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FINAL

DATA EVALUATION REPORT

SUMILARV

Study Type: Mutagenicity: Gene Mutation in Cultured Chinese Hamster V79 Cells

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
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Contract Number: 68D10075
Work Assignment Number: 1-122
Clement Number: 93-104
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATION

MUTAGENICITY STUDIES

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Review Section IV, Date: 2/10/93
Toxicology Branch I/HED (H-7509C)

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutations in cultured Chinese hamster V79 cells

EPA IDENTIFICATION Numbers:

P.C. Code: 129032

CASWELL Number: None

MRID Number: 421783-16

TEST MATERIAL: Sumilarv

SYNONYMS/CAS Number: S-31183; pyriproxyfen; 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether; 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine; $C_{20}H_{19}NO_3$/95737-68-1

SPONSOR: Sumitomo Chemical Co., Ltd., Osaka, Japan

STUDY NUMBER: 207; Reference Number NNT-00-0067

TESTING FACILITY: Sumitomo Chemical Co., Ltd., Osaka Japan

TITLE OF REPORT: Sumilarv--In Vitro Gene Mutation Test of S-31183 in V79 Chinese Hamster Cells

AUTHOR: S. Kogiso

REPORT ISSUED: April 26, 1990

CONCLUSIONS--EXECUTIVE SUMMARY: Sumilarv (S-31183) at nonactivated doses ranging from 10 to 300 µg/mL and S9-activated doses ranging from 3 to 100 µg/mL was evaluated for the potential to induce forward gene mutation at the HGPRT locus in Chinese hamster V79 cells. Two independently performed assays were conducted. In both trials, the highest nonactivated dose (300 µg/mL) was insoluble and the highest S9-activated dose (100 µg/mL) was cytotoxic. There was, however, no evidence that the test material induced a mutagenic response. We conclude, therefore, that sumilarv (S-31183) was adequately tested and found to be nonmutagenic in this test system.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2a) for genetic effects Category II, Gene Mutations. It is, however, recommended that the future submission of studies performed with this cell line contain historical control data.

A. MATERIALS:

1. Test Material: Sumilarv (S-31183)

Description: Pale yellow viscous liquid; the chemical structure was also provided (see DER 93-105).
Identification number: Lot number: PYG-87074
Purity: 95.3%
Receipt date: Not reported
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material storage conditions were not reported; solutions of the test material were prepared in DMSO immediately prior to use.

2. Control Materials:

Negative: None

Solvent/concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMSO to yield a final concentration of 200 µg/mL.

Activation (concentrations, solvent): 9, 10-Dimethyl-1,2-benzanthracene was prepared in DMSO to yield a final concentration of 5 µg/mL.

3. Activation: S9 derived from 7-week old male Sprague-Dawley

_____ Aroclor 1254	__x__ induced	__x__ rat	__x__ liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
__x__ other (Kanechlor-400)		_____ other	

The S9 homogenate was prepared by the performing laboratory and the S9 mix contained the following components:

S9 mix composition:

Component	Concentration
NADPH	4 mM
Glucose 6-phosphate	5 mM
$MgCL_2$	5 mM
KCL	33 mM
Hepes buffer (pH 7.2)	4 mM
S9	30%

Note: One milliliter of the S9 mix was added to 9.0 mL of serum free Eagle's minimum essential medium (EMEM).

4. Test Cells: Mammalian cells in culture

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

Properly maintained? Storage conditions for the stock cultures were not reported.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Not reported.

5. Locus Examined:

_____ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)

__x__ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) 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:

(a) Preliminary cytotoxicity assay: Seven concentrations (0.3, 1, 3, 10, 30, 100, and 300 µg/mL) were evaluated in the absence and presence of S9 activation.

(b) Mutation assay: Two nonactivated and two S9-activated assays were performed; doses were as follows:

(1) Nonactivated conditions:

Initial trial: 10, 30, 100, and 300 µg/mL.

Repeat trial: As above for the initial nonactivated trial.

(2) S9-activated conditions:

Initial trial: 3, 10, 30, and 100 µg/mL.

Repeat trial: As above for the initial S9-activated trial.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound or negative and positive controls for:
__5__ hours (nonactivated) __5__ hours (activated)

(b) After washing, cells were cultured for __7__ days (expression period) before cell selection.

(c) After expression, 3x10^5 cells/dish (10 dishes) were cultured for __7__ days in selection medium to determine numbers of mutants; 100 cells/dish (5 dishes) were cultured for __6__ days in nonselection medium to determine plating efficiency.

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria: The test material was considered positive if: (a) the mutation frequencies (MFs) of the treatment groups were >3-fold higher than the corresponding vehicle control group and higher than "the highest historical control data" (not provided), and (2) the response was both dose related and reproducible.

