2/10/93

# FINAL

DATA EVALUATION REPORT

SUMILARV

Study Type: Mutagenicity: Salmonella typhimurium/Escherichia coli/Mammalian Microsome Preincubation Mutagenicity Assay

Prepared for:

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Environmental Protection Agency
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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/Escherichia coli/mammalian microsome preincubation mutagenicity assay

EPA IDENTIFICATION Numbers:

P.C. Code: 129032

CASWELL Number: None

MRID Number: 421783-15

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: 153; Reference Number NNT-80-0034

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

TITLE OF REPORT: Sumilarv--Reverse Mutation Test of S-31183 in Bacterial Systems

AUTHOR: S. Kogsio

REPORT ISSUED: April 23, 1988

CONCLUSIONS--EXECUTIVE SUMMARY: Under the conditions of two independently performed microbial/mammalian microsome preincubation assays, six doses of sumilarv (S-31183) ranging from 10 to 5000 µg/plate +/-S9 were not cytotoxic or mutagenic in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, TA100 or in Escherichia coli strain $WP_2$ uvrA. The highest nonactivated dose (5000 µg/plate) was insoluble. Based on these findings, it was concluded that sumilarv was tested over an appropriate range of concentrations with no evidence of a mutagenic effect in a well-conducted study.

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

A. MATERIALS:

1. Test Material: Sumilarv (S-31183)

Description: A physical description was not provided; however, the chemical structure was reported.
Identification Number: Lot number: PTG-86011
Purity: 97.2%
Receipt date: Not reported
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material storage conditions and the frequency of test material preparation were not reported.

2. Control Materials:

Negative: None

Solvent/concentration: DMSO/0.1 mL/plate

Positive:

Nonactivation:

| | | |
|---|---|---|
| Sodium azide | 0.5 µg/plate | TA1535 |
| Methylmethane sulfonate | 200 µg/plate | TA100 |
| 2-Nitrofluorene | 2 µg/plate | TA1538 |
| | 1 µg/plate | TA98 |
| ICR-191 | 1 µg/plate | TA1537 |
| N-ethyl-N'-nitro-N-nitrosoquanidine | 2 µg/plate | $WP_2$ uvrA |

Activation:

| | | |
|---|---|---|
| 2-Aminoanthracene | 2 µg/plate | TA1535 |
| | 80 µg/plate | $WP_2$ uvrA |
| Benzo(a)pyrene | 5 µg/plate | TA1537, TA1538, TA98, TA100 |

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

S9 mix composition:

| Component | Amount/mL of S9 mix |
|---|---|
| Phosphate buffer (pH 7.4) | 100 mM |
| Glucose 6-phosphate | 1.7 mg |
| NADPH | 3.62 mg |
| NADH | 3.05 mg |
| $MgCl_2$ | 8 mM |
| KCl | 31.5 mM |
| S9 | 0.1 mL |

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538
list any others: E. coli $WP_2$ uvrA

Test organisms were properly maintained? Not reported.
Checked for appropriate genetic markers (rfa mutation, R factor)? Not reported.

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Six doses (10, 50, 100, 500, 1000 and 5000 µg/plate) were evaluated with or without S9 activation in all tester strains except S. typhimurium TA1538.

(b) Mutation assay:

(1) Initial: Six doses (10, 50, 100, 500, 1000, and 5000 µg/plate) were evaluated in the presence and absence of S9 activation; all tester strains were used. Duplicate plates were prepared per dose, per strain, per condition.

(2) Confirmatory: As above

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
_____ Standard plate test
__x__ Pre-incubation (20) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe)

2. Preliminary Cytotoxicity: The procedures used in the preliminary cytotoxicity assay were not reported.

3. Mutagenicity Assay: The test material, solvent or positive controls were delivered in 0.1-mL volumes along with 0.1 mL of the appropriate bacterial strain and 0.5 mL of 100 mM sodium phosphate buffer (pH 7.4) or 0.5 mL of the S9 mix to top-agar tubes. The tubes were mixed and preincubated with shaking for 20 minutes at 37°C. Following

pretreatment, 2 mL of molten agar, supplemented with 0.05 mM histidine, 0.05 mM biotin, and 0.05 mM tryptophan were added to each tube. The contents of the tubes were mixed and poured over Vogel Bonner E minimal glucose agar plates; cultures were incubated for 65 hours at 37°C and revertant colonies were counted.

4. Evaluation Criteria: The test material was considered positive if a ≥2-fold increase in mutant colonies of any strain was observed and the effect was dose related.

5. Protocol: A protocol was not provided.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: No data were presented for the preliminary cytotoxicity assay. The report stated that the test material was insoluble at the highest nonactivated dose (5000 µg/plate). There was, however, no reduction of the background lawn of growth or revertant colonies at any concentration with or without S9 activation. Based on these findings, doses ranging from 10 to 5000 µg/plate were selected for the two independently conducted nonactivated and S9-activated preincubation mutation assays.

