2/10/93

FINAL

DATA EVALUATION REPORT

SUMILARV

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in HeLa Cells

Prepared for:

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Work Assignment Number: 1-122
Clement Number: 93-105
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MUTAGENICITY STUDIES

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Date: 2/10/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in HeLa cells

EPA IDENTIFICATION Numbers:

P.C. Code: 129032

CASWELL Number: None

MRID Number: 421783-17

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: SMO 298; Reference Number NNT-91-0053

TESTING FACILITY: Huntingdon Research Centre, Ltd., Cambridgeshire, England

TITLE OF REPORT: Sumilarv--Assessment of Unscheduled DNA Repair Synthesis in Mammalian Cells After Exposure to S-31183

AUTHORS: Henderson, L.M., and Proudlock, R.J

REPORT ISSUED: July 29, 1988

CONCLUSIONS--EXECUTIVE SUMMARY: Sumilarv (S-31183) at doses ranging from 0.1 to 204.8 µg/mL was evaluated for the potential to induce unscheduled DNA synthesis (UDS) in two independently performed nonactivated and S9-activated assays using HeLa cells. Results indicated that the highest dose tested (204.8 µg/mL +/-S9) was insoluble; marked cytotoxicity was achieved at nonactivated levels ≥6.4 µg/mL and at S9-activated concentrations ≥51.2 µg/mL in both trials. There was, however, no evidence that the test material induced a genotoxic response. We conclude, therefore, that sumilarv (S-31183) was adequately tested and found to be nongenotoxic in this test system.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms. It is, however, recommended that future submissions contain historical control data.

A. MATERIALS:

1. Test Material: Sumilarv (S-31183)

Description: Pale yellow viscous liquid; the chemical structure was also provided.
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 was stored at room temperature, protected from light; the frequency of test material solution preparation was not reported.

2. Indicator Cells: HeLa S3 epithelioid cells were obtained from Flow Laboratories, Ltd. Cells were maintained in Eagle's Minimum Essential Medium (EMEM) containing Earle's salts, 15% fetal calf serum, and antibiotics. Prior to use cells were resuspended at a density of 5×10^4 cells/mL in fresh EMEM.

3. Control Substances:

Negative: None

Solvent/concentration: DMSO/100 µL/culture

Positive:

Nonactivation: 0.02, 0.04, 0.08, 0.16, and 0.32 µg/mL 4-nitroquinoline-1-oxide (4-NQO)

S9 Activation: 2.5, 5, 10, 20, and 40 µg/mL 2-aminoanthracene (2-AA)

4. Activation: S9 derived from 6-8-week old male Sprague-Dawley

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ 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
0.15 m KCL	0.2 mL
Glucose 6-phosphate	0.036 g
NADP	0.005 g
Distilled water	0.4 mL
S9 fraction	0.4 mL

5. Test Compound Concentrations Used:

   (a) Trial 1: The 12 doses initially evaluated with and without S9 activation were 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, 102.4, and 204.8 µg/mL. Cells exposed to levels ranging from 0.1-3.2 µg/mL -S9 and 0.1 to 102.4 µg/mL +S9 were scored.

   (b) Trial 2: Doses equivalent to those used in Trial 1 were assayed; cells exposed to 0.1-1.6 µg/mL -S9 and 0.1-102.4 µg/mL +S9 were scored.

B. TEST PERFORMANCE:

1. Exposure: Aliquots of prepared cell suspensions (5×10^4 cells/mL) were dispensed into multi-well tissue culture dishes containing glass coverslips. Cultures were placed in a humid environment for 96 hours and refed arginine-deficient medium over a 72-hour period. Tritiated thymidine (final concentration, 5 µCi/mL), S9 mix for the activated test, and the appropriate concentration of the test material and solvent or positive controls were added. At the conclusion of a 180-minute exposure, coverslips were washed, fixed, stained and mounted for autoradiography.

2. Preparation of Autoradiographs: Slides were coated with Kodak AR-10 and dried sequentially at room temperature and at 4°C in the dark to allow exposure of the photographic emulsion to the radioactive isotope. Autoradiographs were developed in Kodak D19, rinsed, fixed, air dried and coded.

