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SC-19192: AN EVALUATION OF MUTAGENIC POTENTIAL
EMPLOYING THE HOST-MEDIATED ASSAY IN THE RAT

P-T NO. 1029H72

FINAL REPORT

Submitted to

Searle Laboratories
Chicago, Illinois



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HAZLETON LABORATORIES

a subsidiary of Environmental Sciences Corporation

SPONSOR: Searle Laboratories

DATE: August 28, 1972

MATERIAL: SC-19192

LOT NO: 3R-A-7273

SUBJECT: FINAL REPORT
An Evaluation of Mutagenic Potential Employing the Host-Mediated
Assay in the Rat
Project No. 700-269

SUMMARY

SC-19192 was administered orally to four groups of 10 male albino rats each, for five consecutive days at dose levels of 0.25, 0.5, 1.0, and 2.0 g/kg/day, given in three equally divided doses. One group of 10 rats served as a control and received the vehicle, and a sixth group served as a positive control and received a single dose of 100 mg/kg of dimethylnitrosamine intraperitoneally on the fifth day only. Following the final dose, the animals were inoculated with Salmonella typhimurium, G-46, by intraperitoneal injection. Three hours later the organisms were recovered, and the peritoneal washings evaluated for the presence of mutant organisms.

There was a dose-related increase in the incidence and severity of soft feces among the groups of animals treated with SC-19192 for five days, with two very high dose animals dying during this period. Body weight gains and food consumption were likewise, decreased for treated animals.

Subsequent evaluation of the mutation frequencies from rats treated with SC-19192 showed no significant alterations from that observed for the negative control animals. Dimethylnitrosamine, employed as a positive control, was shown to be a potent mutagen in this test system, evoking a mutation frequency eight times that of the control group.



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INTRODUCTION

The purpose of this study was to evaluate the mutagenic potential of SC-19192 employing the host-mediated assay in the rat.

MATERIAL

Identification SC-19192; Lot No. 3R-A-7273.

Description White, lumpy powder with no noticeable odor.

Received June 6, 1972.

Purity Assumed to be 100% active ingredient.

METHOD

Animal Groups

Sixty healthy male albino rats of the Purina Caesarean-derived strain, approximately 12 weeks of age at initiation of treatment, were selected at random and divided into the following groups.



<u>Group No.</u>	<u>Treatment</u>	<u>No. of Animals</u>	<u>Dosage Level*</u>
1	Vehicle Control	10	40 ml/kg/day
2	Positive Control (Dimethylnitrosamine)	10	100 mg/kg**
3	SC-19192	10	0.25 g/kg/day
4	SC-19192	10	0.5 g/kg/day
5	SC-19192	10	1.0 g/kg/day
6	SC-19192	10	2.0 g/kg/day

* Administered in three equally divided doses daily for five days.

** Administered intraperitoneally as a single dose on the final (5th) day of treatment for the other groups.

Compound Preparation

The SC-19192 was prepared fresh daily as a 10% aqueous suspension (w/v) in a Tween 80-water (1:99) solution (v/v). The positive control material (dimethylnitrosamine, Eastman, Lot No. 691-1) was prepared as a 100 mg/ml solution in sterile distilled water.

Inneculation and Recovery Procedure

Thirty minutes after the last dosing, all treated and control rats received 2 ml. of a tryptone broth suspension (approximately 8×10^8 CFU/ml) of Salmonella typhimurium, strain G-46, a histidine auxotroph, by intraperitoneal injection. Three hours after such inneculation, each rat was sacrificed by cervical dislocation, the peritoneal cavity opened aseptically and washed with 2 ml. of sterile saline. As much fluid as possible was then removed from the peritoneal cavity, and the sample kept on ice until cultured.



Dilution and Plating

Each sample was plated on Spizizen's Minimal Medium* for the mutant cell count, and on tryptone agar for the total cell count.

Dilution blanks containing 4.5 ml. sterile saline were prepared in advance. Tenfold serial dilutions were made of each sample (0.5 ml. + 4.5 ml.) yielding a concentration series from 10^0 (undiluted peritoneal exudate) through 10^{-7} .

For enumerating total bacterial counts, the 10^{-5} , 10^{-6} , and 10^{-7} dilutions were plated on tryptone agar: 3 plates/sample, 0.2 ml. sample/plate. Each sample was spread over the surface of a 100 mm. plate using a bent glass rod which had been immersed in 95% ethanol and flamed just prior to use.

In plating for the total mutant counts, the 10^0 and 10^{-1} dilutions were used. Additionally the 10^{-2} dilution was also plated for the Group No. 2 (positive control) animals. For the undiluted washings only three plates were used, due to limited volume available, with three plates used for each additional dilution. The plating procedure was identical to that followed for the tryptone agar plates. Determination of mutant organisms was characterized by growth upon the histidine-free medium.