4. Protocol: None presented

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Test: Compound precipitation was observed in cultures treated with the highest dose (300 µg/mL +S9). At this level in the nonactivated phase of the cytotoxicity test, 75% of the cells survived. With S9 activation, survival was dose dependent and ranged from 7% at 300 µg/mL to >100% at doses ≥1 µg/mL. Based on these results, dose ranges selected for the mutation assays were 10-300 µg/mL -S9 and 3-100 µg/mL +S9.

2. Mutation Assays: Representative results from the two independently performed nonactivated and S9-activated mutation assays with the test material are presented in Table 1. In agreement with the preliminary findings, 300 µg/mL -S9 was insoluble and slightly cytotoxic. There was, however, no indication of a mutagenic response over the concentration range selected for the nonactivated trials. In the presence of S9 activation, ≥12% of the cells survived the 5-hour treatment with 100 µg/mL S-31183. Percent survival for the remaining S9-activated concentrations was dose dependent in both trials and ranged from ~56% at 30 µg/mL to ~91% at 3 µg/mL. The results further show that S9-activated S-31183 was not mutagenic. The slight increase in the MF at 100 µg/mL +S9 in the repeat trial (11.3x10^{-6} mutants/colony forming unit) was not considered by our reviewers to be suggestive of a genotoxic response. The increase was not seen in the

TABLE 1. Representative Results of the Chinese Hamster V79 Cell Forward Gene Mutation Assays with Sumilarv (S-31183)

Substance	Dose/mL	S9 Activation	Relative Percent Plating Efficiency (After Treatment)	Total Mutant Colonies per 10 Dishes	Absolute Percent Plating Efficiency (at Selection)	Mutation Frequency $x10^{-6}$[a]
Solvent Control						
Dimethyl sulfoxide	1%	-[b]	100	12	76.8±7.0	5.2±5.3
	1%	-[c]	100	22	76.2±10.4	9.6±5.4
	1%	+[b]	100	16	89.0±12.2	6.0±4.7
	1%	+[c]	100	10	71.8±9.3	4.6±4.9
Positive Control						
Ethyl methanesulfonate	200 µg	-[b]	105	444	75.2±3.0	196.8±26.7
	200 µg	-[c]	110	421	76.2±4.4	184.2±34.0
9,10-Dimethyl-1,2-benzanthracene	5 µg	+[b]	78	535	74.2±11.3	240.3±26.6
	5 µg	+[c]	71	541	72.8±6.2	247.7±30.9
Test Material						
Sumilarv (S-31183)	100 µg[d]	-[b]	91	17	82.6±7.7	6.9±6.0
	300 µg[e]	-	61	13	76.2±5.8	5.7±5.9
	100 µg[d]	-[c]	104	12	77.6±4.6	5.2±5.7
	300 µg[e]	-	91	6	71.6±5.9	2.8±3.9
	30 µg[d]	+[b]	56	24	85.8±9.2	9.3±6.1
	100 µg	+	12	11	83.4±7.2	4.4±5.1
	30 µg[d]	+[c]	57	4	76.6±3.7	1.7±3.0
	100 µg	+	16	28	82.6±11.2	11.3±7.3

[a]Mutation Frequency (MF) = $\frac{\text{Total Mutant Colonies}}{\text{No. of Cells Plated } (3x10^6) \text{ x Plating Efficiency}}$ x 100.

[b]Initial assay

[c]Repeat assay

[d]Results for lower doses (10 and 30 µg/mL -S9 and 3 and 10 µg/mL +S9) did not suggest a mutagenic effect.

[e]Compound precipitation was reported at this concentration.

initial trial and was confined to this dose. Similarly, the calculated MF approximated the expected spontaneous frequency for CHO V79 cells ($\leq 1 \times 10^{-5}$ mutants/colony forming units).[1] By contrast, the nonactivated (200 µg/mL EMS) and S9-activated (5 µg/mL DMBA) positive controls induced powerful mutagenic responses in both trials.

Based on the overall findings, the study author concluded that "S-31183 is not mutagenic under the conditions tested."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the study author's interpretation of the data was correct. In both the presence and absence of S9 activation, sumilarv (S-31183) was assayed to insoluble (300 µg/mL -S9) or cytotoxic (100 µg/mL +S9) levels but failed to induce a mutagenic response in two independently performed studies. In addition, the sensitivity of the assay to detect mutagenesis was adequately demonstrated by the results obtained with 200 µg/mL EMS -S9 and 5 µg/mL DMBA +S9 in both trials. We conclude, therefore, that sumilarv (S-31183) was adequately tested and found to be nonmutagenic in this in vitro mammalian cell gene mutation assay. It is, however, recommended that future submissions of studies performed with this cell line contain historical control data.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A signed quality assurance statement dated April 26, 1990 was provided.)

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.2a) for genetic effects, Category I, Gene Mutations.

[1]Bradley, M.O., Bhuyan, B., Francis, M.C., Langenbach, R., Peterson, A., Huberman, E. (1981). Mutagenesis by chemical agents in V79 Chinese hamster cells: A review and analysis of the literature. Mutat. Res. 87:81-142.