3. Grain Counting: The number of silver grains overlaying the nuclei of 100 non-S-phase cells was counted for each treatment and control culture. Net nuclear grain counts were determined by subtracting the cytoplasmic grain count of a corresponding area adjacent to each nucleus from the nuclear grain count. The number of S-phase nuclei having >3 grains/nucleus was recorded.

4. Evaluation Criteria: The assay was considered positive if a reproducible and statistically significant increase in the number of nuclear grains over compound-treated cells was observed when compared to the concurrent controls.

5. Statistical Analysis: The results were analyzed for significance (p values of <0.05, <0.01, or <0.001) by one-way analysis of variance (ANOVA).

6. Protocol: A protocol was not provided.

C. REPORTED RESULTS: The highest dose assayed in both trials (204.8 µg/mL) was insoluble in the presence or absence of S9 activation. Under nonactivated conditions, doses ≥6.4 µg/mL (initial trial) and ≥3.2 µg/mL (repeat trial) were severely cytotoxic; cells exposed to these concentrations were not scored for UDS. Cytotoxicity was also observed at the highest nonactivated levels scored for the incorporation of ^{3}HTdR (3.2 and 1.6 µg/mL in the initial and repeat trials, respectively) (Table 1). No significant increases in UDS occurred in any treatment group of either trial. The elevated number of average net nuclear grains reported for 3.2 µg/mL in the initial trial was not reproduced in the repeat assay. Our reviewers did, however, note the relatively wide variation in the average solvent control counts (-6 to 29 net nuclear grains).

In the presence of S9 activation, net nuclear grain counts for the solvent-treated groups were more consistent between trials (-30 and -22 net nuclear grains in the initial and repeat trials, respectively). However, the study authors stated that "-30 grains per 100 nuclei" was an unusually low vehicle control results and attributed the significant increases in UDS seen at several doses in the initial S9-activated trial (Table 2) to the low background counts. We tend to agree with the study authors that the significantly increased average net nuclear grain counts observed in the first trial were not biologically meaningful. Gross nuclear grain counts for the majority of treatment groups showing a statistically significant increase were either comparable to or lower than the control. The data further suggest that cytotoxicity, as indicated by the general trend of decreasing cytoplasmic grain counts with increasing doses of the test material, was responsible for the increased net yield of nuclear grains. In the repeat trial, the increases in net nuclear grains observed at doses ranging from 3.2 to 51.2 µg/mL resulted from test material cytotoxicity.

Based on the overall findings, the study authors concluded that "S-31183 has shown no evidence of causing DNA damage in this in vitro test for mutagenic potential."

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the results of the two independently performed HeLa cell UDS assays conducted with sumilarv (S-31183) provided no convincing evidence of a genotoxic effect. We further assess that the test material was evaluated over a range of concentrations that included an insoluble level (204.8 µg/mL +S9), markedly cytotoxic doses (≥3.2 µg/mL -S9; ≥51.2 µg/mL +S9) and noncytotoxic doses. Additionally, the sensitivity of the test system to detect genotoxicity was clearly demonstrated in both trials by the significant dose-related results obtained with the nonactivated positive control

TABLE 1. Representative Results of the Nonactivated HeLa Cell Unscheduled DNA Synthesis Assays with Sumilarv (S-31183)

Substance	Dose	No. of Scored Nuclei	Grains per 100 Cells[a]	Cytoplasmic Grains per 100 Cells[a]	Average Net Nuclear Grains 100 Cells[a]	Percent Cells with ≥3 Net Nuclear Grains[a]
Solvent Control						
Dimethyl sulfoxide	100 µL	1000[b]	130	136	-6	1.3
	100 µL	1000[c]	146	117	29	1.5
Positive Control[d]						
4-Nitroquinoline-1-oxide	0.02 µg/mL	200[b]	1647	166	1481*	100.0
	0.02 µg/mL	200[c]	1924	104	1820*	100.0
Test Material						
Sumilarv	1.6 µg/mL[e]	200[b]	138	143	-5	2.0
	3.2 µg/mL[f,g]	200	152	128	25	1.0
	0.8 µg/mL[e]	200[c]	112	121	-9	0.5
	1.6 µg/mL[f,g]	200	109	111	-3	0.5

