

2/10/93

FINAL

DATA EVALUATION REPORT

SUMILARV

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

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
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Arlington, VA 22202

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Contract Number: 68D10075
Work Assignment Number: 1-122
Clement Number: 93-103
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GUIDELINE SERIES 84: MUTAGENICITY
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MUTAGENICITY STUDIES

EPA Reviewer: Irving Mauer, Ph.D.

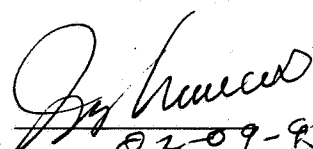
Immediate Office

Health Effects Division (H-7509C)

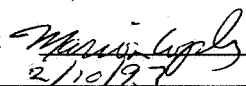
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Date: 02-09-93

Signature: 

Date: 2/10/93

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;
C20H19NO3/95737-68-1

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 $\mu\text{g}/\text{plate}$ +/-S9 were not cytotoxic or mutagenic in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, TA100 or in Escherichia coli strain WP₂ uvrA. The highest nonactivated dose (5000 $\mu\text{g}/\text{plate}$) 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	<u>0.5</u>	µg/plate TA1535
Methylmethane sulfonate	<u>200</u>	µg/plate TA100
2-Nitrofluorene	<u>2</u>	µg/plate TA1538
	<u>1</u>	µg/plate TA98
ICR-191	<u>1</u>	µg/plate TA1537
N-ethyl-N'-nitro-N-nitrosoquanidine	<u>2</u>	µg/plate WP ₂ uvrA

Activation:

2-Aminoanthracene	<u>2</u>	µg/plate TA1535
	<u>80</u>	µg/plate WP ₂ uvrA
Benzo(a)pyrene	<u>5</u>	µg/plate TA1537, TA1538, TA98, TA100

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

<u> </u> Aroclor 1254	<u> x </u> induced	<u> x </u> rat	<u> x </u> liver
<u> </u> phenobarbital	<u> </u> noninduced	<u> </u> mouse	<u> </u> lung
<u> </u> none		<u> </u> hamster	<u> </u> other
<u> x </u> other (Kanechlor-400)		<u> </u> other	

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

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S9 mix composition:

<u>Component</u>	<u>Amount/mL of S9 mix</u>
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 WP₂ 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	
<u>Solvent Control</u>									
Dimethyl sulfoxide	-	0.1 mL	8	7	12	30	77	18	
	+	0.1 mL	11	25	37	48	68	17	
<u>Positive Controls</u>									
Sodium azide	-	0.5 µg	388	--	--	--	--	--	
ICR-191	-	1.0 µg	--	1288	--	--	--	--	
2-Nitrofluorene	-	1.0 µg	--	--	--	520	--	--	
Methyl methane-sulfonate	-	2.0 µg	--	--	536	--	--	--	
	-	200.0 µg	--	--	--	--	445	--	
N-ethyl-N'-nitro-N-nitrosoguanidine	-	2.0 µg	--	--	--	--	--	428	
2-Aminoanthracene	+	2.0 µg	179	--	--	--	--	--	
Benzo(a)pyrene	+	80.0 µg	--	--	--	--	--	610	
	+	5.0 µg	--	169	218	674	954	--	
<u>Test Material</u>									
Sumilarv (S-31183)	-	5000 µg ^{b,c}	10	14	13	29	87	18	
	+	5000 µg ^c	15	32	27	37	79	21	

^aAverage counts from duplicate plates^bCompound precipitation reported at this level.^cResults 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)

Revertants per Plate of Bacterial Tester Strains ^a								
Substance	S9 Activation	Dose/ Plate	TA1535	TA1537	TA1538	TA98	TA100	WP ₂ uvrA
<u>Solvent Control</u>								
Dimethyl sulfoxide	-	0.1 mL	14	14	7	31	81	16
	+	0.1 mL	9	36	32	49	68	18
<u>Positive Controls</u>								
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
<u>Test Material</u>								
2-Aminoanthracene	+	2.0 µg	143	--	--	--	--	--
	+	80.0 µg	--	--	--	--	--	451
Benzo(a)pyrene	+	5.0 µg	--	149	118	433	558	--
<u>Test Material</u>								
Sumilarv (S-31183)	-	5000 µg ^{b,c}	7	9	8	31	66	21
		5000 µg ^c	8	26	25	39	66	19

^aAverage counts from duplicate plates^bCompound precipitation reported at this level.^cResults for lower doses (10, 50, 100, 500, or 1000 µg/plate +/-S9) did not suggest a mutagenic effect.

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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.