoxicity assay:
Nonactivated and activated conditions: 100, 250, 500, 750, 1000, 1500, 2000, 4000 µg/mL

First mutation assay:
Nonactivated conditions: 400, 600, 800, 1000, 1200, 1400, 1600 µg/mL
Activated conditions: 400, 600, 800, 1000, 1200, 1300, 1400, 1500, 1600 µg/mL

Second mutation assay:
Nonactivated conditions: 600, 800, 1000, 1200, 1400, 1500, 1600 µg/mL
Activated conditions: 400, 600, 800, 1000, 1200, 1300, 1400, 1500, 1600 µg/mL

B. TEST PERFORMANCE

1. Cell treatment

a. Cells exposed to test compound, negative/solvent or positive controls for: 5 hours (nonactivated) 5 hours (activated)

b. After washing, cells cultured for 6 days (expression period) before cell selection:

c. After expression, 3×10^5 cells/dish (8 dishes/ group) were cultured for 6-8 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 6-8 days without selective agent to determine cloning efficiency.

2. Statistical methods

The mutant frequencies were analyzed using a weighted analysis of variance and a weighted recursive regression, both with Poisson derived weights. Mutant frequencies based on less than five plates and/or on a relative survival to treatment and/or a relative population growth and/or an absolute cloning efficiency below 10% are not included in the statistical analysis.

All acceptable groups were included in the weighted analysis of variance followed by pairwise comparisons to the solvent control ($\alpha = 0.05$) using the Dunnett test (Dunnett, 1955). The regression analysis was performed on the basis of the actual test material concentrations thereby omitting the positive, negative and solvent controls. If there was a significant concentration related increase of the mutant frequency at $\alpha = 0.05$ in the main analysis, the highest concentration was dropped and the analysis repeated. The process was repeated until $p>0.05$.

3. Evaluation criteria

Colonies were fixed, stained with Giemsa and counted to determine the number of 6-TG resistant colonies in the mutation assay dishes and the number of surviving colonies in the cloning efficiency dishes. The mutant frequency was expressed as the number of 6-TG resistant mutants per 10^6 clonable cells.

To be used in the assessment, mutant frequencies must be based on at least five dishes per culture and relative survival, relative population growth and absolute cloning efficiency must be at least 10%. Criteria for a positive response are a concentration-related, reproducible (within and between independent trials) two to three-fold increase in mutant frequency over the solvent control value. No significant change in osmolality compared to the solvent control can occur in test material treated cultures. Results are considered equivocal if one or more concentrations induce a reproducible and biologically relevant increase in mutant frequency in all trials but in the absence of a positive dose-response.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

Eight concentrations of KBR 3023 ranging from 100 to 4000 µg/mL were tested, with and without S9-mix, in a preliminary cytotoxicity assay with all plating in triplicate. No change in pH of the medium occurred at any test material concentration; however, a precipitate was seen at 4000 µg/mL both with and without S9-mix. KBR 3023 was cytotoxic at concentrations of 1000 µg/mL (absolute cloning efficiency = 49.5%) and higher in the absence of S9-mix and at concentrations of 1500 µg/mL (absolute cloning efficiency = 16.7%) and higher in the presence of S9-mix. The test material was completely cytotoxic at 2000 and 4000 µg/mL with and without S9-mix. An upper concentration of 1600 µg/mL was chosen for the mutation assays both with and without S9-mix. Results of the cytotoxicity test without and with S9-mix are presented in Appendix Tables 1 and 2, respectively (MRID 45231801, pp. 30 and 31). A list of abbreviations used in the tables is included in the Appendix.

B. MUTAGENICITY ASSAY

Two independent mutation assays were conducted using duplicate cultures (eight dishes per culture) at each KBR 3023 concentration. Seven concentrations of KBR 3023 ranging from 400 to 1600 µg/mL were tested without S9-mix in the first assay and seven concentrations ranging from 600 to 1600 µg/mL were tested without S9-mix in the second assay. Nine concentrations of KBR 3023 ranging from 400 to 1600 µg/mL were tested with S9-mix in both assays. In the absence of S9-mix, cytotoxicity (based on growth relative to the solvent control) became apparent at concentrations of 1400 µg/mL and higher. Cytotoxicity was excessive at 1600 µg/mL in both cultures in both assays and also in one of two cultures each at 1400 and 1500 µg/mL in the second assay. Cells were not cloned for mutagenicity evaluation in these cultures. In the presence of S9-mix, both

cultures at 1600 μg/mL in both assays and one culture at 1500 μg/mL in the first assay and both cultures at 1500 μg/mL in the second assay were not cloned due to cytotoxicity.

There were no statistically significant increases in the mutant frequency seen in either assay, with or without S9-mix. The solvent control values without S9-mix (0.6 - 1.5 mutants per 10^6 surviving cells) and with S9-mix (0.0 - 3.3 mutants per 10^6 surviving cells) were within the testing laboratory's historical ranges and the positive control values were significantly increased over the respective solvent control values. Results from the first and second mutation assays without activation are presented in Appendix Tables 3 and 4, respectively (MRID 45231801, pp. 32 and 33). Results from the first and second mutation assays with activation are presented in Appendix Tables 5 and 6, respectively (MRID 45231801, pp. 34 and 35).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an **Acceptable** study. KBR 3023 was tested to cytotoxic concentrations, proper experimental protocol was followed and the negative, solvent and positive control values were appropriate. There was no evidence of a mutagenic effect at any KBR 3023 concentration tested, with or without added mammalian metabolic activation.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

APPENDIX

(MRID 45231801)

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

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