[a]Average value of duplicate cultures for the positive controls and test material doses; average value of ten replicate cultures for the solvent controls; calculated by our reviewers.
[b]Results from the initial trial
[c]Results from the repeat trial
[d]Five levels of the positive control were assayed, and the lowest dose was selected as representative; all other concentrations induced significant increases in UDS.
[e]Results for lower levels (0.1, 0.2, and 0.4 µg/mL in both trials, and 0.8 µg/mL in the initial trial) did not provide convincing evidence of a genotoxic response.
[f]Cytotoxicity was reported at this concentration.
[g]Higher doses (6.4, 12.8, 25.6, 51.2, 102.4, and 204.8 µg/mL in both trials and 3.2 µg/mL in the repeat trial) were severely cytotoxic.

NOTE: The highest assayed concentration (204.8 µg/mL) was reported to be insoluble.

*Significantly increased compared to the vehicle control ($p<0.001$) by ANOVA

TABLE 2. Representative Results of the S9-Activated HeLa Cell Unscheduled DNA Synthesis Assays with Sumilarv (S-31183)

Substance	Dose	No. of Scored Nuclei	Grains per 100 Cells[a]	Cytoplasmic Grains per 100 Cells[a]	Average Net Nuclear Grains 100 Cells[a]	Percent Cells with ≥3 Net Nuclear Grains[a]
Solvent Control						
Dimethyl sulfoxide	100 µL	1000[b]	161	191	-30	1.4
	100 µL	1000[c]	158	180	-22	1.8
Positive Control[d]						
2-Aminoanthracene	5.0 µg/mL	200[b]	349	192	158***	16.5
	2.5 µg/mL	200[c]	321	186	135**	9.5
Test Material						
Sumilarv	0.4 µg/mL[e]	200[b]	209	198	12*	2.0
	0.8 µg/mL	200	167	161	6*	0.5
	1.6 µg/mL	200	164	137	27**	2.5
	3.2 µg/mL	200	152	149	3*	0.5
	6.4 µg/mL	200	150	158	-7	0.0
	12.8 µg/mL	200	136	105	31**	1.5
	25.6 µg/mL[f]	200	135	139	-4	1.5
	51.2 µg/mL[f]	200	133	126	7*	2.0
	102.4 µg/mL[f,g]	200	113	111	3*	0.5
	3.2 µg/mL[e]	200[c]	161	171	-10	1.0
	6.4 µg/mL	200	167	158	9	1.0
	12.8 µg/mL	200	180	162	18	3.5
	25.6 µg/mL	200	162	137	25*	3.0
	51.2 µg/mL[f]	100	166	136	30	2.0
	102.4 µg/mL[g]	200	132	138	-5	0.5

[a]Average value of duplicate cultures for the positive controls and test material doses; average value of ten replicate cultures for the solvent control

[b]Results from the initial trial

[c]Results from the repeat trial

[d]Five levels of the positive control were assayed, the lowest dose showing a highly significant increase in UDS was selected as representative.

[e]Results for lower levels (0.1 and 0.2 µg/mL in both trials and 0.4, 0.8, and 1.6 µg/mL in the repeat trial) did not provide convincing evidence of a genotoxic response.

[f]Cytotoxicity reported at this concentration; only one culture at 51.2 µg/mL in the second trial could be scored.

[g]The highest assayed concentration (204.8 µg/mL) was severely cytotoxic and insoluble in both trials.

*Significantly increased compared to the vehicle control ($p<0.05$) by ANOVA

**Significantly increased compared to the vehicle control ($p<0.01$) by ANOVA

***Significantly increased compared to the vehicle control ($p<0.001$) by ANOVA

(0.02-0.32 µg/mL 4-NQO) and S9-activated positive control (2.5-40 µg/mL 2-AA). We conclude, therefore, that the study is acceptable. It is, however, recommended that historical background control data be submitted with future studies since the UDS assay is infrequently performed in HeLa cells, particularly in combination with autoradiographic procedures.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement signed and dated June 22, 1988.)

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms. It is, however, recommended that future submissions contain historical control data.