2. Mutation Assays: Representative data from the initial and confirmatory mutation assays with the test material are presented in Tables 1 and 2, respectively. Results from both trials were in good agreement and indicated that 5000 µg/plate of nonactivated sumilarv precipitated. There was, however, no indication of a cytotoxic or mutagenic response in any strain either in the presence or absence of S9 activation. Our reviewers noted the relatively high revertant counts for strain TA1537 and the relatively low revertant counts for strain TA100 plated with the the S9-activated solvent control in both trials. However, the outcome of the study was unaffected. All strains, including TA1537 and TA100, responded in the expected manner to the appropriate direct-acting or promutagenic positive controls in both trials. Based on the overall results, the study author concluded, that "S-31183 is not mutagenic under the conditions tested."

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. Both in the presence and absence of S9 activation, sumilarv was tested over an appropriate range of concentrations but failed to induce a mutagenic effect. In addition, the response of all tester strains to the appropriate nonactivated and S9-activated positive control demonstrated that the assay had an adequate level of sensitivity to detect a mutagenic response. It was concluded, therefore, that sumilarv was negative in this microbial test system.

TABLE 1. Representative Results of the Initial Preincubation Microbial Mutagenicity Assay with Sumilarv (S-31183)

| Substance | S9 Activation | Dose/ Plate | Revertants per Plate of Bacterial Tester Strains[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 | $WP_2$ uvrA |
| **Solvent Control** | | | | | | | | |
| Dimethyl sulfoxide | - | 0.1 mL | 8 | 7 | 12 | 30 | 77 | 18 |
| | + | 0.1 mL | 11 | 25 | 37 | 48 | 68 | 17 |
| **Positive Controls** | | | | | | | | |
| Sodium azide | - | 0.5 µg | 388 | -- | -- | -- | -- | -- |
| ICR-191 | - | 1.0 µg | -- | 1288 | -- | -- | -- | -- |
| 2-Nitrofluorene | - | 1.0 µg | -- | -- | -- | 520 | -- | -- |
| | - | 2.0 µg | -- | -- | 536 | -- | -- | -- |
| Methyl methane-sulfonate | - | 200.0 µg | -- | -- | -- | -- | 445 | -- |
| N-ethyl-N'-nitro-N-nitrosoguanidine | - | 2.0 µg | -- | -- | -- | -- | -- | 428 |
| 2-Aminoanthracene | + | 2.0 µg | 179 | -- | -- | -- | -- | -- |
| | + | 80.0 µg | -- | -- | -- | -- | -- | 610 |
| Benzo(a)pyrene | + | 5.0 µg | -- | 169 | 218 | 674 | 954 | -- |
| **Test Material** | | | | | | | | |
| Sumilarv (S-31183) | - | 5000 µg[b,c] | 10 | 14 | 13 | 29 | 87 | 18 |
| | + | 5000 µg[c] | 15 | 32 | 27 | 37 | 79 | 21 |

[a]Average counts from duplicate plates
[b]Compound precipitation reported at this level.
[c]Results for lower doses (10, 50, 100, 500, or 1000 µg/plate +/-S9) did not suggest a mutagenic effect.

TABLE 2. Representative Results of the Confirmatory Preincubation Microbial Mutagenicity Assay with Sumilarv (S-31183)

| Substance | S9 Activation | Dose/ Plate | Revertants per Plate of Bacterial Tester Strains[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA1535 | TA1537 | TA1538 | TA98 | TA100 | $WP_2$ uvrA |
| Solvent Control | | | | | | | | |
| Dimethyl sulfoxide | - | 0.1 mL | 14 | 14 | 7 | 31 | 81 | 16 |
| | + | 0.1 mL | 9 | 36 | 32 | 49 | 68 | 18 |
| Positive Controls | | | | | | | | |
| Sodium azide | - | 0.5 µg | 304 | -- | -- | -- | -- | -- |
| ICR-191 | - | 1.0 µg | -- | 1370 | -- | -- | -- | -- |
| 2-Nitrofluorene | - | 1.0 µg | -- | -- | -- | 395 | -- | -- |
| | - | 2.0 µg | -- | -- | 611 | -- | -- | -- |
| Methyl methane-sulfonate | - | 200.0 µg | -- | -- | -- | -- | 302 | -- |
| N-ethyl-N'-nitro-N-nitrosoguanidine | - | 2.0 µg | -- | -- | -- | -- | -- | 316 |
| 2-Aminoanthracene | + | 2.0 µg | 143 | -- | -- | -- | -- | -- |
| | + | 80.0 µg | -- | -- | -- | -- | -- | 451 |
| Benzo(a)pyrene | + | 5.0 µg | -- | 149 | 118 | 433 | 558 | -- |
| Test Material | | | | | | | | |
| Sumilarv (S-31183) | - | 5000 µg[b,c] | 7 | 9 | 8 | 31 | 66 | 21 |
| | | 5000 µg[c] | 8 | 26 | 25 | 39 | 66 | 19 |

[a]Average counts from duplicate plates
[b]Compound precipitation reported at this level.
[c]Results for lower doses (10, 50, 100, 500, or 1000 µg/plate +/-S9) did not suggest a mutagenic effect.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement dated April 23, 1988 was provided.)

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