All plates were incubated at 37°C ; tryptone plates for 18 hours, and minimal agar plates for 40 hours.

* See appendix for composition.



Scoring

The following procedure was employed to calculate mutation frequency.

$$\frac{\text{No. of Colonies/Plate}}{\text{No. of Plates}} \times 5 = \text{CFU/ml* of sample plated}$$

$$\text{CFU/ml} \times 1/\text{Dilution Factor} = \text{CFU/ml in Undiluted Exudate}$$

$$\text{Mutation Frequency (MF)} = \frac{\text{Total Mutant Cells}}{\text{Total Cells}}$$

RESULTS

General Observations - In-Life Phase

The incidence and frequency of soft feces was increased in a dose-related trend from the low level through the very high dose level. This was observed in 2/10 low dose rats for a single day (usually Day 5) and increased in frequency to where it was recorded for 10/10 very high dose rats on all five days of the study. Two very high level rats died during the treatment period, one on Day 3 and one on Day 5.

One low level rat died following intraperitoneal injection of the indicator organism, probably the result of a faulty injection.

Body weight and food consumption data are presented in Figure No. 1. Rats treated with SC-19192 exhibited weight losses (mean weight losses ranged from 14 to 23 grams) during the five day period which exceeded the very slight mean weight loss (4 grams) shown by the negative control. Treated animals

* Colony forming units.



Figure No. 1 - Mean body weight and food consumption data (\pm s.d.) recorded during the five-day treatment period

Group (Treatment)	Mean Body Weight (g.)		Mean Food Consumption (g.)
	Day 0	Day 5	Five Days
1 (Negative Control)	276 \pm 22	270 \pm 21	91 \pm 14
2 (Positive Control)	280 \pm 16	297 \pm 17	123 \pm 16
3 (Low Level)	282 \pm 8	268 \pm 24	83 \pm 20
4 (Mid Level)	281 \pm 22	265 \pm 25	84 \pm 22
5 (High Level)	283 \pm 10	260 \pm 13	79 \pm 14
6 (Very High Level)	279 \pm 19	262 \pm 23	76 \pm 19

likewise, showed lower food consumption than did the controls. Body weight and food consumption data for the positive control group revealed values greater than that experienced by the negative controls which can probably be related to the lower frequency of handling of these animals during the five day period.

Growth of Indicator Organisms

Recovery of viable organisms indicated that injected Salmonella typhimurium did not replicate optimally in vivo. However, in most cases, sufficient organisms were recovered to determine the incidence of mutant organisms per rat. Total cell counts are presented in Table No. 1, Page No. 9. Statistical analysis of total cell counts reveal no significant differences between groups.

Mutation Frequency Indices

Calculated mutation frequency indices for individual animals are shown in Table No. 1. Group mean indices (\pm s.d.) are shown in Figure No. 2. Although not optimal, it is apparent that replication of organisms was entirely adequate to detect a mutagenic agent. Dimethylnitrosamine was shown to be a potent mutagen in this test system, evoking a mutation frequency 18 times that of the control group.

Figure No. 2 - Mean Mutation Frequency Indices for Rats Treated with SC-19192

<u>Group (Treatment)</u>	<u>Mean MF\pms.d.</u> <u>($\times 10^{-7}$)</u>	<u>MFt/MFc*</u>
1 (Negative Control)	1.26 \pm 0.64	1.00
2 (Positive Control)	23.36 \pm 10.58 ^{S+}	18.53
3 (Low Dose)	2.90 \pm 6.33	2.12
4 (Mid Dose)	0.83 \pm 0.84	0.65
5 (High Dose)	1.34 \pm 1.21	1.06
6 (Very High Dose)	2.19 \pm 1.88	1.73

* MF of Experimental Sample
MF of Control Sample

Value is 1.00 for Control Sample

S+ = Statistically higher than control, according to Student's t-test;
p < 0.01.



Evaluation of the mutation frequency data revealed no significant increases in the mutation frequency among animals treated with SC-19192.

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NOTE: The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Table No. 1 - Total cell counts, mutant cell counts, and mutation frequencies for individual animals treated with SC-19192 dimethylnitrosamine, or which served as control

<u>GROUP NO.</u>	<u>RAT NO.</u>	<u>TOTAL CELLS CFU/ml OF EXUDATE</u> ($\times 10^7$)	<u>MUTANT CELLS CFU/ml OF EXUDATE</u>	<u>MUTATION FREQUENCY</u> ($\times 10^{-7}$)
1 (Control)	1	5.6	8.3	1.5
	2	2.3	3.3	1.4
	3	6.0	15.0	2.5
	4	12.9	21.5	1.6
	5	5.2	8.3	1.6
	6	32.3	50.0	1.5
	7	14.4	11.6	0.8
	8	33.3	28.3	0.8
	9	59.0	35.0	0.6
	10	29.8	10.0	0.3
Mean \pm s.d.		20.1 \pm 18.1	19.1 \pm 14.7	

Table No. 1 - Continued

<u>GROUP NO.</u>	<u>RAT NO.</u>	<u>TOTAL CELLS CFU/ml OF EXUDATE</u> ($\times 10^7$)	<u>MUTANT CELLS CFU/ml OF EXUDATE</u>	<u>MUTATION FREQUENCY</u> ($\times 10^{-7}$)
2 (Positive Control)	11	2.3	101	43.9
	12	13.6	216	15.8
	13	23.0	261	11.3
	14	2.1	23	10.9
	15	20.5	545	26.6
	16	14.8	420	28.4
	17	7.6	185	24.3
	18	1.5	45	30.0
	19	28.5	840	29.5
	20	53.0	685	12.9
Mean \pm s.d.		16.7 \pm 15.8	332.1 \pm 280.3	

Table No. 1 - Continued

<u>GROUP NO.</u>	<u>RAT NO.</u>	<u>TOTAL CELLS CFU/ml OF EXUDATE</u> (x 10 ⁷)	<u>MUTANT CELLS CFU/ml OF EXUDATE</u>	<u>MUTATION FREQUENCY</u> (x 10 ⁻⁷)
3 (Low Dose)	21	6.6	13.0	1.9
	22	25.3	18.0	0.7
	23	4.9	8.0	1.6
	24	11.4	225.0	19.7
	25*	-	-	-
	26	53.3	18.0	0.3
	27	29.3	6.6	0.2
	28	20.8	15.0	0.7
	29	22.3	13.0	0.6
	30	37.8	15.0	0.4

Mean \pm s.d.

23.5 \pm 15.5 36.8 \pm 70.7

* Animal died following injection of the indicator organism.

Table No. 1 - Continued

<u>GROUP NO.</u>	<u>RAT NO.</u>	<u>TOTAL CELLS CFU/ml OF EXUDATE</u> (x 10 ⁷)	<u>MUTANT CELLS CFU/ml OF EXUDATE</u>	<u>MUTATION FREQUENCY</u> (x 10 ⁻⁷)
4 (Mid Dose)	31	15.7	11.5	0.7
	32	23.8	5.0	0.2
	33	23.1	8.0	0.3
	34	6.6	20.0	3.0
	35	20.1	8.0	0.4
	36	12.0	16.5	1.4
	37	24.5	15.0	0.6
	38	33.5	18.0	0.5
	39	28.5	8.0	0.3
	40	17.5	15.0	0.9
Mean ±s.d.		20.5 ± 7.9	12.5 ± 5.1	

Table No. 1 - Continued

<u>GROUP NO.</u>	<u>RAT NO.</u>	<u>TOTAL CELLS CFU/ml OF EXUDATE</u> ($\times 10^7$)	<u>MUTANT CELLS CFU/ml OF EXUDATE</u>	<u>MUTATION FREQUENCY</u> ($\times 10^{-7}$)
5 (High Dose)	41	20.1	15.0	0.7
	42	55.1	28.0	0.5
	43	15.0	16.5	1.1
	44	54.0	33.3	0.6
	45	23.0	23.0	1.0
	46	6.2	8.0	1.3
	47	119.0	21.5	0.2
	48	8.6	10.0	1.2
	49	5.2	13.0	2.5
	50	5.3	23.0	4.3
Mean \pm s.d.		31.2 \pm 36.1	19.13 \pm 8.1	

Table No. 1 - Continued

<u>GROUP NO.</u>	<u>RAT NO.</u>	<u>TOTAL CELLS CFU/ml OF EXUDATE</u> ($\times 10^7$)	<u>MUTANT CELLS CFU/ml OF EXUDATE</u>	<u>MUTATION FREQUENCY</u> ($\times 10^{-7}$)
6 (Very High Dose)	51	6.8	23.0	3.4
	52	13.6	28.0	2.0
	53	56.0	20.0	0.4
	54	15.1	20.0	1.3
	55	36.6	21.5	0.6
	56*	-	-	-
	57	36.1	11.5	0.3
	58**	-	-	-
	59	1.7	8.0	4.7
	60	3.7	18.0	4.8
Mean \pm s.d.		21.2 \pm 19.5	18.8 \pm 6.4	

* Animal found dead on Day 3.

** Animal found dead on Day 5.

APPENDIX

Spizizen's Minimal Medium

4X Salt Solution:

$(\text{NH}_4)_2 \text{SO}_4$	8.0 gram
K_2HPO_4	56.0 gram
KH_2PO_4	24.0 gram
Na Citrate	4.0 gram
Mg SO_4	0.8 gram
H_2O	qs to 1 liter

Sterilize by autoclaving (121°C/15 min.)

Medium:

4X Salt Solution	250 ml
5.0% Glucose (sterile)	100 ml
1.5% Bacto-agar (sterile)	650 ml